IDENTIFICATION OF PEPTIDASES GENERATING CONSTITUTIVE
COLLAGENOLYTIC ACTIVITY OF GE OBACILLUS THERMOLEOVORANS
DSM15325
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Background
While the main biological importance of collagen degradation for prokaryotic pathogens is tightly connected with virulence, thermophilic bacteria use collagenolysis when exploit collagen for nutrition. To date the view that collagen catabolism by thermophilic bacteria is a result of a cascade of synergistically active inducible peptidases is prevailing. Usually this model of collagen degradation omits the thermophilic bacteria constitutively produced collagenolytic peptidases. The constitutive collagenolytic activity of thermophilic bacteria was never deliberately analyzed and the understanding of constitutive peptidases importance for collagen hydrolysis in vivo is obscure, what reduces the overall fundamental understanding of collagenolysis biological roles for prokaryotes.

Objectives
Detect hydrolytic activity of Geobacillus thermoleovorans DSM15325 constitutively produced collagenolytic peptidases. Identify detected hydrolases by mass spectrometry.

Methods
G. thermoleovorans DSM15325 was cultivated in modified M9 medium up to late exponential growth phase when the total secretome proteins were precipitated and analyzed by SDS-PAGE. The evaluation of collagenolytic peptidases activity was performed by gelatin zymography and azocollysis. Detected peptidases were excised from acrylamide gel and subjected to mass spectrometry analysis. Obtained enzyme sequences were analyzed in silico.

Conclusions
The Bacillolysin (protein ID AEV20496.1), Pz-peptidase A (protein ID BAD99433.1) and B (protein ID BAD99434.1) were identified as peptidases generating constitutive collagenolytic activity of G. thermoleovorans DSM15325.
Background

Bacteria from genus *Cronobacter* are opportunistic pathogens associated with rare but severe infections mainly among neonates. Based on the epidemiological studies and molecular typing methods such as MLST is obvious that *C. sakazakii*, *C. malonaticus* and *C. turicensis* are prevalent in neonatal infections and particularly sequence types ST4, ST7 and ST19 are considered as an attribute of virulence strains.

Objectives

The aim of this study was to map membrane of virulent strains being as the gateway to the host organism and to contribute to the knowledge of virulence features of this genus.

Methods

We applied several techniques for membrane proteins isolation such as the whole cell membrane isolation by phenol extraction method, the membrane sub-fractionation into outer membrane, periplasm and inner membrane, and the immunoprecipitation using polyclonal antibodies against whole *Cronobacter sakazakii* cells. The isolated proteins were consequently identified by mass spectrometry and peptide mass fingerprinting method.

Conclusions

Membrane proteins play a pivotal role in bacterial pathogenesis thus a complex study of membrane proteome of pathogens is invaluable. Here we present membrane proteomes report of the strains considered as pathogenic focusing on virulence traits determination.
ANALYSES OF ACTIVATED SLUDGE METAPROTEOMES OF A WASTEWATER TREATMENT PLANT DURING WINTER AND SUMMER PERIOD

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Background
Molecular biological approaches (i.e. community fingerprinting, metagenome sequencing) revealed that only a small portion of the microbial community typically found in wastewater treatment plants (WWTP) can be cultivated in the laboratory. In order to understand its function without isolating single strains, metaproteomics analysis has been applied to activated sludge samples.

Objectives
The impact of changes in temperature (winter and summer period) on metaproteomes of activated sludge samples derived from a full-scale WWTP was investigated to enable a detailed description on functional level.

Methods
For reduction of sample complexity prior to MS analysis, different fractionation techniques including reversed phase liquid chromatography (RP-LC), sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE), and liquid isoelectric focusing were combined and compared regarding effort and quality of resulting protein identifications. The derived spectra were identified using the UniProtKB/Swiss-Prot protein database. Additionally, identified KEGG ontologies (Kyoto Encyclopedia of Genes and Genomes) and Enzyme Commission numbers were used to plot proteins hits into pathway maps of the central carbon and nitrogen metabolism.

Conclusions
As expected, the high resolving but most time consuming three-dimensional approach yielded the highest amount of protein identifications. The results revealed taxonomic differences in the abundance of specific classes, such as Beta- and Gammaproteobacteria, in activated sludge collected during winter and summer period. Fractions of eukaryotic proteins decreased from winter to summer period, indicating a better degradation performance during summer period due to increasing process temperature. Additionally, more proteins associated to the nitrogen
metabolism, like hydroxylamine dehydrogenase or ammonia monooxygenase, were identified in summer period.
CALCIUM-TRYPTOPHAN INTERACTIONS IN CALCIUM-BINDING PROTEINS

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Background
Calcium ion is the important structural element in bacterial cells and is also involved in cell signaling. Also, the calcium binding may enable protein folding and enzymatic activity. Aromatic amino-acids in proteins can stabilize the native protein structure via pi-pi interactions and may also play a role in calcium binding by cation-pi interactions. Terbium (Tb³⁺) is the phosphorescent lanthanide that may be used as a calcium binding analog. Tb³⁺ increases the quantum yield as it binds to calcium binding sites (in proteins, DPA), which enables spectroscopic ion binding measurements. In our previous studies of FrpC protein of Neisseria meningitidis we found the calcium binding as a driving force for FrpC folding. The two Trp residues of FrpC also play an important role as stabilizing factor in calcium binding and enzymatic activity, the autocatalytic cleavage.

Objectives
In order to estimate the importance of calcium-Trp interactions in proteins, we analyzed PDB crystal structures possessing both Trp residues and bound calcium.

Methods
In all calcium-containing-protein-structures in PDB (n=6403) relative Ca/Trp positions were analyzed (392000 cases). FrpC studies: Steady-state fluorescence spectroscopy.

Conclusions
We confirmed that in calcium-binding enzymatic domain of FrpC, calcium is bound in the close proximity to Trp residues that supports the calcium-Trp interaction. Using PDB database search, we found that such calcium-Trp position is rather rare among protein structures and that calcium is most frequently positioned toward nitrogen hetero-atom in indol.
Background

Streptomyces are filamentous Gram positive soil bacteria of industrial interest due to their ability produce two-thirds of all known antibiotics as well as other so-called "secondary metabolites" useful to human health or agriculture. A reverse correlation between the content in TriAcylGlycerol (TAG) and the ability to produce antibiotics was recently demonstrated. In the presence of glucose, the lipid content of S. coelicolor is less than 10% its dry cell weight (DCW) indicating that its TAG content is very low and abundantly produces antibiotics whereas the total lipid content of S. lividans is up to 30% its DCW and does not produce antibiotics. Interestingly, in the presence of glycerol, the two strains have the same lipid content (about 30% of their DCW).

Objectives

In order to understand the molecular basis of these phenotypical differences between these two Streptomyces species, we developed a novel gel-free shotgun proteomic approach.

Methods

This strategy involves a simple protein extraction followed by proteolysis in presence of trypsin and LysC and LC-MS/MS analysis by Q-exactive MS (Thermo Fisher). We performed a three-factor analysis of variance that included: strain factor (S.lividans/S.coelicolor); carbon source factor (glucose/glycerol), and time factor (36h/48h/72h).

Conclusions

More than 2500 proteins were identified corresponding approximately to 30% of the genome. This study showed different pattern of expression of genes of central carbon and lipids metabolism as well as antibiotics biosynthesis, upon growth on glucose or glycerol. These results revealed some potentially interesting genes to be genetically modified for further improvement of antibiotics and/or TAG production by Streptomyces species.
THE SMALL PROTEIN SCO2038 CONTROLS STREPTOMYCES COELICOLOR DIFFERENTIATION BY MODULATING TRYPTOPHAN BIOSYNTHESIS

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Background

In Streptomyces coelicolor amino acid metabolism is an important clue of the morphological and physiological differentiation program and, differently from other bacteria, the expression of amino acid biosynthetic genes is not subjected to end-product negative regulation. In some amino acid biosynthetic gene clusters, such as tryptophan, histidine and proline, small orfs (about 100-300 nucleotides) were identified. These small orfs, such as sco2038, encode proteins whose cellular role have to be elucidated to highlight possible novel and crucial molecular mechanisms controlling amino acid synthesis and, thus, differentiation program.

Objectives

The aims of this work are:

1. the understanding of the effects exerted by tryptophan on primary metabolism, morphological differentiation and antibiotic production;

2. the study and characterization of the SCO2038 function as modulator of tryptophan biosynthesis.

Methods

- Differential proteomic analysis based on 2D-DIGE and MS procedures.
- SEM analysis.
- Generation and characterization of sco2038 mutants
- Identification of potential SCO2038 interaction partners by pull down assay coupled with MS identification and Bacterial Adenylate Cyclase Two Hybrid System.
- qRT-PCR analysis.

Conclusions
The obtained results revealed that tryptophan controls the expression of metabolic and regulatory proteins and promotes aerial mycelium formation, spores production and actinorhodin antibiotic biosynthesis. Moreover, the small orf sco2038, encodes a 7 KDa protein playing a key role in modulating tryptophan biosynthesis and thus, morphological differentiation. In the light of these results we propose to rename sco2038 as \textit{trpM}, the gene encoding the tryptophan biosynthesis Modulator TrpM.
**SLP PROFILES IN ENDEMIC AND NON-ENDEMIC CLOSTRIDIUM DIFFICILE PCR-RIBOTYPES**

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**Background**

*Clostridium difficile* is divided into approximately 400 PCR-ribotypes, some of them are widespread and associated with severe disease. *C. difficile* PCR-ribotypes 014/020 and 002 are the most common among endemic PCR-ribotypes.

Many bacteria, including *C. difficile*, express surface layer protein (Slp). This protein forms surface-exposed proteinaceous layer which mediates interactions with the external environment, i.e adhesion to host cells as in case of *C. difficile*. Two Slp proteins with molecular weights (MWs) of 32-38 kDa (low MW) and 42-48 kDa (high MW) are expressed and vary in size in different *C. difficile* strains. Slp typing is described, but not widely used.

**Objectives**

The aim of the study was to compare slp profiles of two endemic PCR-ribotypes (014/020 and 002) and selection of non-endemic PCR-ribotypes.

**Methods**

Eighty-eight *C. difficile* isolates from 21 different PCR-ribotypes were included. Extraction of Slp proteins was performed by using low-pH glycine solution and further analyzed by SDS-PAGE.

**Conclusions**

Five different slp profiles (1-5) were identified. Slp profiles 1 and 2 were the most frequent and present in the majority of PCR-ribotypes. Endemic and non-endemic
PCR-ribotypes shared 3 out of 5 slp profiles. Two remaining slp profiles were present only in one strain each.

Our results indicate that slp profile does not contribute significantly to *C. difficile* colonization properties and endemicity.
Background

The aim of the project is to develop biomarkers to help distinguish between the inner and the outer membrane of *Escherichia coli*. The fluorescent proteins mCherry, *E. coli* Flavin binding Fluorescent Protein and superfolder GFP, were used as biomarkers. These were directed to the inner or outer membrane using signal sequences from lipoproteins or by fusion with membrane proteins. The fusion partners were the outer membrane protein A (OmpA), the artificial TAT-lipobox signal sequence for transport to and lipid anchoring in the outer membrane, the major coat protein of phage pf3 that is inserted into the inner membrane, and mistic, a integral inner membrane protein from *Bacillus subtilis*.

Objectives

The aim of the project is to develop a bacterial strain (*E.coli* BL21 DE3) with optimized expression levels of the fluorescent markers for co-localization studies of bacterial membrane proteins.

Methods

The methods used in the project include molecular biology (cloning, PCR), microbiological (culturing, recombinant protein expression) and biochemical (cell fractionation, membrane isolation) techniques. In addition, the project will also use analytical methods such as fluorometry and fluorescence microscopy.

Conclusions

The fluorescent marking of the two membranes makes membrane fractions easier to detect after membrane separation. It also help to assess the quality of the separation procedure.
CHARACTERISATION OF THE BSA TYPE THREE SYSTEM SECRETOME OF BURKHOLDERIA PSEUDOMALLEI USING HYPER-SECRETING MUTANTS

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Background
Many Gram-negative bacteria utilise Type III secretion systems (T3SS’s) to deliver effector proteins into target host cells where they hijack cellular processes for their own benefit. T3SS’s form an injectosome with proteins spanning both membranes of the bacterium and an external needle through which effector proteins are delivered into the target cell. *B. pseudomallei*, the causative agent of the tropical disease melioidosis in humans and animals, possesses three distinct T3SS’s, of which the Bsa system has been shown to play a role in invasion of non-phagocytic cells, escape from the endocytic compartment and virulence in murine models of melioidosis. To date few proteins have been proven to be secreted by the Bsa apparatus and, by analogy with other pathogens that deploy T3SS’s, it is likely that there are many other effectors awaiting discovery.

Objectives

To characterise the total secretome of *B. pseudomallei* in standard laboratory media and the repertoire of Bsa-secreted effector proteins.

Methods

We have determined the effector secretome of the Bsa T3SS using hyper-secreting mutants of coupled with iTRAQ, a gel free quantitative proteomics technique.

Conclusions

Our study provides one of the most comprehensive core secretomes of *B. pseudomallei* described to date and identified 26 putative Bsa-dependent secreted proteins that may be considered candidate effectors.
Background
The protist parasite *Trichomonas vaginalis* causes one of the most common non-viral sexually transmitted disease in humans, trichomoniasis. Previous studies indicate that neutrophils, leukotriene B\(_4\) (LTB\(_4\)) and interleukin-8 (IL-8) are found in the vaginal discharges and vaginal smears of patients infected with *T. vaginalis*.

Objectives
IL-8 is often associated with inflammation, also known as neutrophil major chemotactic factor, causing them to migrate toward the site of infection by *T. vaginalis* and start innate immune response to defense. Previous studies focused on the profiling of chemoattractants from neutrophils stimulated by *T. vaginalis* or *T. vaginalis* ESPs, but very little is known about the cell-cell interaction between *T. vaginalis* and neutrophil. We hypothesize that both *T. vaginalis* ESPs and direct contact with neutrophil can induce the release of chemoattractants in the vagina.

Methods
In this study, we confirmed that both *T. vaginalis* and ESPs can induce neutrophil to release IL-8. We also used 2-Dimensional SDS-PAGE to investigate the differentially expressed ESPs from *T. vaginalis* before and after contact with neutrophil. A total of 16 differentially expressed protein spots were identified by MALDI-TOF-MS, and discussed the possible roles of identified proteins in the biology of host-parasite interactions.

Conclusions
We established a representative 2-DE map of ESPs of *T. vaginalis* induced by neutrophil and identified differentially expressed protein that may promote cytoadherence and long-term colonization while ESPs from neutrophil may be related to implicate in the first line of host defense.
COMPARATIVE PROTEOMICS ANALYSIS FOR THE IDENTIFICATION OF TARGETS OF THE TMK1 MAP KINASE IN THE MYCOPARASITIC FUNGUS TRICHODERMA ATROVIRIDE

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Background

Mycoparasitic species of the filamentous fungus Trichoderma are commercially applied as biological control agents against fungal plant pathogens. The mycoparasitic interaction of Trichoderma with the host fungus comprises specific recognition events leading to activation of "molecular weapons" in the mycoparasite which are involved in host attack and lysis and result in utilization of the host fungus as a nutrient source for the mycoparasite.

Investigations on the underlying intracellular signal transduction pathways of Trichoderma atroviride revealed the involvement of a Mitogen-activated protein kinase (MAPK) which is essential for triggering the mycoparasitic Response. Mutants missing the tmk1 gene show reduced mycoparasitic activity against host fungi.

Objectives

The aim of the presented study was the identification of target proteins being regulated by the Tmk1 MAPK pathway upon host recognition. To this end, T. atroviride wild-type and the delta-tmk1 mutant were co-cultivated with the host fungus Rhizoctonia solani and Trichoderma mycelia were harvested from the confrontation zone upon direct interaction between the two fungi and from respective un-induced (self-confrontation instead of confrontation with the host) controls.

Methods

Two-dimensional Difference Gel Electrophoresis (2-D DIGE) was applied for comparatively analysing the proteomes of the four samples with the aim to find mycoparasitism-relevant targets of the Tmk1 MAPK.
Conclusions

Bioinformatic analyses revealed 60 proteins being regulated in the wild-type response (WT vs. *R. solani* minus WT vs. WT) and 126 proteins being host-induced Tmk1 targets (WT vs. *R. solani* minus delta-tmk1 vs. *R. solani*), of which 30 and 60 proteins, respectively, were identified by mass spectrometry.
PROTEOMICS ANALYSIS OF THE B. SUBTILIS INNER SPORE MEMBRANE

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Background

The endospore is the dormant form of \textit{Bacillus subtilis} and many other Firmicutes. By sporulation these spore formers can survive very harsh physical and chemical conditions. Yet they need to go through germination to return to their growing form. The spore inner membrane (IM) has been shown to play an essential role in triggering the initiation of germination; however, its protein composition was not comprehensively characterized yet.

Objectives

To isolate IM from \textit{B. subtilis} spores and to characterize the \textit{B. subtilis} spore IM proteome.

Methods

The spores were chemically decoated and enzymatically deprived of the cortex. Subsequently the core was mechanically disrupted and the IM fraction was collected by differential centrifugation. Tryptic peptides, acquired from in-gel digestion, were analyzed using ion trap LC-MS/MS, which led to identification of the spore IM proteins.

Conclusions

We have adapted the spore IM isolation protocol to proteomics studies. Over 900 proteins could be identified from the \textit{B. subtilis} spore IM preparations, in which ca. one-third were predicted to be membrane proteins. In addition to the previously known IM proteins, a number of novel IM proteins were identified which are likely to provide new insights into the IM protein composition, its functions and in particular into the spore germination machinery.
Background

The presence of plasmids carrying class 1 integrons in a collection of psychrotolerant enterobacteria isolated from Fildes Peninsula was previously established. In these plasmids, we could identify a DNA region with three ORFs (intI1, dfrA14 and hyp, encoding a hypothetical protein) highly similar to a fragment originally identified in plasmid pKOX105 from Klebsiella oxytoca (HM_126016).

Objectives

DNA sequence analysis of plasmids purified from some of these enterobacterial isolates.

Methods

Plasmid DNA from isolates CDTR5, CN11 and HP19 were purified with Gene Plasmid Midprep Elute® HP (Sigma-Aldrich Co, MO) and sequenced in a Ion 314TM Chip on a Ion PGMTM System (Life Technologies,) at the IIBCE. The reads were assembled using CLC Genomics Workbench v6.5 (CLC bio). Annotation of pHP19 contigs and comparison with plasmids pECL_A and pKOX105 were done using RAST server (http://rast.nmpdr.org/rast.cgi).

Conclusions

Sequence analysis of pHP19 showed a region of 7181 nts highly similar (99% identity by Blastn) with a region of pKOX105 (54641pb). This contig includes the intI1 gen and other ORFs encoding a DNA-cytosine methyltransferase and a probable membrane protein.

The sequences of other plasmidic regions were similar to those of Enterobacter cloacae ATCC 13047 plasmid pECL_A (IncFII) (199562pb) (NC_014107), including genes for replication, stability and conjugation. Other cluster genes related with mercury, arsenic, tellurite, nickel and copper resistance identified were identical to those found in pECL_A. Thus this plasmid is likely a mosaic of portions of plasmids
that have been characterized in mesophilic enterobacteria, showing that HGT between these bacteria and the Antarctic microbiota is rather common.
C-DI-GMP CONTROL MODULES CONFINED WITHIN MOBILE ELEMENTS: THE CASE OF ICEACATY.2

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Background

The intracellular nucleotide c-di-GMP has recently emerged as one of the central elements of the signal transduction network linking perception of environmental or intracellular cues to specific alterations in cellular function. In response to different input signals, the opposing activities of diguanylatecyclases (DGCs) and phosphodiesterases (PDEs) control the cellular concentration of c-di-GMP. Inside the cell, c-di-GMP binds a currently unknown number of effector molecules that subsequently interact with cognate target components to produce a variety of output phenotypes (e.g. transition from planktonic to biofilm). Given the multiplicity of c-di-GMP related genes in most bacterial genomes and their scattered distribution within the genomes, the elements that belong to the same c-di-GMP control module are difficult to uncover.

Recently, an integrative conjugative element -ICEAcaTY.2- has been characterized in the extreme acidophile Acidithiobacillus caldus ATCC 51756. ICEAcaTY.2 is an actively excising element of widespread occurrence in a collection of At. caldus strains, that behaves as a cohesive heritable unit. Bioinformatic analysis of ICE.2-type elements has revealed that c-di-GMP synthesis/degradation functions and putative c-di-GMP binding effectors and targets co-occur within the element.

Objectives

To gain insight into the participation of c-di-GMP genes encoded by ICEAcaTY.2 as a functional control module.

Methods

To test this hypothesis the expression of the c-di-GMP genes encoded by ICEAcaTY.2 was quantified by real-time PCR under different growth conditions (e.g. planktonic, attached, stressed).

Conclusions

Transcriptional behavior of key c-di-GMP module components in attached cells and upon DNA damaging conditions support the hypothesis and further suggest that this control module could be critical for biofilm development and DNA exchange.
ENVIRONMENTAL ISOLATES OF V. PARAHAEMLYTOICUS CARRY SXT/R391 INTEGRATIVE CONJUGATIVE ELEMENTS

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Background

SXT/R391 Integrative conjugative elements (ICEs) are self-transmissible mobile genetic elements able to confer adaptive features to bacterial hosts, including Vibrionaceae. They share a conserved genetic scaffold encoding their own conjugation, integration, excision, and regulatory machinery, interspersed with variable DNA clusters located in conserved hotspots.

Objectives

In silico analysis of 96 V. parahaemolyticus genomes was performed to identify Integrative Conjugative Elements (ICEs) of the SXT/R391 family.

Methods

Four novel ICEs were detected in environmental non-pandemic V. parahaemolyticus strains isolated in China, Spain, and Malaysia (2002-2008). ICE sequences were annotated using the RAST annotation pipeline and comparative analysis was performed with the Artemis Comparative Tool, BLASTN and BLAST-PSI. Given their unique genetic contents they were named: ICEVpaSpa1 (~111 kb), ICEVpaMal1 (~70 kb), ICEVpaChn4 (~66 kb), ICEVpaChn5 (~86 kb). We identified gene sequences from other ICEs as well as unique genetic features such as a set of heat-shock proteins and chaperones likely to respond to environmental stress (ICEVpaSpa1); genes with transpeptidase domains belonging to the beta-lactamase TEM family (ICEVpaSpa1); putative type 1 restriction/modification systems (ICEVpaChn5, ICEVpaSpa1); type III restriction-modification systems (ICEVpaMal1); ars operon encoding an efflux system mediating arsenic resistance (ICEVpaMal1, ICEVpaChn4); and an antibiotic resistance cluster carrying floR, strAB and sul2 genes (ICEVpaChn5).

Conclusions

V. parahaemolyticus ICEs exhibit significant genetic polymorphisms, sign that the structure of each ICE has been shaped by frequent recombination, with genetic
content likely dependent on the specific locale in the environment from which it was originally isolated.
FEMS-1251
Mobile genetic elements

OBTENTION OF HYPERACTIVE INTEGRASES BY EVOLVING SYNONYMOUS GENES
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Background

Integrons are gene recruitment platforms that allow for rapid bacterial evolution, playing a major role in the acquisition of antimicrobial resistance genes. The integrase catalyzes the reaction between $\text{attC}$ and $\text{attI}$ sites. However, its activity is finely tuned to preferentially process $\text{attI} \times \text{attC}$ and $\text{attC} \times \text{attC}$ reactions, as they allow for the acquisition and rearrangement of cassettes, rather than the $\text{attI} \times \text{attI}$ reaction. Being a less frequent phenomenon, the structural basis of $\text{attI}$ recognition has yet remained elusive.

Objectives

We have conducted directed evolution experiments on the integrase to obtain hyperactive mutants for the $\text{attI} \times \text{attI}$ reaction. In order to explore a broader evolutionary landscape of the integrase we have recoded the protein into two alternative, yet synonymous, alleles of the $\text{intI1}$ gene.

Methods

In a first round of experiments we obtained among the three alleles 8 mutations conferring an increase in recombination rates. We reinserted all mutations into the three wt alleles, obtaining a 100-fold increase in recombination efficiency. Re-evolving these alleles yielded new mutants with recombination rates for $\text{attI} \times \text{attI}$ comparable to those of the wild type protein for the $\text{attI} \times \text{attC}$ reaction. Along these experiments we obtained landscape-specific mutations and the re-evolution of a codon to a residue beyond its evolutionary landscape in any of the three starting alleles.

Conclusions

We have successfully explored an enlarged evolutionary landscape of the integrase allowing us to obtain hyperactive molecules, while shedding light on the structural features that are important for the recognition of the $\text{attI}$ site.
Mobile genetic elements

CONJUGATIVE TYPE IVB PILUS RECOGNIZES LIPOPOLYSACCHARIDE STRUCTURE OF RECIPIENT CELLS TO INITIATE PAPI-1 PATHOGENICITY ISLAND TRANSFER IN PSEUDOMONAS AERUGINOSA

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Background

Horizontal gene transfer is an important mechanism in Pseudomonas aeruginosa for adapting to a wide range of environments and, in particular, for acquiring virulence determinants which are mostly carried on pathogenicity islands. One of the largest pathogenicity island of P. aeruginosa is PAPI-1, a 108-kb island which is known to be transferred from a donor to a recipient strain through a type IVb pilus. However, the PAPI-1 acquisition mechanism has not been elucidated to date.

Objectives

We aimed at investigating the mechanism of PAPI-1 acquisition in recipient cells.

Methods

To determine the receptor for conjugative type IVb pilus, a standard PAPI-1 transfer assay described by Carter et al. (2010) was carried out using PA14TnC2 as a donor and 38 PAO1 mutants with an altered lipopolysaccharide (LPS) biosynthesis pathway as recipients. The loss of receptor for conjugative type IVb pilus in PAO1 mutants can result in a significant decrease of PAPI-1 transfer. The conjugative pilus receptor was then confirmed with a competition assay by adding outer-membrane and LPS preparations into the standard mating assay.

Conclusions

Transfer efficiency of PAPI-1 to the AlgC mutant producing a truncated LPS core was reduced by three orders of magnitude compared to that of wild-type PAO1, indicating that LPS acts as a receptor for conjugative type IVb pilus. The homopolymer of D-rhamnose, or rhamnan structure was confirmed as a specific receptor for conjugative type IVb pilus. Further investigation will define if P. aeruginosa that have acquired
PAPI-1 specify a surface exclusion mechanism by LPS modification preventing further recognition by conjugative pilus.
REGULATION OF THE MOBILITY OF THE LVH-GENOMIC ISLAND OF LEGIONELLA PNEUMOPHILA

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Background

Horizontal gene transfer (HGT) and mobile genetic elements (MGEs) are major generators of genome plasticity and diversity. Certain of these elements can exist in the Legionella pneumophila (Lp) genome in an excised or integrated form and encode for type IV secretion systems (T4SS) and/or conjugation systems. Intriguingly, each of these elements encodes a homologue of CsrA (carbon storage regulator). CsrA is a global regulator of virulence in L.p. The Lvh-region is one of these MGEs encoding a T4ASS and a CsrA homologue.

Objectives

Deciphering the mechanisms of mobility of the Lvh

Methods

Distribution of the Lvh was analyzed by comparative genomics of 50 L.p strains, the mobility and regulation of transfer were analyzed by mutagenesis, conjugation experiments, Q-PCR, RNA-Seq and whole genome sequencing of the transconjugants

Conclusions

The Lvh mobility is mediated by a phage-like integrase. It is transferable at a rate of $10^{-4}$ and inserts specifically in the tmRNA gene in all analysed transconjugants. Using a ΔdotA strain we show that the Dot/Icm T4SS is implicated in the conjugation of the lvh-region, as the transfer rate drops to $10^{-7}$. Conjugation experiments in a Δihf mutant (Integration host factor), a nucleoid-associated protein revealed its role in the mobility of the Lvh. Most interestingly the putative RNA-binding protein LvrC (CsrA homologue) affects the mobility of Lvh by conjugation in specific growth conditions. Thus, The mobility of the predicted self-transmissible Lvh-genomic island is regulated by a complex network of global (IHF) and specific regulatory proteins (LvrC) and depends largely on the Dot/Icm T4SS.
Background

Three ancient Acinetobacter lwoffii strains were aseptically isolated from East-Siberian permafrost sediments and carbon-14-dated to 15,000-30,000 years and 2-3 million years before present.

Objectives

Investigate the structure and properties of plasmids from ancient Acinetobacter lwoffii strains, and the presence of various heavy metal and antibiotic resistance genes on those plasmids.

Methods

Complete genomic sequences (average coverage >30) of three strains were obtained using pyrosequencing method. 13 plasmid sequences were assembled and manually analysed using bioinformatics software and public databases and verified with PCR amplification of regions where ambiguous results occurred.

Conclusions

Each strain carried at least two plasmids with their sizes varied between 6 kb and 287 kb. Three largest plasmids designated pKLH208 (287 kb), pKLH211 (190 kb) and pKLH220 (130 kb) belonged to ED23-35, ED45-23 and VS15 strains respectively. Each of these plasmids encoded similar replication and segregation systems and 12 to 18 IS-elements of IS1, IS4, IS5, IS6 and IS66 families with almost twice as many IS-elements with interrupted tnp genes, suggesting frequent transposition processes. Apart from two different mercury resistance operons found only on pKLH208 and pKLH211, large plasmids encoded operons conferring resistance to cobalt, zinc, cadmium, chrome and arsenical compounds, several copies of copper and iron intake regulating operons, urea degrading operons, aadA (streptomycin/spectinomycin) and tetA-tetR (tetracycline) antibiotic resistance genes. We found that 11 of 13 identified plasmids, including two large plasmids pKLH208 and pKLH220, encoded either
mobilisation or conjugation and type IV secretion systems and are potentially capable of transferring horizontally.
COMPARISON OF INTEGRON ATT C SITES: THEIR STRUCTURAL FEATURES DIFFERENTIALLY INFLUENCE THE FREQUENCY AND ORIENTATION OF CASSETTE INTEGRATION
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Background

The integron is a bacterial recombination system for capturing and rearranging gene cassettes through site-specific recombination, which plays a role in the rise of antibiotic multiresistance in Gram-negative bacteria. The distinctive property of integron recombination sites flanking the cassettes (attC) is that they have almost no sequence conservation. Instead, their single-stranded DNA possesses conserved structural features, and attC sites are recombined as a folded form of the bottom DNA strand. Contrary to the bottom strand, the recombination of the top strand would integrate the cassette in the opposite orientation, preventing the expression of its gene from the promoter provided by the integron platform.

Objectives
In order to understand the basis of this specificity, we tested the recombination of several attC sites, and selected 3 of them for further analysis.

Methods
For these sites, we mutated their structural features and quantified the changes in frequency and orientation of cassette integration for the corresponding bottom and top strands.

**Conclusions**

We observed that some of the structural features, such as the extrahelical bases (EHBs), play similar roles for all of the tested sites, but the extent to which they influence the frequency and the orientation of cassette integration differs among \textit{attC} sites. Yet other structural features, such as the unpaired central spacer (UCS), have very different, sometimes even opposing effects depending on the \textit{attC} site. Our study suggests that the structural requirements for \textit{attC} site recognition are rather relaxed, and that the control of cassette integration might be governed by more complex mechanisms than previously assumed.
POSSIBLE ROLE OF A MOBILE GENETIC ELEMENT IN TRANSLATIONAL MODULATION

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Background
A consortium of microorganisms adapted to grow in acidic environments participate in the bioleaching of minerals. The facultative aerobic bacterium Acidithiobacillus ferrooxidans is a Gram negative, chemolithotrophic, autotroph that utilizes either Fe²⁺ or sulfur reduced compounds as electron donors.

Objectives
We are interested in the role of non-coding RNAs (sRNAs and tRNAs) and RNAses encoded in a mobile genetic element in translation control.

Methods

By means of bioinformatic analyses 37 genes encoding tRNAs were detected in the mobile element ICEAfe1 from A. ferrooxidans strain ATCC 23270. Encoded tRNAs are aminoacylated in vivo in A. ferrooxidans, although they are transcribed at low levels.

Two strategies were used for their identification of sRNAs: 1. Bioinformatic prediction of transcription units in intergenic regions (IGR) of A. ferrooxidans genome and comparison to RNA deep sequencing data. 2. The identification of sRNAs that bind to the RNA chaperone Hfq were identified by RNA deep sequencing. Currently we are working in the validation of target mRNAs of the identified sRNAs. At least two snRNAs encoded in the ICEAfe1 were validated.

Four type II toxin-antitoxins systems specific for strain ATCC 23270 were identified in the in the ICEAfe1. All four toxins encoded an RNAse that is counteracted by the
Conclusions

Different genes encoding possible factors that modulate translation have been identified in the integrative conjugative element ICEAfe1 from Acidithiobacillus ferrooxidans. Whether these systems effectively modulate translation in this organisms is currently under investigation.
A NEW SHUTTLE-EXPRESSION SYSTEM TO GENERATE POLYHISTIDINE-TAGGED FUSION PROTEINS IN STAPHYLOCOCCUS AUREUS AND ESCHERICHIA COLI

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Background

A better understanding of the metabolism of S. aureus is needed. However, only few tools are available for production of tagged fusion proteins.

Objectives

To develop S. aureus-E. coli shuttle vectors for the expression of target genes and rgs-his₆ codon fusions.

Methods

The strong constitutive promoter of S. aureus type 1 capsule biosynthetic gene 1A (P_{cap}) upstream of a multiple cloning site (MCS) and codons for rgs-his₆ were synthesized and inserted into pBUS1 background. The MCS contained a Bgcl site to generate C-terminal RGS-His₆ translational fusions of cloned genes through a simple cut-religation method. Using PCR-based techniques, two vectors were constructed that contain the P_{cap}-MCS-rgs-his₆ sequence (pBUS1-P_{cap}-HC and pTSSCm-P_{cap}) and two promoter-less variants suitable for cloning genes including their own promoter (pBUS1-HC and pTSSCm). The plasmids contained the E. coli origin ColE1, the terminator sequence rrnB(T1)₅ and the tetracycline resistance marker tet(L) for S. aureus and E. coli. Gram-positive replicon was improved through either complementation of the minimum pAMα1 replicon from pBUS1 with the single strand origin oriL from pUB110 or substitution with a pT181-family replicon. Plasmid stability, copy numbers and recombinant protein synthesis was analyzed in S. aureus and E. coli.

Conclusions

The new shuttle vectors displayed increased copy numbers and segregational stability in S. aureus. Feasibility of rgs-his₆ codon-fusion, gene expression and protein purification were demonstrated in S. aureus and E. coli using the macrolide, lincosamide, streptogramin B resistance gene erm(44). The new His-tag expression...
system represents a helpful tool for the direct analysis of target gene function in staphylococci.
THE PRESENCE OF TEMPERATE BACTERIOPHAGES IN UROPATHOGENIC ESCHERICHIA COLI STRAINS

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Background

Horizontal gene transfer vectors, including bacteriophages, promote the spread of a broad spectrum of genes, among them some encoding medically relevant traits such as virulence factors. In Escherichia coli (E. coli), temperate bacteriophages have been shown to promote the spread of shiga toxin genes across shigatoxigenic strains of the species¹.

Objectives

Here we aim to assess the presence of lysogenic bacteriophages in uropathogenic E. coli (UPEC) strains, and to investigate a possible correlation with the virulence factor profiles of the examined strains.

Methods

Twenty UPEC strains were screened for the presence of 15 different virulence-associated traits (fimH, papC, papGII, sfaDE_focIC, afaBC_draBC, hlyA, cnf1, usp, ibeA, iucD, iroCD, iroN, fyuA and ireA) by PCR amplification of target genes, and were subsequently assigned virulence scores by counting possession of these genes. Additionally, strains were screened for the presence of lysogenic bacteriophages by induction with UV light. Data were analysed using Student’s t-test.

Conclusions

We have found that out of the 20 screened strains, nine possessed UV-inducible lysogenic bacteriophages. Furthermore, data analysis has shown that the strains harbouring UV-inducible lysogenic bacteriophages also possessed a significantly higher virulence score than the strains lacking UV-inducible bacteriophages (p < 0.01). The results of this preliminary study imply that temperate bacteriophages might have a potential role in virulence factor transfer across UPEC strains.

Reference
Background
Transposable elements (TEs) are components of nearly all bacterial genomes. They promote structural changes in DNA, which may result in different phenotypes. We focused on the elements of the Tn3 family – the most abundant group of bacterial transposons.

Objectives
Our studies shed a new light on Tn3 family, which is much more diverse than previously thought. We showed that Tn3 elements comprise (i) a composite transposon generated by two copies of a non-composite cryptic transposon, as well as (ii) simple non-autonomous elements (TIMEs - Tn3-derived inverted-repeat miniature elements) and (iii) their derivatives. We have provided for the first time evidence that some of the non-autonomous transposons may contain, in their core regions, functional plasmid replication and stabilizations systems, which can be transferred among replicons co-residing in a bacterial cell. The transposition events may therefore significantly influence structures of bacterial plasmids leading to generation of molecules exhibiting novel properties. Bioinformatic analyses of the nucleotide sequences of several bacterial genomes revealed the presence of large elements originating from the Tn3-family non-composite transposons, which acquired additional genetic information, thus forming a kind of transposable genomic islands.

Methods
Standard methods of molecular biology were used.

Conclusions
The obtained results indicate the powerful role of the Tn3-family TEs in the mobilization to transposition of segments of genomic DNA. Such shuffling of genetic information, may significantly influence the structures of bacterial genomes. Huge diversity of the identified elements allows to draw conclusions concerning the high dynamics of evolution of this group of TEs.
CULTURE-INDEPENDENT RECOVERY OF IS1216 COMPOSITE TRANSPOSONS FROM ORAL METAGENOMIC DNA.

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Background

A composite transposon is a transposon consisting of two insertion sequences (ISs) flanking a DNA segment which often carries antibiotic resistance (AR) genes. If both IS elements are the same then it is possible that one copy of the IS element plus the intervening DNA segment can excise as a circular form. This circular molecule has been called an “Unconventional Circularizable Structure” (UCS) or “Translocatable Unit” (TU). IS1216 composite transposons have been shown to carry various AR genes. In addition UCSs have also been found containing IS1216.

Objectives

To screen for IS1216 composite transposons and IS1216 containing UCSs in human oral metagenomic DNA.

Methods

PCR primers were designed to amplify in the outward direction from IS1216. The IS1216 PCR products were then cloned and analysed.

As our PCR strategy would also detect UCSs, PCR primers were designed to amplify outward from the intervening DNA region to determine if each IS1216 amplicons was derived from a UCS circular structure.

Conclusions

We found four different IS1216 composite transposons and confirmed the presence of two novel UCSs in the oral metagenomic DNA. One of them contains an antiseptic resistance gene and one contains a universal stress response protein encoding gene.

This is the first report of a PCR strategy to amplify the DNA segment on the composite transposons in metagenomic DNA, and is also the first time that UCSs have been found in metagenomic DNA. This PCR strategy can be used to identify
novel AR genes associated with mobile genetic elements in metagenomes from any environment.
Mobile genetic elements

DETECTION AND CHARACTERISATION OF INTEGRON GENE CASSETTES IN HUMAN ORAL METAGENOMIC DNA.
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Background

An integron is a genetic element, which is able to capture gene cassettes and direct the expression of open reading frames (ORFs) contained within them. They are involved in the dissemination of antibiotic resistance genes (ARGs) in many pathogens. The oral cavity is a reservoir of ARGs; however, these have not been shown previously to be present on an integron cassette.

Objectives
To investigate the presence of integrons and associated gene cassettes in the human oral cavity by using a PCR-based approach.

Methods
Different primer sets were tested on oral metagenomic DNA obtained from both the UK and Bangladesh, including novel primers based on the Treponema denticola integrase and attC sequences, and previously published primers targeting various conserved regions on different integrons. The resulting metagenomic amplicon libraries were then sequenced and analysed.

Conclusions

A diverse array of ORFs presents on gene cassettes and associated with integron integrases have been identified. The cassettes contain ORFs predicted to confer antibiotic resistance and adaption to environmental stresses including a putative chloramphenicol acetyltransferase, bleomycin binding protein, multidrug transporter, cof-like hydrolase, competence and motility related proteins.

This is the first study confirming the presence of integron gene cassettes in oral metagenomic DNA. The predicted proteins are likely to carry out a multitude of functions however the function of the majority is as yet unknown. We hypothesise that the oral cavity may be a rich source of diverse integron cassettes because it is such a variable physicochemical and stressful environment for its bacterial inhabitants.
Background

We address the central role of a functionally related group of transposable elements called “HuH elements” in structuring and shaping bacterial genomes. We have identified and characterised a widespread class of IS (IS200/IS605 family) fundamentally different from classical TEs: they use obligatory single strand DNA intermediates and have ends with subterminal imperfect palindromes (IP) which are recognised and bound by their transposases. These transposases are members of a larger “HuH” endonuclease superfamily. The protein binds the subterminal IP located some distance from the cleavage sites. Remarkably, cleavage sites are not recognised directly by the protein but by short “guide” sequences 5’ to the IP foot. Recognition involves a network of canonical and non-canonical base interactions similar to those found in RNA structures.

Objectives

We have demonstrated the importance of the lagging strand template for activity of some members and our in silico genomic analysis suggests that all IS200/IS605 family members have evolved a mode of transposition that exploits ssDNA at the replication fork.

Methods

Some members of this group appear to have been domesticated to perform important roles in the prokaryotic cell: such as homing endonucleases in some group I prokaryotic introns and as enzymes responsible for proliferation of short intergenic multicopy palindromic regulatory sequences called REPs.

Conclusions

The results provide an overview of the role of “HuH” transposases and their associated mobile elements in shaping the prokaryotic genome.
FUNCTIONAL CHARACTERIZATION OF AN INOSITOL METABOLISM ENCODING GENE CLUSTER CONTAINED IN ICEEFM1 OF ENTEROCOCCUS FAECIUM

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Background
Whole genome sequencing revealed the presence of an integrative conjugative element, ICEEfM1 in a clinical Enterococcus faecium (Efm) isolate (E1504) with an insertion of a cluster of 10 genes putatively involved in inositol metabolism.

Objectives
Functional characterization of the inositol metabolism (iol) gene cluster.

Methods
A cre-lox recombination system was used to construct a markerless iolD deletion mutant in strain E1504. The phenotype was determined by growth on minimal medium with inositol as carbon source (M-ino). The genetic organization of the gene cluster was determined by operon and promoter mapping and expression was determined after growth in rich and minimal medium in the presence and absence of myo-inositol. An in vivo mouse gut-colonization model was used to compare colonization abilities of E1504-wildtype and iolD mutant.

Results. The iolD deletion resulted in abolished growth in M-ino. The gene cluster is organized as an operon of 10 genes with its own promoter. Expression was only observed in the presence of myo-inositol and absence of other carbon sources. No difference was observed in gut-colonization abilities between wild-type and mutant.

Conclusions
The 10 gene insertion in ICEEfM1 confers Efm the ability to grow when only inositol is available as carbon source. Due to the availability of additional other sugars the iol gene cluster did not provide a selective advantage in the in vivo mouse model. Possibly the gene cluster was acquired in an environment where carbon sources were limited and only inositol was present like e.g. plants and soil.
GENETIC AND LIFE-HISTORY TRAITS DETERMINING LYSOGENY AND THE DISTRIBUTION OF PROPHAGES

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Background
Bacterial viruses (phages) are ubiquitous and have a strong impact on microbial population dynamics. Temperate phages may become prophages upon cell infection, producing lysogenic hosts and thus contributing to the evolution of bacterial gene repertoires.

Objectives
We identified and studied 2246 prophages among the genomes of 1196 bacterial species to pinpoint the genetic and life-history traits associated with lysogeny and how they shape the distribution of prophages among bacteria.

Methods
Surprisingly, whereas lysogens have larger genomes and are more often pathogens, they are not different in terms of cell volume or defense systems, like CRISPR-Cas. Importantly, growth-related trade-offs seem to be key to the decision of lysogeny. We propose that lysogeny is more adaptive when hosts show large variations in the resources they can provide. Accordingly, bacteria with low minimal doubling times, which are associated with strong variations in growth rates and cell volume, show remarkably high rates of lysogeny. This fits theory suggesting that lysogeny is a strategy of slow vertical reproduction in waiting for future more efficient horizontal reproduction. This strategy has important consequences for bacterial evolution: we find that lysogens have much more diverse gene repertoires, after controlling for other factors, showing the key role of lysogeny in bacterial adaptation.

Conclusions
Our work shows a complex dependency of bacterial genetic diversification on host-parasite interactions and the life-history traits of both bacteria and phage. It allows establishing coarse-grained predictions of the abundance and distribution of prophages and explains the paucity of prophages in certain bacterial clades.
CONTRIBUTION OF INSERTION SEQUENCE ELEMENTS TO THE GENOME PLASTICITY OF CUPRIAVIDUS METALLIDURANS AE126

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Background
Mobile genetic elements play a significant role in bacterial evolution. Insertion Sequence (IS) elements are of specific interest as they constitute an importing driving force for genome plasticity. *Cupriavidus metallidurans* AE126 (type strain CH34 cured of pMOL30) harbors 19 distinct IS elements from nine IS families, reaching a total of 52 intact IS copies. *C. metallidurans* AE126 displays in the presence of toxic zinc concentrations spontaneous mutations resulting in an increased zinc resistance.

Objectives
The objective of this study is: (i) to determine the contribution of IS elements to genome adaptation of AE126 after zinc stress and (ii) to examine the putative stress-specific inducible transposition of IS elements.

Methods
Zinc resistant AE126 derivatives were screened by PCR and mutations were identified by sequencing. Promoter transcription of IS elements was analyzed with the promoter probe vector pGLR1.

Conclusions
All derivatives had a base substitution/indel (45%) or an insertion of an IS element (55%) in the regulatory locus of *cnr* (cobalt-nickel resistance). Seven IS elements were identified to be transposed into the regulatory locus of *cnr*. Three elements were found more often with respect to their copy number and transcription of their promoters were induced by supplementing zinc or cadmium to the growth medium but not nickel or cobalt. Some AE126 derivatives, e.g. with an ISRme5 element inserted in the same orientation as *cnrYXH*, are pre-adapted as they evolved to ameliorate resistance to other stress challenges. Pre-adaptation could putatively be explained by the increased transcription of the mutated *cnr*-operon and concomitant co-transcription of ISRme5.
Objective To investigate the distribution and characteristics of vibrio seventh pandemic island-I (VSP-I) in Vibrio cholerae. Method All V. cholerae genome sequences in GenBank were downloaded and screened for VSP-I island using VSP-I (VC0175~VC0185) of strain N16961 as the reference sequence. MUMmer software was used to analyze single nucleotide polymorphism (SNP); OrthMCL software was used for homologous gene analysis; finally the BLAST software was used to search sequence variation and functional annotation. Conclusions VSP-I widely exists in the genomes of all the seventh epidemic V. cholerae O1 strains and its sequence is highly conserved; Among the 105 V. cholerae O1 strains, the VSP-I sequences of 94 strains were completely identical, the VSP-I sequences of 11 strains had 1~14 SNP loci, respectively. The VSP-I exists also in the genomes of two non-O1/O139 strains. However, their VSP-I sequences contain some insertion and deletion (INDEL) comparing to that of O1 strains. VSP-I had 9 conserved genes totally. Among them, VC0183 existed in all strains, but its length of the non-O1/O139 strains was different to the O1 strains. Five (VC0181~VC0185) of 9 genes were involved in its transfer and integration. And VC0178 encoding potato glycoprotein analogues and VC0180 encoding protein hydrolase may be related to virulence. As a specific genetic cluster within the seventh cholera pandemic strains, VSP-I has been transferred to the non-O1/O139 strains, and variation of its sequence has been occurred as well.
Background

The antimicrobial activity of silver is well documented, however its mechanism of action is not well understood. It is unclear whether the effects of silver nanoparticles (Ag-NPs) and Ag⁺ ions are similar or have different antimicrobial mechanisms.

Objectives

The objective was to compare the damages caused by Ag-NPs and silver nitrate (AgNO₃) on gram-positive and gram-negative bacteria by transmission electron microscopy.

Methods

Ag-NPs were synthesized by reduction of AgNO₃ with sodium citrate. *Pseudomonas aeruginosa* ATCC 15442 and *Staphylococcus aureus* ATCC 6538 were treated with Ag-NPs or AgNO₃ at 37 °C for 2 h. The cells were fixed with 2.5% glutaraldehyde for 24 h and post-fixed in 1% osmium tetroxide for 2 h at 25 °C, washed once in 0.1 M PBS and dehydrated by sequential transfer in a graded series of ethanol solutions. Fine sessions were cut with diamond blades using ultramicrometer and double stained with uranyl acetate and lead citrate saturated and observed using the transmission electron microscope with EDX detector (FEI Tecnai-G2-20, 2006) available in microscopy center of Federal University of Minas Gerais.

Conclusions

The Ag-NPs affected severely *P. aeruginosa*, forming a condensation region in the center of the cell and a clear zone around. According to the EDX spectrum, the condensation region is composed mainly of silver, sulfur and phosphorus. However, these elements were not detected in the clear area. Damages to *S. aureus* were similar to those caused on *P. aeruginosa*, however, milder form. Damages caused by AgNO₃ were similar to those caused by Ag-NPs.
FEMS-0903
New antimicrobial mechanisms

LIPID PEROXIDATION IS NOT RELATED TO THE ANTIMICROBIAL MECHANISM OF SILVER NANOPARTICLES AGAINST PSEUDOMONAS AERUGINOSA AND STAPHYLOCOCCUS AUREUS
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Background
The antimicrobial activity of silver nanoparticles (Ag-NPs) may be related to the formation of reactive oxygen species (ROS). A common feature among the different ROS is the ability to cause some oxidative damage in cells. The lipids are the main targets during oxidative stress.

Objectives
The objective was to determine whether the Ag-NPs cause oxidative stress in the lipids of membranes of Pseudomonas aeruginosa or Staphylococcus aureus.

Methods
The Ag-NPs were synthesized according Monteiro et al. (2009), with some modifications. The lipid peroxidation analysis was performed according to Becerra et al. (2006), with some adjustments. Samples containing 50 µg/ml Ag-NPs or AgNO₃ were inoculated with 1.0 x 10⁸ CFU/ml of P. aeruginosa or S. aureus and incubated at 37°C for 1 h. Then was added 1 ml of 35% trichloroacetic acid (w/v). After 20 min, the samples were treated with 1 ml of 0.5% thiobarbituric acid (w/v) and heated at 80°C for 30 min, then cooled and centrifuged. The absorbance was determined at 535 nm. The controls with water and 2,2'-azobis (2-methyl-propionamide) dihydrochloride (AAPH) were made. A standard curve of malondialdehyde was obtained by hydrolysis of 1′1′3′3′-tetraetoxipropano.

Conclusions
The concentration of malonaldehyde, a product of lipid peroxidation, in P. aeruginosa and S. aureus cells treated with Ag-NPs and AgNO₃ was not different (p > 0.05) from cells treated with water, however, was lower from the cells treated with AAPH, a ROS generator. This indicates that lipid peroxidation is not involved in the antimicrobial activity of Ag-NPC and AgNO₃.
Background

Diamond is a material with interesting properties, such as chemical inertness, hardness, high transparency and good biocompatibility with eukaryotic cells. These qualities make this material very promising for various bio-medical applications such as biosensors, articular implant coatings, tissue-engineering and regenerative medicine. Several published works also indicate that diamond thin films, due to their tailored surface properties, could possess anti-adhesive or even antimicrobial properties.

Objectives

The objectives of our work is to develop and standardize the methods for diamond thin film deposition on glass substrate, to develop and optimize the methods for testing of antibacterial properties of diamond surfaces, to assess these properties and to compare the influence of diamond surface terminations on their possible antibacterial character.

Methods

We used diamond films CVD deposited by microwave plasma CVD process. *Escherichia coli* was chosen as the model bacterium. Bacterial biofilm was cultivated on diamond-coated glass in CDC biofilm reactor. Fluorescence microscopy and ATP assay was used to quantify bacterial growth on diamond surfaces.

Conclusions

We managed to deposit diamond thin films of standard properties on glass over large areas (1x3”). We successfully developed the continuous cultivation method for biofilm growth and testing of antibacterial properties of diamond thin films under different conditions. We compared the antibacterial potential of diamond films with different surface terminations (H, O and F).
This work was supported by funding from Czech Science Foundation (GACR 15-01687S).
New antimicrobial mechanisms

SUSCEPTIBILITY OF FOOD CONTAMINATING MICROBIAL COMMUNITIES TO ATMOSPHERIC COLD PLASMA AND IMPLICATIONS FOR RESISTANCE-PROOF ANTI-MICROBIAL TREATMENT

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Background

Atmospheric cold plasma (ACP) has received increasing attention as a novel anti-microbial treatment, which may be less prone to resistances. Containing ions, free electrons, UV light and reactive species, plasma can cause damage to microbial cell walls and/or intra-cellular components.

Objectives

To determine the anti-microbial efficacy of ACP on food-contaminating microorganisms and their ability to develop resistance to plasma treatment.

Methods

The inactivation of a range of common microbial food contaminants using di-electric barrier discharge ACP was investigated and cultures of S.aureus and E.coli were subjected to repeated sub-lethal plasma treatment. The background microflora of plasma-treated meat products was analysed over 2-4 week storage and community profiling was performed using PCR-amplification and denaturing gradient gel electrophoresis (DGGE) of 16s RNA.

Conclusions

Microorganisms displayed varying susceptibility to ACP, with C.jejuni and B.thermosphacta showing rapid inactivation whereas E.coli, L.monocytogenes and S.aureus required longer treatment times. Repeated selection of sub-lethally treated colonies did not increase resistance in short-term adaptation studies. The background microflora of meat products was reduced up to 1log over 2-4 week storage following treatment and PCR-DGGE revealed differences in plasma tolerance between microbial populations. In an era of growing anti-microbial resistances, resistant-proof alternative treatments are gaining significance. ACP has shown anti-microbial effects on a variety of microorganisms and acts through a range of physico-chemical effects that may prevent the development of resistances. Such novel anti-microbial strategies have implications not only in the food industry but also the medical sector but will require long-term adaptation studies to ensure resistance-proof.
**NEW ANTIMICROBIAL MECHANISMS**

**SELF DECONTAMINATING PHOTOCATALYTIC TEXTILES**

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**Background**

Healthcare textiles are known to be able to transfer pathogens.

**Objectives**

To provide new antimicrobial textiles, we functionalized decontaminating photocatalytic textiles by the innovative LBL (Layer-by-Layer) technique. Photocatalysis deals with the activation of TiO₂ semiconductor particles by UVA, which produces highly Reactive Oxygen Species responsible for the microorganisms inactivation.

**Methods**

The LBL coating is based on successive application of positively charged layers and negatively charged layers. To improve the adhesion of the first layer on the fibers, the textile was first submitted to a hydrolysis using KOH solution. Negatively-charged TiO₂ particles with a polyethylenimine (PEI) polycation layer were alternatively spraying onto textiles. Another functionalization was also performed by adding an interfacial layer of PEI before building bilayers of alternatively positively-charged TiO₂ particles with a poly(styrene sulfonate) (PSS) polyanion. These sequence were repeated 1-5-10 times until (PEI/TiO₂)ₙ, or PEI/(PSS/TiO₂)ₙ bilayers were achieved. The antibacterial properties of functionalized textiles against *E. coli* were determined after 15-30-60min of solar light radiation (226W/m² of visible light).

**Conclusions**

Whereas no antibacterial effect was observed for the textiles pretreated by KOH and kept in the dark, we respectively obtained a 2log reduction for the textiles functionalized with only one layer of PEI and a 6log reduction with addition of a single layer of TiO₂ (PEI/TiO₂)₁. For the PEI/(PSS/TiO₂)ₙ textiles, antibacterial activity is function to solar light duration and also depends of the number of bilayers. The best antibacterial activity (4log reductions) was obtained for PEI/(PSS/TiO₂)₁₀ under 60min of solar light radiation.
New antimicrobial mechanisms

DETERMINING GROWTH INHIBITION MECHANISMS OF THE ANTIMICROBIAL AGENT FREE NITROUS ACID (FNA) FROM THE TRANSCRIPTOME RESPONSE OF PSEUDOMONAS AERUGINOSA PAO1
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Background

Free nitrous acid (FNA) has recently been demonstrated as an antimicrobial agent to a range of microorganisms. However, the antimicrobial mechanism of FNA is largely unknown.

Objectives

Consequently, we aim to gain a systematic understanding of the bacteriostatic/bactericidal effects of FNA. This was examined on Pseudomonas aeruginosa PAO1, an organism with intrinsic resistance to various antibiotics and disinfectants.

Methods

P. aeruginosa was grown under anaerobic denitrifying conditions and when FNA was added (0.05 mg N/L) growth temporarily stopped. From cultures either exposed or not exposed to added FNA, RNA was extracted and transcripts were detected by HiSeq-Illumina sequencing. Transcripts were quantified and those exhibiting ≥2.5 or ≤-2.5 fold abundance change were designated as differentially expressed in response to FNA.

Conclusions

In response to FNA 177 genes showed increased transcription while 471 genes exhibited deceased transcript levels. Genes coding ribosome proteins showed significantly decreased transcript levels, implicating that protein biosynthesis was severely inhibited by FNA. Respiration was likely inhibited, as lowered substrate utilization coincided with decreased transcript levels of genes coding for denitrification. In contrast, genes encoding nitric oxide reductase were up regulated, which possibly functioned to remove toxic NO derived from the added nitrite. In the response, genes of the tricarboxylic acid cycle were less expressed, while enzymes of the glyoxylate shunt had increased levels. Evidently the cells would generate less reducing power through these cycles. This first investigation elucidating the
mechanisms of FNA effect on *P. aeruginosa*, contributes underpinning knowledge for potential application of this novel antimicrobial agent.
ELUCIDATION OF THE MODE OF ACTION OF A NEW ANTI-PSEUDOMONAL COMPOUND

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Background

*Pseudomonas aeruginosa* is a gram-negative pathogen that causes severe infections in immunocompromised patients and is responsible for 10 – 15% of the hospital-acquired infections worldwide. Due to the emergence of multidrug-resistant *P. aeruginosa* strains, there is an urgent need for the development of new antibiotics with activity against *P. aeruginosa*. We recently identified a new antibacterial compound, SPI003, which inhibits growth of various gram-positive and gram-negative pathogens, including *P. aeruginosa*.

Objectives

In this study, we sought to unravel the mode of action of SPI003, using *P. aeruginosa* as a model organism.

Methods

In order to unravel the mode of action of SPI003, different approaches were used. First, a library of *P. aeruginosa* mutants was screened to identify mutants that are sensitive or resistant to SPI003. Second, spontaneous resistant mutants were generated by plating *P. aeruginosa* cells on agar plates supplemented with SPI003 at a final concentration of 5 x MIC. Third, a transcriptome analysis was carried out on exponentially-growing cells of *P. aeruginosa* treated with the compound. Finally, the membrane permeability of *P. aeruginosa* was examined after exposure to SPI003 using established assays.

Conclusions

Our study shows that SPI003 exhibits a fast membrane-damaging activity, which is likely to be the primary cause of its antibacterial activity.
INVESTIGATION OF ATMOSPHERIC COLD PLASMA AGAINST ESCHERICHIA COLI MUTANTS

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Background

Atmospheric cold plasma (ACP) is effective against a wide range of pathogenic microorganisms with promising application in food and medical industry. Generation of reactive oxygen species (ROS) plays a crucial role when air/oxygen containing gases are used. As a mixed group of oxidative reactive species generated by ACP, there are limited publications about the cellular regulation system under plasma pressure.

Objectives

To investigate the effect of the plasma stress on the microbial inactivation, intracellular reactive oxygen species and genetic regulation.

Methods

Escherichia coli BW 25113 and its isogenic mutants in soxR, soxS, oxyR, rpoS and dnaK genes were suspended in phosphate buffered saline (PBS) at 10^8 CFU/ml. Samples were then treated with high voltage plasma (80 kVRMS) in a sealed package for 1, 3 and 5 min following 0, 1 and 24 h post-treatment storage. Intracellular ROS were measured using fluorescence spectrophotometer (Biotek synergy, 480/530nm) with the probe 2′, 7′- dichlorofluorescein diacetate immediately after storage.

Conclusions

Without post-treatment storage time, the absence of RpoS and OxyR led to higher reduction after treatment. However, DnaK showed its repairing function correlated to cell survival with 24 h, while it has no effect on reducing intracellular ROS level. SoxS became important with both 1 and 24 h storage, which is not observed in SoxR. It implied their different characteristics as subunits. Overall, the cell response against plasma generated oxidative stress could be divided to short term and long term, which are dominated by oxidative stress deletion genes and damage repairing genes.
NEW ENVIRONMENTAL-FRIENDLY COMPOUNDS TO COMBAT CITRUS CANKER

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Background
The culture of citrus fruits is constantly under the threat of plant diseases. One of them is citrus canker, which is caused by the bacterial plant pathogen Xanthomonas citri subsp. citri (Xac). Current modes of prevention of Xac infection and spreading include spraying trees with copper containing bactericides and eradication of infected and healthy trees in a certain radius around. Despite these efforts, about 300 Million tons of fruit per year were lost during the last decade.

Objectives
In addition to being inefficient, copper containing bactericides negatively impact soil quality since copper accumulates in the environment. Therefore, more sustainable ways to combat citrus canker are urgently needed. Recently, a class of plant-derived semi-synthetic compounds, the alkyl gallates, has shown promising activity against Xac [1] and the bacterial cell division protein FtsZ has been found to be their target (unpublished).

Methods
Here, we present in vitro results on the activity of the alkyl gallates against FtsZ from different bacteria. We also discuss the ideal alkyl chain length of a lead gallate candidate as a platform for chemical synthesis of new compounds with enhanced antibacterial activity.

Conclusions
Our initial results show that the development of novel environmental-friendly antibacterials to combat Xac is feasible. The efficacy of these compounds in the field will be evaluated and their toxicity to plants and animals will be determined. We expect that the compounds can be used as a sustainable alternative to prevent citrus canker and they will contribute to control the spread of Xac in citrus plants.
FEMS-2414
New antimicrobial mechanisms

MECHANISMS OF ANTIBACTERIAL ACTION OF CATIONIC PEPTIDE WARNERIN
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Background
Rapid development of antibiotic resistance of bacteria necessitates the search for new natural compounds inhibiting the growth and functioning of pathogenic microflora.

Objectives
To study the antibacterial mechanisms of warnerin, a new peptide from lantibiotic family that demonstrate the sequence of events causing the death of attacked bacterial cells.

Methods
Outcomes of warnerin attack of Staphylococcus epidermidis 33 GISK (Moscow) were analyzed using the combination of different methods such as polarography (respiration), spectrography (intracellular potassium loss), fluorescence assay of membrane potential dynamics and hydroxyl radicals’ formation, electron and atomic-force microscopy of cell structure damages

Conclusions
Peptide introduction into the bacterial incubation medium results in the increase in their oxygen consumption followed by rapid cellular release of potassium ions and sharp decline of intracellular ATP concentration. Apparently, the disturbance of ATP-linked electron transport along the respiratory chain favors their accumulation on iron-sulfur centres. This results in increase of free Fe²⁺ ions and as a consequence, to the elevation of hydroxyl radical concentrations. Peptide inhibition of ATP formation is accompanied by marked proton accumulation on the outer side of bacterial membrane, particularly in its adjacent layers of peptidoglycane. This facilitates the autolytic hydrolase extrusion from the binding sites and to uncontrolled total bacteriolysis that is revealed by microscopic data. Results of investigation evidence for the pluripotent antibacterial action of warnerin that point to expressed prospects for its practical use.

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FUNCTIONALIZATION OF PROTOPORPHYRIN IX WITH ANTIMICROBIAL PEPTIDES TO ENHANCE PHOTOANTIMICROBIAL CHEMOTHERAPY (PACT).

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Background
Photodynamic antimicrobial chemotherapy (PACT) combines activities of light, photosensitizing compound (PS) and oxygen, to eradicate microorganisms through the oxidation-related mechanisms. To obtain more specific photodynamic action, PS can be functionalized with various types of adducts. Presented approach combines activity of a photosensitizer and antimicrobial peptides (AMPs).

Objectives
Protoporphyrin IX (PPIX) covalently conjugated with antimicrobial peptides: PEX or CAM, was analyzed for its spectral properties and antimicrobial action. Bacterial strains used in the study were Staphylococcus aureus Newman (MSSA) and COL (MRSA).

Methods
Peptides were synthesized using the methodology Fmoc/tBu on polystyrene resin and purified by HPLC. MIC values were determined by the broth dilution method in BHI medium in the darkness. Absorbance spectra screening was performed using the EnVision™ microplate reader (PerkinElmer) within 350-700 nm range. PACT was conducted using the LED lamp (SecureMedia, Poland) emitting incoherent red light ($\lambda_{\text{max}}$ 627 nm). Viability of bacteria was calculated counting colony forming units (CFU).

Conclusions
Combination of pure compounds showed no influence on PPIX spectral properties and AMPs activity by means of MIC values and showed synergistic effect of PPIX and CAM in PACT experiments. PS-AMP conjugates reveal weaker antimicrobial activity by means of MIC values, as compared to pure peptides. Conjugation of peptides to PPIX changed its spectral properties, visualized by hypsochromic shift of absorbance spectra, hyperchromic effect in Soret band and hypochromic effect in Q bands. These observation implied weaker/no photodynamic activity induced by red light, which was confirmed experimentally, however indicated efficient activity induced by blue light.
Background
Antimicrobial efficacy of silver nanoparticles (AgNPs) was proved and broad applications were proposed so far. This study shows the potential mechanism of AgNPs action towards opportunistic pathogen Pseudomonas aeruginosa.

Objectives
Study of the AgNPs effect on selected P. aeruginosa virulence factors.

Methods

P. aeruginosa PAO1 was used in this study. Silver nanoparticles were obtained from Nano-Tech (Warsaw, Poland). Minimal inhibitory concentration of silver nanoparticles that inhibit biofilm formation (MBIC) was estimated by standard two-fold dilution method. Swimming motility was examined on 0.3% agar plates and swarming motility on 0.6% agar plates supplemented with 0.05–0.5xMBIC AgNPs. Twitching motility across the glass surface was analysed after staining with crystal violet. Pyoverdine production was estimated in 48-hour PAO1 biofilms grown in microtitre plates. Biofilm formation was studied after crystal violet staining and cell survival was determined in Live/Dead Assay (Promega). Statistical analysis of the results in comparison to control sample without AgNPs was performed.

Conclusions

Strong antibacterial effect of AgNPs was observed. MBIC was 4 microgram mL\(^{-1}\). There was significant decrease from 22% to 40% in swimming and from 22% to 29% in swarming motility in samples containing 0.1–0.5xMBIC of AgNPs in comparison to P. aeruginosa PAO1 incubated without nanoparticles. No influence of AgNPs on twitching motility was observed. Pyoverdine production was significantly inhibited by AgNPs in concentration of 0.5 MBIC. The inhibition of biofilm formation by 44% was also observed. Hence, it was shown that silver nanoparticles affect several virulence factors of P. aeruginosa including swarming, swimming motility, pyoverdine production and biofilm formation.
Background

Microorganisms associated with eukaryotic hosts are involved in various physiological functions, including chemical defense and immune responses. Production of bioactive molecules such as antibiotics and antifouling substances by associated bacteria, is one of the mechanisms to protect the host against pathogens, predation and surface colonization.

Objectives

In this study, extracts from 10 bacterial strains isolated from the blue mussel, *Mytilus edulis* (Kiel Fjord, Baltic Sea, Germany), were screened for their antimicrobial activity against two panels of test microorganisms: a standard panel (*Escherichia coli*, *Bacillus subtilis* and *Candida albicans*) and environmental panel composed of strains isolated from the mussels (*Pseudomonas veronoi*, *Bacillus pumilus* and *Shewanella baltica*).

Methods

To identify isolated strains, PCR amplification of 16S rRNA was carried out and bioactivity tests were modified according to Schneemann et al. (2010) to evaluate the interaction of isolated strains with the test microorganisms.

Conclusions

Inhibiting effects varied considerably among the test microorganisms, with *P. veronoi* showing the most pronounced antibacterial activity against both the standard and the environmental panels. Moreover, the Gram-positive bacteria, and particularly *B. pumilus*, were generally more susceptible than Gram-negative strains, whereas no strain was found to inhibit growth of *E. coli*. These findings suggest that bacteria associated with the blue mussels may be involved in preventing pathogen invasions and that these hosts represent a promising source for the isolation of
antibiotic-active bacteria.
Background

Cryptococcosis is a potentially fatal systemic mycosis, which is caused by *Cryptococcus neoformans*(Cn) and *C. gattii* (Cg). This disease is an opportunistic infection for AIDS. *Cryptococcus* is able to cause three types of infections, cutaneous cryptococcosis, pulmonary cryptococcosis and cryptococcal meningitis. The treatment recommended is the intravenous amphotericin B with flucytosine administered orally. This drug combination causes severe side effects and yeast-resistance, encouraging the search for innovative anti-*Cryptococcus* agents.

Objectives

The objectives of this work were synthesis of chalcone and hydroxychalcones, determination of antifungal activity against Cn and Cg strains, evaluation of cytotoxicity against mammalian cell lines, and evaluation of the inhibitory cell adhesion activity of Cn onto pneumocytes.

Methods

The chalcone and hydroxychalcones were synthesized by Claisen-Schmidt condensations. The anti-*Cryptococcus* activity was evaluated by microdilution test performed in 96-well plates, using CLSI-M27-A2 document. This assay demonstrated the values of Minimum Inhibitory Concentration (MIC) and Minimum Fungicidal Concentration (MFC) to five strains; Cn-ATCC90012, and four clinical isolates CnS, CnI, CnR and Cg118. The cytotoxicity was evaluated by MTT colorimetric assay, using five mammalian cell lines, A549, HepG2, MRC-5, U87-MG and NOK.

Conclusions

The hydroxychalcones exhibited potent antifungal activity, demonstrating MIC and MFC values ranging from 1.95 to 15.6µg/mL, which were related to high inhibition of Cn adhesion onto pneumocytes. In MTT assay, at 100µg/mL, these compounds were not toxic for all cell lines. We conclude that hydroxychalcones are antifungal hits, with relative selectivity for yeast, and their mode of action is related to inhibition of yeast adhesion onto host-cells.
IN VIVO CLUSTER FORMATION OF NISIN AND LIPID II IS CORRELATED WITH MEMBRANE DEPOLARIZATION

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Background

Nisin and related lantibiotics can kill bacteria in two ways: by pore formation, which leads to membrane depolarization, or by sequestering Lipid II, which interferes with cell wall synthesis. Literature shows that during sequestration, nisin and Lipid II cluster into non-physiological domains, which are visible with fluorescence microscopy. Recently we observed that a reported 'non-pore' forming nisin mutant clusters LipidII, but also depolarizes the membrane (Lages et al., 2013, Environm. Microbiol. 15, 3272).

Objectives
To further investigate the relation between LipidII clustering and membrane depolarization.

Methods
Microscopy, fluorescent assays to detect membrane integrity.

Conclusions

We used in vivo assays to investigate how and when nisin forms pores, depolarizes the membrane, and segregates Lipid II into domains. By comparing the activity of pore-forming and pore-deficient mutants of nisin we show that clustering of nisin and Lipid II only occurs when (i) membrane integrity is reduced by pore formation and (ii) when the membrane is completely depolarized. Furthermore, pore-forming variants of nisin were also able to kill L-forms – which grow without a cell wall – whereas non-pore forming lantibiotics only kill bacteria with a cell wall. Our results have important implications for the mechanism-of-action of nisin. Because cluster formation never occurred with non-pore-forming lantibiotics, we suggest that lantibiotics either kill by pore-formation, or by occlusion of Lipid II for incorporation into the cell wall.
Background

Combinatory effect between conventional antimicrobial drugs and phytochemical products can be a new strategy to increase effectiveness of antibiotics.

Objectives

We aimed to verify synergism between essential oils compounds from plants and antimicrobial drugs for methicillin-resistant Staphylococcus aureus (MRSA).

Methods

Five essential oils compounds (citronellol, geraniol, eugenol, terpineol and cinnamaldehyde) were tested against 10 strains of S. aureus MRSA. Mueller-Hinton agar (MHA) plus 0.5% Tween 80 was mixed with each compound (¼ of Minimal Inhibitory Concentration (MIC90%)). Nine antimicrobial drugs discs (oxacillin (1 µg), gentamicin (10 µg), erythromycin (15 µg), sulfazotrim (25 µg), vancomycin (30 µg), penicillin G (10 U) levofloxacin (5 µg), tetracycline (30 µg) and linezolid (30 µg)) were laid on culture medium inoculated with S. aureus. Assays were performed in duplicate. Mann-Whitney test was used as statistical. Time kill curves were performed between EOC and AD for positive cases. Treatments were incubated with 10^5 CFU/mL of MRSA strain in MH broth. Media without EOC and/or AD was used as control. Aliquots were removed at different time intervals between 0 and 24h. Viable colonies were counted after incubation (24 h/ 37ºC) in order to obtain CFU/mL.

Conclusions

The present study showed a promising way of treatment for diseases caused by MRSA as well as against other bacterial species, because time kill curves for EOC an AD demonstrated additive activity. Thereby, these compounds can be used in combination with conventional antimicrobials.
SENSITIVITY OF MDR ACINETOBACTER BAUMANNII WOUND ISOLATES TO RUMEX SANGUINEUS, RUMEX CRISPUS AND URTICA DIOICA EXTRACTS

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Background
Acinetobacter baumannii is a pleomorphic, non-motile bacterium and etiological agent of various infections in immunocompromised patients. It has been characterized as a novel, drug resistant and rapidly emerging clinical pathogen with increasing prevalence. Regarding recent multi-drug resistant A. baumannii strains emergence, it is urgent to examine alternative antimicrobial agents, including various plant extracts.

Objectives
The aim of this study was to determine in vitro antimicrobial activity of Rumex crispus, Rumex sanguineus and Urtica dioica extracts against multi-drug resistant A. baumannii wound isolates.

Methods
The broth microdilution assay was used to determine minimal inhibitory concentrations (MIC) and minimal bactericidal concentrations (MBC) of 8 plant extracts obtained from different parts of the examined plants. For antibacterial activity testing 20 multiple resistant A. baumannii wound isolates and 2 reference strains (ATCC BAA-747 and ATCC 19606) were used.

Conclusions
Urtica dioica possessed no effect against A. baumannii, while Rumex sanguineus and Rumex crispus extracts showed significant antibacterial activity against all examined isolates. Bacteriostatic effect was obtained with concentrations 1 - 4 mg mL⁻¹ with median value 2 mg mL⁻¹, while bactericidal activity was achieved with a slightly higher concentrations of Rumex extracts (1 to 8 mg mL⁻¹ with median 4 mg mL⁻¹). The obtained result confirmed earlier detected antibacterial effect of R. crispus extracts against A. baumannii, while anti-A. baumannii activity of R. sanguineus extracts was detected for the first time. Accordingly, the plants from the genus Rumex are valuable source of bioactive compounds against this bacterium and antimicrobial activity of dominant extract components should be further examined.
Background
Staphylococcus aureus infections are becoming increasingly difficult to treat due to antibiotic resistance. Of particular concern are the community-associated methicillin-resistant S. aureus (CA-MRSA) strains such as USA300. Virulence of CA-MRSA is regulated by the agr quorum-sensing system, which in response to agr-encoded autoinducing peptides confines exotoxin production to the stationary growth phase with concomitant repression of surface-expressed adhesins. In need of novel treatment options, anti-virulence therapy, targeting virulence factors rather than viability, has been proposed. Solonamide B was recently isolated from a marine bacterium as a putative anti-virulence compound that markedly reduced expression of the toxins α-hemolysin and the phenol-soluble modulins.

Objectives
To further strengthen the anti-virulence candidacy of Solonamide B and synthesized analogues.

Methods
Chemically synthesized solonamides and lactam analogues were validated for interference with agr virulence gene regulation, both quantitatively and qualitatively, using reporter fusion strains. The compounds' influence on biofilm formation and fibronectin binding of S. aureus were assessed as examples of complications related to agr negative strains; and lastly, toxicity of the compounds and the immune modulating capacity of solonamide-treated S. aureus were assessed on PBMCs, T-cell populations and DCs via flow cytometry.

Conclusions
We find that structural differences between solonamide analogues interfere differentially with agr, and that they do not display toxicity or influence immune cell activity and integrity. Importantly, our results also dismiss the major concern that application of compounds mimicking an agr-negative state may lead to adverse
interactions with host factors and immune cells.
Background

Preparation of liposome surface modified by various materials such as RNA, DNA or Proteins has been developed for several decades.

Objectives

We would like to investigate targeting ability of lysosomal membrane proteins related to killing bacteria through modifying the liposome surface to check the possibility of as antimicrobial agents whereby we tried to find specific membrane proteins.

Methods

Firstly, we used mixtures of lipid, cholesterol and lysosomal membrane protein isolated from lysosomes extracted from *S. cerevisiae* treated by H$_2$O$_2$ and NH$_4$Cl to modify liposome surface for antimicrobial activity after making liposomes prepared with hydration method. Liposomes were modified by lysosomal membrane proteins with two different methods in order to confirm the targeting ability.

Conclusions

The results showed that the antimicrobial activity was not shown without modification of liposome surface but it was better cell mortality in type 1 than type 2 method. Based on these results, we analyzed whole lysosomal membrane proteins by 2-DE assay to find specific proteins associated with antimicrobial activity and constructed recombinant *S. cerevisiae*. And then, we found the genes (HSP70) related to antimicrobial activity and prepared liposomes modified by lysosomal membrane proteins extracted from lysosomes isolated from recombinant *S. cerevisiae* tagged with GFP. The result showed that the antimicrobial activity of liposomes modified with Type1 method was better than control. Therefore, lysosomal membrane proteins for targeting bacteria will play an important a role.

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Research Program for agriculture Science & Technology Development (Project No:PJ01052701) Rural Development Administration, Republic of Korea.
Background
Lantibiotics and microcins form different groups of posttranslationally modified bacterial peptides mainly produced by Gram-positive and Gram-negative bacteria, respectively. Most of these peptides exhibit a potent antimicrobial activity, more active even than some antibiotics. Maturation of a peptide begins with a propeptide which is guided throughout specific modification events due to leader peptide (an amino acid sequence recognized by particular modification biomodule). The leader peptide is proteolitically removed in the last steps of peptide modification making the modified peptide active. Posttranslational modifications endorse lantibiotics and microcins with high target-specific activities and stability against proteolysis.

Objectives
To design plug-and-play biomodules for production of novel peptides containing both, lantibiotic- and microcin-specific posttranslational modifications.

Methods
We used a synthetic biology approach to design biomodules. We utilized peptide modification systems cloned under different controlled promoters to assure gradual and temporal activity of designed biomodules. Codon optimized synthetic genes of designed model peptides to be modified were employed together with modification machineries.

Conclusions
This is a unique case where posttranslational modifications from two different peptide classes are fused together into a single peptide chain in vivo. Here, we also show the possibilities of employing chimeric leader peptides for substrate recognition and procession by particular posttranslational modification machineries.
Background
Silver and its compounds have strong antimicrobial activities for bacteria, fungus, and viruses. Recent advances in researches made on metal nanoparticles appear to revive the use of silver nanoparticles (SNPs) for antimicrobial applications. It has been shown that SNPs prepared with a variety of synthetic methods have effective antimicrobial activity.

Objectives
In this work, silver nanoparticles (Ag NP) with 5% silver chloride (NaNoRa\textsuperscript{2}), were used to evaluate the antimicrobial activity against various bacteria Gram-positive, Gram-negative and yeasts.

Methods
Silver nanoparticles (NanoRa\textsuperscript{2}) were synthesized with silver chloride to 5%, by the Sol-Gel method. Silver nanoparticles were characterized by SEM (Scanning Electron Microscope). Silver nanoparticles (NanoRa\textsuperscript{2}) were tested for different Gram-positive bacteria as \textit{Enterococcus faecalis}, \textit{Staphylococcus aureus}, \textit{Staphylococcus epidermidis}, and Gram-negative bacteria such as \textit{Klebsiella pneumoniae}, \textit{Escherichia coli}, \textit{Pseudomonas aeruginosa}, \textit{Proteus mirabilis} and \textit{vulgaris}, as well as the yeast \textit{Candida albicans}. The antibacterial activity of the silver nanoparticles was evaluated following the guidelines for the Kirby-Bauer diffusion method according to the CSLI.

Conclusions
Nanoparticles have a particle size of 100nm. The spectrophotometric profile showed characteristic IR peaks, links titanium dioxide that have the NanoRa\textsuperscript{2}. The silver nanoparticles were able to inhibit the growth of Gram-positive, Gram-negative and yeasts from 10 ppm and 50 ppm. In the case of \textit{Staphylococcus aureus} methicillin resistant as well as \textit{Candida albicans}, the silver nanoparticles at a concentration of 50 ppm, larger zones of inhibition were showed.
P113-DERIVED PEPTIDES AS NOVEL INHIBITORS FOR DRUG-RESISTANT CANDIDA SPP.

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Background

Candida spp., major opportunistic pathogens, infects immunocompromised people and is responsible for life-threatening infections. The individuals commonly affected include those with oral candidiasis of HIV-positive patients, cancer patients undergoing radiotherapy, and xerostoma patients. Candida infections may further result in severe systemic infection and convert multiple organ failure (1). Moreover, Candida spp. can develop resistance to multiple antifungal drugs by over-expression of drug resistance-related targets.

Objectives

In this study, we developed a patented anti-fungal peptide P-113 to overcome the emerging issue of drug-resistance in Candida infections. We also modified P-113 by tandemly repeating sequences to improve the candidacidal efficiency.

Methods

The P-113, derived from human histatin-5 protein, retains a full antibacterial activity and exhibits a wide spectrum of activity in vitro against both bacteria and fungi (2). Several drug- (azoles-) resistant clinical strains including C. albicans, C. krusei, C. glabrata, and C. tropicalis were isolated from oral candidiasis patients. We investigated the susceptibility of these drug-resistant strains to P-113 by candidacidal assay (3).

Conclusions

The results showed that P-113 was able to kill these drug-resistant strains, suggesting a promising candidacidal activity of P-113 toward drug-resistant Candida spp. To improve the candidacidal activity, we designed P-113-derived dimeric and trimeric peptides which contain 2- and 3-time tandem repeats. The two tandem repeats improved the candidacidal potency of the peptides, and the dimer and trimer
have IC$_{50}$ values against *C. albicans* of 0.5 and 0.4 µM, respectively, compared with the monomer P-113 (IC$_{50}$ = 2.3 µM). P-113 and its derivatives show a potential for the development of novel therapeutics against *Candida* infections.
New antimicrobials for resistant organisms

SYNERGISM OF FLORFENICOL IN COMBINATION WITH ANALOG THIAMPHENICOL AGAINST STAPHYLOCOCCUS HYICUS, STREPTOCOCCUS SUIS, AND PASTEURELLA MULTOCIDA


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Background
Florfenicol (FF) and thiamphenicol (TAP) are amphenicol antibiotics commonly used in veterinary and aquiculture practice with broad-spectrum antibacterial activity. Previous study found that FF and TAP exhibited synergistic effect against Staphylococcus aureus (including methicillin-resistant) clinical isolated strains.

Objectives
To further investigate the spectrum of synergistic activities of FF and TAP combination.

Methods
The antibacterial activities of FF and TAP combination against a total of 69 strains of Salmonella enterica, Pasteurella multocida, Pseudomonas aeruginosa, Riemerella anatispestifer, Streptococcus suis, and S. hyicus were evaluated by checkerboard assay and time-kill study. For protective effects of combination therapy on chicken, FF and TAP alone or in combination were administered intramuscularly 30 mins prior to the intramuscular infection of P. multocida.

Conclusions
The checkerboard assay showed additive effect (0.5 ≤ fractional inhibitory concentration (FIC) index ≤ 1) of FF/TAP combination in 75% of P. multocida, 53% of S. suis, 50% of S. hyicus, 33% of P. aeruginosa and 11% of S. enterica isolates that also include FF and TAP-resistant strains. In addition, 8 strains of P. multocida, S. suis, and S. hyicus were further demonstrated in the time-kill study confirming synergism between these two drugs. It also appeared that the combination enhanced the bacteriostatic effect to bactericidal on the 3 bacteria. Most notably, the P. multocida-challenged chickens were effectively protected by FF/TAP combination at lower dosages. Present study demonstrated the potentiality of a novel combination regimen of the same class of antibiotics against P. multocida infections.
NEW ANTIMICROBIALS FROM ACTINOALLOMURUS

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Background

These past few years have been changing dramatically the landscape of infectious diseases, showing that investment on drug discovery should not be disregarded as a steady emergence and spread of antibiotic resistance keeps occurring and giving origin to more multi-drug resistant strains. One possible source to enhance the antibacterial arsenal is to look on what Nature has been using to solve the very same problem. With the myriad of species waiting to be scrutinized, it is wise to give priority to those less exploited but phylogenetically related to known producers.

Objectives

This project is part of a screening program designed to assess the metabolic potential of the recently described genus *Actinoallomurus*, and to deliver new molecular structures.

Methods

The program includes small-scale fermentations followed by in vitro and in vivo bioactivity tests on fermentation broths extracts. Positive samples are then subjected to a combination of bioassays and chemical analysis (HPLC, MS and NMR and a query within ABL (a proprietary antibacterial database) to determine the novelty of active compounds and discard those already known.

Conclusions

Our study shows *Actinoallomurus* carries the biosynthetic machinery to create a vast range of different active molecules. The dereplication process of 200 *Actinoallomurus* microbial extracts revealed the potential to produce a broad range of chemical classes, from lantipeptides to new hyperhalogenated angucyclines or aromatic polyethers. The diversity of the clusters of secondary metabolism found in the sequenced genome of an *Actinoallomurus*, seems to corroborate the theory that this genus may become an important producer of new active molecules.
New antimicrobials for resistant organisms

OPSONOPHAGOCYTOSIS RESPONSE IN MICE IMMUNIZED WITH CIH RECOMBINANT PROTEIN FROM STAPHYLOCOCCUS AUREUS

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Background

The human pathogen Staphylococcus aureus is responsible for many hospital-associated and community-acquired infections. The emergence of methicillin-resistant S. aureus (MRSA) and vancomycin resistant S. aureus strains have prompted research into staphylococcal vaccines and preventive measures. Staphylococcal clumping factor A (ClfA), iron surface determinant B (IsdB) and gamma hemolysin B (HlgB) are key virulence factors and opsonic activity plays an important role in successful vaccine development against S. aureus infections.

Objectives

In this report, the BALB/c mice were immunized with a recombinant protein (CIH) composed of selected antigenic regions of ClfA, IsdB and HlgB of S. aureus. After the immunization, opsonophagocytic activity assay (OPK) was performed to all serum samples.

Methods

After exposing the opsonized bacteria with peritoneal macrophages, the phagocytic capacity of macrophages exposed with serum from mice immunized with recombinant protein CIH showed a significant increase as compared with macrophages exposed with serum from mice immunized with PBS (p = 0.002).

Conclusions

Our finding indicated, that immunization of BALB/c mice with recombinant protein CIH promotes the phagocytosis of S. aureus and enhances the clearance of the in vivo pathogen.
Background

Due to the resistance of microorganisms to antibiotics, studying alternative methods such as plant extracts becomes very important.

Objectives

Evaluating the in vitro antifungal activity of Persea americana extract on C. albicans biofilm and its cytotoxicity in macrophage culture (RAW 264.7).

Methods

For the determination of the minimum inhibitory concentration (MIC), microdilution in broth (M27-A2 standard) was performed. Thereafter, the concentrations of 12.5, 25, 50, 100 and 200 mg/mL (n=10) with 5 min exposure were analyzed on mature biofilm in microplate wells for 48 h. Saline was used as control (n=10). After treatment, biofilm cells were scraped off and dilutions were plated on Sabouraud dextrose agar. After incubation (37°C/48 h), the values of colony forming units per milliliter (CFU/mL) were converted to log10 and analyzed (ANOVA and Tukey Test, 5%). The cytotoxicity of the P. americana extract was evaluated on macrophages by MTT assay.

Conclusions

Results: The MIC of the extract was 6.25 mg/mL and with 12.5 mg/ml there was elimination of 100% of planktonic cultures. Regarding the biofilms, a significant reduction (p

Conclusion: P. americana extract showed antifungal activity against C. albicans biofilm, whereas the concentration of 50 mg/mL presented the best results.
Evaluation of Myrtus Communis and Ziziphus Leaves Antimicrobial Activity on IMP-Type Metallo-Beta-Lactamase Producing Pseudomonas Aeruginosa Strains


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Background

Pseudomonas aeruginosa is one of the leading nosocomial pathogens. In recent years, inappropriate use of antibiotics is the cause of untreatable ‘superbugs’ and high death rates in clinics in worldwide. One of the mechanisms of resistance to carbapenem antibiotics in P. aeruginosa is metallo-β-lactamases (MBLs) production that hydrolyzes all carbapenems that are the last generation of drugs for treatment of infections caused by MDR P. aeruginosa.

Objectives

The aim of this study is evaluating the effects of Myrtus communis leaves and ziziphus leaves methanolic extract on IMP-Type metallo-beta-lactamase producing P. aeruginosa strains.

Methods

This experimental study was carried out on hospitalized burn patients during 2012-2013. Antibiotics and extracts susceptibility tests were performed by disc diffusion and broth microdilution methods. MBL detection was performed by Combination Disk Diffusion Test (CDDT). The bla(VIM) and bla(IMP) genes were detected by PCR and sequencing methods.

Conclusions

It was found that among 83 imipenem resistant P. aeruginosa strains, 48 (57.9%) were MBL producers. PCR and sequencing methods proved that these isolates were positive for blaIMP-1 genes, whereas none were positive for bla (VIM) genes. All MBL-producing P. aeruginosa were resistant to Meropenem, Imipenem, Ceftazidime, Amikacin, Tobramycin, Ciprofloxacin, Aztreonam, Piperacillin/Tazobactam, Ceftriaxone, Cefepime and Carbenicillin. The result of MIC for imipenem was 128(µg/ml) for all strains. The MIC (mg/ml) and MBC (mg/ml) results of Myrtus
*communis* was 6.25 and 12.5 (mg/ml) for all isolates, *respectively* Although the ziziphus leaves methanolic extract represent any significant result on this isolates.
FEMS-2578
New antimicrobials for resistant organisms

IDENTIFICATION AND CHARACTERIZATION OF A NOVEL CRYPTIC CATIONIC ANTIMICROBIAL PEPTIDE FROM THE THIRD DOMAIN OF LIFE

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Background

The huge increasing of bacterial strains resistant to classical antibiotics is threatening the public health security, thus the need of developing new antimicrobials has become critical. Cationic Antimicrobial Peptides (CAMPs) represent suitable alternatives, since they do not lead to selection of resistant strains. Indeed, CAMPs act by disrupting the membrane integrity and/or by altering its potential, so that, to acquire resistance bacteria need to remodulate the membrane composition. Such modifications could be detrimental to bacterial cells, thus dramatically reducing the onset of resistant strains.

Objectives

Although proteins involved in the immune response of multicellular eukaryotes represent a natural source of CAMPs, new methodologies for their identification are required to encounter the rising demand of more active and stable CAMPs.

Methods

A powerful bioinformatics tool has been recently set up and used to predict the antibacterial activity of cryptic CAMPs (i.e. embedded in the primary structure of proteins). Given their hydrophobic/cationic nature, sequences of transcription factors were chosen as source of choice for the discovery of new cryptic CAMPs. In particular, our analysis focused on extremely stable proteins from thermophilic Archaea.

Conclusions
Among the identified CAMPs, one from the DNA-binding protein Stf76 (here named PepC) has been structurally and functionally characterized. This peptide has the capability of inducing both membrane fusion and pore formation. Interestingly, the DNA-binding activity was retained opening the way to speculate about novel mechanisms of action. Moreover, its antimicrobial activity has been proved on clinical strains isolated from patients affected by cystic fibrosis.
Background
Marine sponges are an important -and yet understudied- reservoir of new antimicrobials, a majority of which are produced by associated microbes.

Objectives
We tested the antibacterial activity of 110 bacterial strains isolated from two sponges (Halichondria panicea and Hymeniacidon perelvis) collected off rocky substrate in the low intertidal zone at Wimereux, France.

Methods
Strains were isolated on various media (R2A, Brain Heart Infusion (BHI)-agar and Marine agar) and tested against three target clinical strains: Pseudomonas aeruginosa ATCC 27853, Escherichia coli ATCC 25922 and Staphylococcus aureus ATCC 2913. Antagonistic activity was measured in double layer agar assays.

Conclusions
Fifty strains (45%) were able to inhibit the growth of at least one clinical strain (most often S. aureus). Antibacterial-producing strains belonged to Gamma-Proteobacteria (88%, mostly Pseudomonas spp.) and Firmicutes (12%, among which Lactococcus spp. and Vagococcus spp.). The production of antimicrobial substances turned out to be modulated by the medium formulation, its salinity (tested from 0.5 to 3.5%) and the temperature used in antagonism assays (tested from 8 to 25°C). The best results were obtained with BHI-agar, the lowest salinity and highest temperature tested. In all cases, antimicrobial substances were not soluble, as the antagonistic activity was lost when cultures were filtered on 0.2 mm-pore membranes. This study confirmed the potential of sponge-associated microbes for the production of antibacterial substances.
Background

With the emerging threat of infections caused by multidrug-resistant bacteria and scarce prospects of newly introduced antibiotics in the future, bacteriophages (phages) are currently being reconsidered as alternative therapeutics. However, the full breath of phage diversity suitable for treatment of bacterial infections is still largely unexplored.

Objectives

This study aims at providing an overview of the general occurrence of bacteriophages (with therapeutic potential) in natural environments and at devising strategies for their rapid characterization and classification at molecular level.

Methods

Novel phages were isolated from various aquatic systems (e.g. from general and hospital wastewater, activated sludge samples from sewage plants, streams, rivers, ponds and lakes). Environmental samples were pre-incubated with clinical isolates of multidrug-resistant bacteria. Enriched bacteriophages were subsequently obtained with the double agar layer plaque assay. “PhiSigns” (http://www.phantome.org/phisigns/) was used for phage identification based on the detection of signature genes. Genomic comparison of phages was performed based on digitized fluorescent restriction-fragment-length-polymorphism-analysis (fRFLP).

Conclusions

For roughly 50% of tested bacterial strains lytic bacteriophages were found against multidrug-resistant Pseudomonas aeruginosa, Klebsiella pneumoniae and Enterobacter sp. with waste water samples harbouring the highest phage diversity. By contrast no bacteriophages were isolated against multidrug-resistant Staphyloccoccus aureus and Acinetobacter baumanii. PhiSigns combined with fRFLP proofed to be a useful tool for rapid phage identification, which is the base for their proper selection for ultimate whole genome sequencing. S. aureus and A. baumanii phages require additional isolation steps such as FeCl₃-precipitation and/or use of
subinhibitory antibiotic-concentrations for enhancing plaque visibility on agar plates.
Background
Dermatophytes are well known for one of world-wide cause of diseases such as tinea pedis, tinea cruris, and tinea corporis. Dermatophytes infections are increasing of taking antifungal agents, accordingly increased side effects like toxicity and tolerance. That was why searches on natural antifungal medicine have motivated. Many kinds of woody essential oils have antifungal activity, especially essential oils of conifers. *Abies holophylla* Maxim (AH) is widespread in East Asia and its essential oil was known to have effective antifungal actions against *Aspergillus fumigatus*.

Objectives
Therefore, this study was to investigate the antifungal activity of AH essential oil against dermatophytes, such as *Epidermophyton floccosum*, *Trichophyton mentagrophytes* and *Trichophyton rubrum*, and to determine the potential effective compound as dermatitis treatment.

Methods
To evaluate the potential antifungal activities of AH essential oil and its fractions, Minimum Inhibitory Concentration (MIC) measurement and agar dilution method were used with morphological observation. Also, their major constituents were analyzed by GC/MS. The morphological changes of the dermatophytes exposed to the AH essential oil were observed by electron microscopes.

Conclusions
As the results, the highest activities were identified in the fraction containing borneol, limonene, 3-carene, and camphene. Also, morphological observation using electron microscopes (SEM and FEM) showed a dramatic changes of cell wall of dermatophytes exposed to the AH essential oil. In conclusion, AH essential oil and its constituents were expected to be used as antifungal agent or raw material for dermatitis therapy.
Background

Antibiotic resistant *Staphylococcus aureus* is a serious nosocomial pathogen and the major cause of life threatening infections, like burn wound infections. There is an urgent need to overcome antibiotic resistance by developing new antimicrobial treatment. The use of active plant secondary metabolites simultaneously with silver nanoparticles (AgNPs) against *S. aureus* is based on multi-target strategy.

Objectives

The aim was to find an alternative anti-staphylococcal treatment based on the combination of *Drosera binata* secondary metabolites and silver nanoparticles.

Methods

Plant extracts were obtained from *in vitro* cultured *D. binata* tissues. Antibacterial activity was determined by Broth Microdilutions Method. Checkerboard Titration technique was used to study interactions between AgNPs and extracts or secondary metabolites. Composition of the extract was established by the HPLC-MS analysis. The MTT Assay was used to evaluate cytotoxicity of antimicrobial agents towards *in vitro* cultured human keratinocytes.

Conclusions

Both silver nanoparticles and the *D. binata* extract possess antibacterial activity towards various *S. aureus* strains regardless of their antibiotic resistance. The synergistic bactericidal activity was observed when AgNPs and plant extracts were combined. This allowed to significantly reduce the bactericidal concentrations of AgNPs and the extract by 50% and 97%, respectively. Among all extract constituents only the chlorine derivative of plumbagin (ChPL) showed synergistic bactericidal activity with AgNPs. Moreover, reduced bactericidal concentrations of mixtures of
AgNPs with the extract or ChPL were observed to be non-cytotoxic towards human keratinocytes. Obtained results indicate that combination of AgNPs and secondary metabolites from *D. binata* tissues may provide an alternative antimicrobial treatment.
New antimicrobials for resistant organisms

ANTIBACTERIAL AND ANTIFUNGAL ACTIVITY OF NEWLY SYNTHESISED BENZOXABOROLES, BENZOSILOXABOROLES AND PYRIDOXABOROLES
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Background

Benzoxaboroles, and related compounds, are a particularly interesting group of synthetic agents. Their diverse biological activities and widespread applications in medicinal chemistry have undergone intense scientific investigation.

Objectives

We have examined whether new benzoxaborole-based derivatives and their analogues possess antimicrobial activity and are substrates for multi-drug-resistance efflux pumps.

Methods

The antibacterial and antifungal activities of newly synthesised compounds: 3 benzoxaborole-, 3 benzosiloxaborole- and 6 pyridoxaborole-based derivatives were evaluated. Antimicrobial activity was screened using the disc-diffusion test (0.4mg of the derivative was applied to a filter paper disc). The MIC and MBC values were estimated according to CLSI and EUCAST recommendations. During determination of MIC values ± PAbN (80mg/L), the concentrations of tested compounds in the broth medium ranged: 0.39-400mg/L. The 19 reference ATCC strains of Gram-positive, Gram-negative bacteria and yeast were used in this study.

Conclusion:

Generally, the most potent antibacterial and antifungal activities were found for fluorine substituted benzosiloxaboroles – analogues of benzoxaboroles possessing a silicon atom for carbon substitution at the 3-position (MICs 25-400mg/L and 0.78-100mg/L, respectively). Interestingly, the B(OH)\textsubscript{2}-substituted 3-phenylbenzoxaboroles were found to be the most potent against Gram-positive cocci: Staphylococcus sp. (MICs 12.5-50mg/L), Enterococcus sp. (MIC 200mg/L) and yeasts (MIC 50mg/L). Furthermore, we demonstrated that benzoxaboroles were removed by efflux pumps present in Gram-negative bacteria. Of note, only two pyridoxaborole-based compounds were found to be weakly active against Staphylococcus sp.

These data demonstrate structure-activity relationships of the tested derivatives and
highlight the need for further investigations into the antimicrobial properties of new benzoxaboroles and related compounds.
FEMS-2473
New antimicrobials for resistant organisms

APPLICATION OF A HIGH-THROUGHPUT ASPERGILLUS FUMIGATUS CELL WALL STRESS REPORTER SYSTEM: IDENTIFICATION OF SYNTHETIC PEPTIDES INCREASING THE SENSITIVITY FOR ANTIFUNGAL MEDICINES
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Background

Increased resistance to currently used antifungal compounds and the fact that these agents are often harmful to man and environment have resulted in a growing demand for new antifungals, which selectively act on cellular processes that are unique to fungi. To meet this demand, we have established in A. niger a luciferin/luciferase based reporter system for high-throughput screening of natural products for identification of potential new antifungal drugs.

Objectives

Our system allows us to identify compounds that specifically inhibited the fungal cell wall biosynthesis.

Methods

Recently the system has been developed for A. fumigatus and non-Aspergillus species. The system was validated by analyzing its performance with several antifungal drugs which are commonly used in the clinic like Caspofungin, Amphotericin B, Voriconazol (V fend) as well as Nikkomycin. A dose response behavior of the compounds could be demonstrated together with in time different types of stress responses. Importantly, whether the compound tested is fungistatic or fungicidal could be clearly indicated.

Subsequent analysis of different synthetic antimicrobial peptides, HTX1-4, revealed also a moderate effect on cell wall stress induction indicated by an increase in Lux activity.

Conclusions

Interestingly, incubation of these peptides at sublethal concentrations together with some of the antifungal medicines showed a considerable increase in lux activity.
and a significant increase in sensitivity of *A. fumigatus* for these medicines. Growth test with sublethal concentrations of HTX-2 on EBV-LCL, K562 cells and PHA-blasten, and also cytotoxicity- and genotoxicity tests with NCTC2544 cells did not show any negative/toxic effects on these human cell lines.
Background

Lantibiotics are potent peptide antimicrobials active against Gram-positive bacteria, including multidrug resistant pathogens. They display various posttranslational modifications, with lanthionine rings and dehydroamino acids as key signatures of this family of antimicrobials. In addition, some lantibiotics contain other enzymatic modifications that have a role in their activity.

Objectives

Our purpose is to combine different enzymes from diverse lantibiotic production systems in order to hypermodify the model lantibiotic nisin as a proof-of-principle. This can set a methodology for the design and modification of other (antimicrobial) peptides.

Methods

We have combined, in either *Lactococcus lactis* or *Escherichia coli*, the modification machinery of nisin (the dehydratase NisB, the cyclase NisC) with the hydroxylases MibO and CinX from the microbisporicin and the cinnamycin machineries, respectively; the C-terminal decarboxylase GdmD from the gallidermin cluster; and the reductase LtnJ from the lacticin 3147 system.

Conclusions

We have generated nisin derivatives containing hydroxyproline, hydroxyaspartate, a C-terminal aminovinyl-cysteine, or D-alanine in their structure. Moreover we have confirmed that these additional enzymes do not require leader peptide recognition to modify their substrate.

Our results provide an insight for the application of Synthetic Biology for the design and à la carte modification of (antimicrobial) peptides.
New antimicrobials for resistant organisms

THERAPEUTIC MONOCLONAL ANTIBODIES AGAINST THE GLOBALLY SPREAD MULTI-DRUG RESISTANT ESCHERICHIA COLI CLONE ST131-O25B:H4

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Background
The globally spread multi drug resistant E. coli clone ST131-O25b:H4 is responsible for a significant proportion of multi-drug resistant extraintestinal infections. By definition, isolates of this clone express the O25b antigen, whose unique structure has been resolved recently.

Objectives
We aimed to develop humanized monoclonal mAbs against the O25b antigen that may provide an alternative adjunct or stand-alone therapeutic option against this E. coli lineage.

Methods
Murine mAbs against the unique O25b antigen were generated by standard hybridoma technique. Following a primary screening, selected antibodies were subjected to humanization. Specificity and binding characteristics were assessed by immunoblots and ForteBio using purified antigens. Surface staining of ST131-O25b isolates irrespective of the capsular type expressed was shown by flow cytometry. Upon surface binding, the humanized antibodies induced complement mediated bactericidal activity in vitro. Passive immunization with the humanized mAbs elicited high level of protection at low mAb doses in a murine model of bacteremia. All whole cell assays were performed with bacteria grown in standard culture medium as well as in depleted pooled human serum in order to mimic in vivo-like conditions.

Conclusions
Humanized mAbs against E. coli O25b antigen were developed that elicited bactericidal activity in vitro and showed protection in vivo. Prophylactic passive immunization of colonized individuals or adjunct therapy of infected patients by mAbs may replace/substitute antibiotic therapy against this drug resistant clone. Relevant cases could be identified by the co-developed companion diagnostic tool.
Background. The problem of controlling microbial infections remains one of the most serious challenges faced by mankind, despite the development of very effective antimicrobial agents. Bacterial resistance to antibiotics, which for decades were considered to be capable of combating any bacterial infection, actually destroyed hopes for combating pathogenic bacteria. One of alternatives to antibiotic treatment is photodynamic antimicrobial chemotherapy based on visible light-activation of dyes called photosensitizers. In the dark, these photosensitzers are non-toxic or have low toxicity. No bacterial resistance to photosensitzers has been reported to date.

Objectives. The present report is dedicated to the antibacterial activity of the photosensitizers Rose Bengal and Methylene Blue against Gram-positive Staphylococcus aureus. The antimicrobial activity of both free and immobilized photosensitizers was examined.

Methods. Rose Bengal and Methylene Blue were immobilized on polystyrene, polycarbonate and poly(methyl methacrylate) by dissolution in chloroform with further evaporation of the organic solvent and the formation of thin polymeric films. Immobilization of these photosensitzers on polyethylene and polypropylene was performed by dissolution in the melted polymers. The free and immobilized photosensitizers showed high efficiency in eradication of S. aureus as tested by the MIC, the colony-forming units count and measurement of the inhibition zone.

Conclusions. Photodynamic inactivation of S. aureus by Rose Bengal and Methylene Blue opens prospects for developing an alternative to antibiotic treatment of bacterial infections.
Background

Marine organisms are continuously exposed to microbial population and are equipped with effective immune molecules to combat microbial invasion. Antimicrobial peptides are important components of innate defense mechanisms exhibited by animals. These organisms produce a range of AMPs varying greatly in size, structure and other physicochemical properties. Investigating novel antimicrobial peptides from marine organisms can provide new insight into the immune response of these organisms and a possibility of discovering new and effective drugs in medicine/aquaculture.

Objectives
Screening marine organisms for discovering novel antimicrobial peptides

Methods

Total RNA was extracted from haemocytes and cDNA was synthesized. PCR amplification was done using gene specific primers for AMPs. Amplicons were cloned and sequencing of the inserts were done at SciGenom, India. Homology search was performed by using BLASTn and BLASTp at NCBI and physio-chemical characterization by ProtParam Tool. Phylogenetic tree was constructed based on amino acid sequences by Neighbor joining method using MEGA 5.0

Conclusions

Four novel ALF isoforms were characterized, among which two were identified from crabs (Scylla serrata and Portunus pelagicus) and two from shrimp (Fenneropenaeus indicus). Four novel isoforms of crustins were also identified and characterized from Scylla serrata, Scylla tranquebarica, Portunus pelagicus and Fenneropenaeus indicus. Two penaeidin isoforms were characterized from Fenneropenaeus indicus and Penaeus monodon and a hepcidin from Chlorophtalmus bicornis. The marine environment, with its enormous biodiversity remains a largely untapped reservoir of bioactive peptides. Recombinant expression and bioactivity testing of these peptides
will definitely contribute to the design and formulation of potential peptide drugs for application in medical/ aquaculture industry.
New antimicrobials for resistant organisms

RESEARCHANT HUB: THE FIRST ONLINE PLATFORM FOR EXPERIMENTAL COMPOUND SHARING

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Background

Today we witness the global health threat of antimicrobial resistance. This phenomenon relies largely on the erosion of the effective antimicrobials and has drawn the attention of researchers from the traditionally used antimicrobials to the so-called experimental compounds.

Experimental compounds are bioresearch compounds that have been subject to a certain degree of development, but have been put away either due to project de-prioritization or lack of effectiveness against the application in question. They may, however, be very valuable in other applications; for instance, in diseases sharing similar biochemical pathways. These compounds are a rich source of new and, possibly, better antimicrobials. The question is: How can researchers gain access to them? We will discuss our efforts in developing a compound sharing facility and how we will collaborate with the community to facilitate open-innovation models for the antimicrobial compounds discovery.

Objectives

Our goal is to advance research in the field of antimicrobial resistance by re-purposing rare and one-of-the-kind experimental compounds to the whole of academic community. For that, we developed ResearchAnt Hub (RAH). RAH is powered by the ResearchAnt Foundation, which is a not-for-profit organization. RAH is a repository and a platform that fosters researchers worldwide to use and share their experimental compounds.

Methods

Our repository will contain thousands of experimental compounds that were developed or manufactured by privately- and academically-led researchers during the course of their work.

Conclusions

RAH is a unique not-for-profit open-innovation initiative. It represents a major step towards the discovery of new drugs to counteract today’s ever-increasing antimicrobial resistance problem.
Background
Antibiotic resistant human pathogens are diminishing our ability to treat infections and novel antimicrobials are urgently needed to replace our current arsenal of antimicrobials. *Pseudomonas aeruginosa* strains have been shown to produce antimicrobial compounds including bacteriocins, pigments and secondary metabolites that show promise as novel antimicrobial therapeutic agents.

Objectives
To assess the antimicrobial activity of four environmental *P. aeruginosa* isolates from University College London Hospital (UCLH).

Methods
Four *P. aeruginosa* isolates were obtained from various sites in UCLH. Cross streak assays using indicator organisms including *Escherichia coli*, *Staphylococcus aureus* and *Salmonella typhimurium* were conducted to determine the spectrum of inhibition of these *P. aeruginosa* strains. A spot-on lawn assay was used to compare the ability of each strain to inhibit the growth of *S. aureus*. To determine if the *P. aeruginosa* strains secreted compounds that were inhibiting *S. aureus*, this indicator strain was grown in cell free media that the *P. aeruginosa* strains were previously grown in.

Conclusions
Competitive hospital environments may be a rich source of bacteria producing novel antimicrobials. The results of the above experiments show that these four hospital environment acquired *P. aeruginosa* isolates are capable of inhibiting a wide range of bacteria. Some strains had a greater ability to inhibit *S. aureus* than others. All of the *P. aeruginosa* strains secreted inhibiting compounds into the media they were grown in. We are currently characterising the genetic and biochemical basis of these compounds.
EFFECT OF BACTERIOCIN PRODUCED BY STAPHYLOCOCCUS EPIDERmidIS AGAINST MRSA

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- Background: Staphylococcus aureus including methicillin-resistant S. aureus (MRSA) is recognized as a globally important pathogen in food-borne disease. Therefore bacteriocins have been focused on new weapon that are considered safe and effective substances as food additives to prevent food-borne pathogens.
- Objectives: To control MRSA of agricultural product, identify and characterize a antimicrobial peptide (bacteriocin) produced by Staphylococcus epidermidis.
- Methods: S. epidermidis were isolated from leafy green vegetables and identified by API Staph ID test and 16s rRNA sequence analysis. Antimicrobial activities of S. epidermidis against antibiotic resistant S. aureus and other Gram (+) bacteria were tested using agar diffusion assay. Purification of the active compound was achieved using a combination of ammonium sulfate precipitation, dialysis and reverse-phase high-performance liquid chromatography (RP-HPLC). The effects of heat, pH and enzymes on bacteriocin activity were determined using S. aureus CCARM3723 as indicator organism.
- Conclusions: S. epidermidis S-199 strain showed broad and strong antimicrobial activity. Purified antimicrobial peptide was heat-labile but stable in a wide range of pH. And the antimicrobial peptide showed resistance to α-amylase, lipase, and RNase, and DNase. However, antimicrobial activity was lost after trypsin, pronase E, pepsin, α-chymotrypsin, proteinase K, and papin treatment.
New antimicrobials for resistant organisms

ROLE OF PRODIGIOSIN AS A MICROBICIDE
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Background

Number of microbial species are metabolically wired to produce some pigments constitutively. Prodigiosin is such a pigment having prodigious biological activities in accord to its name. Microbicides are compounds that can be applied inside the vagina or rectum to protect against sexually transmitted infections (STIs). Discovery of drugs, targeting multiple steps in multiple diseases is of great interest.

Objectives

To evaluate prodigiosin for its microbicide potential and study its mechanism of actions in restricting sexually transmitted infections like E. coli, C. albicans, HSV, HIV, Trichomonas vaginalis. ii) To check cytotoxicity of prodigiosin against human cells.

Methods

96 well plate assay used for antibacterial assay followed by its biochemical investigations after treatment with prodigiosin at LC30 concentration, cell associated and cell free assays were used to check anti HIV-1 (CXCR4 and CCR5) potential, while ONPG for HSV, cytotoxicity of prodigiosin was determined by MTT assay.

Conclusions

Prodigiosin induces leakage in bacterial cell membrane and simultaneously inhibits activity of few essential enzymes. Prodigiosin inhibits viral entry and cell fusion for HSV and HIV (IC50- 0.01 µg/ml). It also inhibited T. vaginalis (1.2 µg/ml). The use of pigment found on safer side against human cells (PBMC (78±3.1 µg/ml) and HeLa cells (70.2±5.3 µg/ml)). One compound having all these activities is a remarkable feature. Prodigiosin can be formulated as a microbicide (drug) and applied on the affected area with a cream base so as to treat the STIs. In conclusion prodigiosin can be used in treatment modality for protection from STIs.
FEMS-0701
New antimicrobials for resistant organisms

REGULATORY NETWORKS AND ECOLOGICAL CUES THAT CONTROL ANTIBIOTIC PRODUCTION BY ACTINOMYCETES
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Background
Actinomycetes are prolific producers of natural products, including anticancer compounds, antifungals and antibiotics. The treasures that lie hidden in the actinomycete genomes may well be our final resource in the battle against the rapidly emerging infectious diseases associated with multi-drug resistance.

Objectives
We aim to understand the triggers and cues that elicit antibiotic production in the soil as well as in the laboratory. This knowledge is then translated into technologies for the activation and mining of silent pathways.

Methods
To uncover novel antimicrobials, we use a combination of ecological insights, systems biology, NMR-based metabolomics and genome mining to uncover the regulatory mechanisms that control antibiotic production. We then apply this for the discovery of novel antimicrobials.

Conclusions
One major control system revolves around the nutrient sensory protein DasR, which pleiotropically controls antibiotic production. Many other control systems are undoubtedly in place to allow actinomycetes to respond appropriately to challenges by competitors and fluctuations in nutrient composition in the habitat. Furthermore, once a bioactivity is elicited under specific growth conditions, novel approaches are needed to rapidly identify the bioactivity of interest and link it to a specific gene cluster. Molecular and ecological insights to elicit antibiotic production will be discussed, and examples of novel antibiotics identified by these approaches are presented.
ACTIVITY ANALYSIS OF LYSOSOMES AS A FUNCTIONAL CELL ORGANELLE IN SACCHAROMYCES CEREVISIAE

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Background
Lysozmes are functional cell organelles which have antimicrobial and anticancerous activity. Lysosomal enzymes are easily integrated or released when exposed to some stimuli factors in all eukaryotic cells.

Objectives
In order to verify features special function of lysosomes, several conditions were used to stimulate in vitro functions of lysosomes. In addition, we confirm lysosomal safety and activity in vivo for using antimicrobial and anticancerous materials.

Methods
Proteomic analysis of lysosomal enzymes in response to the enhanced and repressed condition for in vitro activity using 2-DE. Among up-regulated protein dots response to oxidative stress, the dot with highest increase was chosen to overexpress in Saccharomyces cerevisiae and then confirm the lysosomal activity. In addition, we injected lysosomes into rat model infected with bacteremia, and then analysis bacterial growth in blood of rat according to the reaction time.

Conclusions
The antimicrobial and anticancerous activity of lysosomes was enhanced in cloned S. cerevisiae in vitro assay. In addition, it is safe to inject lysosome into normal rat. Moreover, when being injected into rat model infected with bacteremia, lysosome showed its ability to inhibit bacterial growth in blood. Therefore, increases in endogenous levels of lysosomes may have various applications such as antimicrobial agents and apoptosis-inducing materials for cancer cells and it is very promising to use the organelles to improve in vitro and in vivo functions.

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FEMS-1529
New antimicrobials for resistant organisms

EFFECT OF ACID TREHALASE (ATH) ON IMPAIRED YEAST VACUOLAR ACTIVITY
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Background
Acid trehalase (ATH) has been suspected to have two different localizations, vacuolar lumen and cell surface, among which the localization at cell surface is believed to be responsible for extracellular trehalose utilization.

Objectives
A lot of efforts were made to confirm the exact localization of ATH in yeast cells. Along with this, the trafficking pathway responsible for the delivery of ATH into yeast vacuoles was also studied with the purpose to provide better control on the transportation of this protein in yeast cells.

Methods
ATH was overexpressed in yeast cells grown on trehalose-containing medium to observe the impact of this enzymes on yeast vacuolar activity. After that, 4 groups of typical proteins representing the 4 main trafficking pathways in yeast were also selected to analyze their expression pattern via 2DE.

Conclusions
The overexpression of ATH was observed to decrease vacuolar activity. Moreover, ATH was confirmed again to locate in both cell surface and vacuoles. Finally, it is interesting to discover that on medium containing trehalose, the ATH's localization at cell surface, but not vacuoles, is prioritized to utilize extracellular trehalose for cell growth. The mechanism behind that is suggested via the down regulation of MVB pathway that sorts ATH into vacuoles.

Acknowledgement: This work was carried out with the support of “Cooperative Research Program for agriculture Science & Technology Development (Project No:PJ01052701)” Rural Development Administration, Republic of Korea.
Background

In the last few decades, the emergence of bacterial resistance to antibiotics has become a common phenomenon. As a consequence, the effectiveness of antibiotic treatment of bacterial infection has progressively decreased. Therefore, it's necessary to develop and test new antimicrobial compounds against multidrug-resistant bacteria. Recently, the employment of metallic nanoparticles has emerged as an alternative to the use of organic compounds as antimicrobial agents: different metals and metalloids nanoparticles have indeed shown a promising bactericidal capability.

Objectives

The aim of the present study was to evaluate the antimicrobial activity of selenium and tellurium nanoparticles of bacterial origin against both planktonic and biofilm culture of three pathogenic strains: Escherichia coli JM109, Pseudomonas aeruginosa PAO1 and Staphylococcus aureus ATCC 25923.

Methods

To measure the antimicrobial activity of these nanoparticles we determined both the minimum biocidal concentration (MBC) and the minimum biofilm eradication concentration (MBEC) using the MBEC assay™. We also observed the effect of increasing concentrations of nanoparticles on biofilm structure using Confocal Microscopy (CLSM).

Conclusions

Our results indicate that both Se⁰ and Te⁰ nanoparticles possess antimicrobial and biofilm eradication activity. In particular Se⁰ nanoparticles exhibited antimicrobial activity at lower concentration. The activity seemed to be dependent on the dimension of the nanoparticles: indeed, the highest activity was shown by the nanoparticles smaller in size. The key observation is that bacteria grown in biofilm mode didn’t exhibit a higher level of resistance against the nanoparticles antimicrobial action. Results described in this study suggest a possible application of these nanoparticles as an effective antimicrobial agent.
FEMS-1881
New approaches for typing

**AN INTEGRATED PLATFORM FOR HIGH-THROUGHPUT WHOLE GENOME MLST AND WHOLE GENOME SNP ANALYSIS.**
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**Background**

With the increasing availability of next generation sequencing technology, whole genome sequencing (WGS) methods are increasingly being used for bacterial typing. Key challenge is the ability to rapidly extract the relevant information from large sequence data files.

**Objectives**

We present two pipelines for high resolution WGS-based molecular typing: whole genome multilocus sequence typing (wgMLST) and whole genome single-nucleotide polymorphism analysis (wgSNP). Both strategies are compared using data from a *Staphylococcus aureus* outbreak.

**Methods**

Using an in-house developed wgMLST schema that extends the core genome schema from Leopold *et al.* (2014), we apply two independent allele calling approaches, an assembly-free and a BLAST-based allele calling algorithm, to determine locus presence and detect allelic variants in a quality-controlled manner. The wgSNP pipeline, tuned to reduce false positives while maximizing resolution, detects SNP variants by mapping the WGS reads to a reference sequence internal or external to the data set.

For both methods, all calculation-intensive data processing steps are performed on the BioNumerics® Calculation Engine, deployed locally or in the cloud.

**Conclusions**

The BioNumerics® 7.6 software and its integrated Calculation Engine offer a powerful platform where both wgMLST and wgSNP can be performed to provide a robust,
portable and high resolution picture of molecular typing data. The polyphasic approach allows for validation both between WGS analysis techniques and traditional techniques such as MLST or PFGE. We illustrate this by comparing core genome MLST (cgMLST), wgMLST and wgSNP, thus establishing a clear picture of the differences in resolution between those analysis techniques.
Background

Tuberculosis is a treatable disease, yet remains a major worldwide health problem. In the last 10 years approximately 80 million people contracted the disease. The development of techniques for rapid diagnosis based in molecular technologies would greatly facilitate worldwide efforts to prevent further spread of the disease. One potential diagnostic sequence, the DR locus, is present in all isolates of M. tuberculosis complex bacteria. It is useful for molecular typing of M. tuberculosis because of its fortuitous absence in non-tuberculosis strains of mycobacteria.

Objectives

In this study, we attempted to combine the specificity of molecular inversion probe (MIP) technology with the sensitivity of pyrosequencing in order to detect a short conserved 18 bp sequence included in DR locus in 25 isolates of M. tuberculosis.

Methods

DNA from 25 M. tuberculosis isolates were extracted and submitted to the MIP reactions. A MIP was designed against the DR sequence of M. tuberculosis strain H37Rv and the MIP reactions were performed; the MIPs were then submitted to pyrosequencing reactions.

Conclusions

We design a molecular inversion probe for a specific region of M. tuberculosis genome. Probing this MIP with 25 M. tuberculosis genomic DNA and determining their sequences by pyrosequencing, we detect a common sequence among all strains of M. tuberculosis. Also, we determine the minimal amount of DNA needed (50 ng) in order to obtain a good readout. By introducing a modification on pyrosequencing
methodology we were successful in detecting M. tuberculosis DNA even in presence of a very small amount of DNA (500 fg).
New approaches for typing

**TYPING OF ENTEROBACTERIACEAE BY ERIC-PCR, REP-PCR AND MALDI-TOF MS METHODS**

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Background: The fast and reliable fingerprinting methods for typing of *Enterobacteriaceae* are widely required in food microbiology for tracking sources and ways of food contamination. ERIC-PCR and REP-PCR genotyping use as markers specific DNA sequences (enterobacterial repetitive intergenic consensus sequences or repetitive extragenic palindromic sequences, respectively) and the changes of their dispersal, detectable by methods. In comparison MALDI-TOF MS phenotyping uses the conformity of protein profiles. As used typing markers mirror the differences among strains only partially, also compared to other features as the antibiotics resistance and biofilm formation, their combination could propose more precise approach.

Objectives: The aim was to compare the discriminatory power of ERIC-PCR, REP-PCR and MALDI-TOF MS fingerprinting methods for different *Enterobacteriaceae* isolates, also with respect to their antibiotics resistance and biofilm formation.

Methods: Fifty six isolates of *Enterobacteriaceae*, including genera *Escherichia*, *Enterobacter*, *Klebsiella*, *Serratia* and *Raoultella*, isolated from food and clinical sources in Czech Republic in 2007-2014, were genotyped by the same ERIC-PCR and REP-PCR methods (maximal length of analysed fragments 3000 bp). MALDI-TOF MS typing was done by the ethanol-formic acid extraction in Bruker Autoflex Speed MALDI-TOF mass spectrometer. The resistance for 12 antibiotics and the ability to form biofilm in different media at 25 °C were tested.

Conclusions: REP-PCR is more discriminative than ERIC-PCR in most species. The discriminatory power of MALDI-TOF MS phenotyping depends on the range of used protein peaks (e.g. application of specific intensity criteria) and the used clustering methods, but never gives the false positive results in comparison to the genotyping methods.
USE OF MATRIX-ASSISTED LASER DESORPTION/IONIZATION- TIME OF FLIGHT (MALDI-TOF) MASS SPECTROMETRY FOR ROUTINE ANALYSIS IN A DRINKING WATER TREATMENT PLANT

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Background
Drinking water treatment plants employ different treatments depending on source water quality to assure adequate chemical and microbiological quality according to the Directive 98/83/EC. The study of bacterial communities throughout the treatment plant could provide a basic understanding of the effects of water processing that can be used to improve the management in drinking water treatment plants.

Objectives
To assess the use of MALDI-TOF MS for routine analysis in a drinking water treatment plant to study bacterial diversity throughout the different treatments in comparison with other methods such as the PhenePlate® system, the API 20E strip and 16S rRNA gene sequencing.

Methods
A total of 366 colonies isolated from different points and seasons throughout the water treatment process including feed water (40), sand filtration (36), ultrafiltration (44) and reverse osmosis (191) were used in the study. The colonies were analyzed by MALDI-TOF MS by direct colony inoculation on the plate. The colonies were also biochemically fingerprinted using the PhenePlate® system, clustered according to their similarity and a representative strain was selected for 16S rRNA gene sequencing and API based identification.

Conclusions
The diversity decreased along the different treatments, with a total of 20 genera being detected. The use of MALDI-TOF MS was reliable compared to the PhenePlate® system and has the advantage of being faster and relatively cheap compared to other technologies such as the 16S rRNA sequencing. The API web was not sufficiently reliable for water monitoring since the identification of the majority of the strains failed compared to the other methods used.
MOLECULAR DIVERSITY OF CANDIDA ALBICANS ISOLATED FROM IMMUNOCOMPROMISED PATIENTS, BASED ON MLST METHOD
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Background
Multilocus sequence typing (MLST) is used as a highly discriminatory method for tracing sources and routes of transmission, and the genetic relatedness of isolates.

Objectives
The aim of the study was to assess the genetic diversity among C. albicans strains isolated from oropharynx and bronchoalveolar lavage (BAL) of immunocompromised patients.

Methods
A total of fourteen epidemiologically unrelated clinical isolates of C. albicans from three hospitals in northern Iran were tested. Seven loci of housekeeping genes were sequenced for all fourteen isolates.

Conclusions
The fourteen isolates were placed in 10 clonal clusters (CC) while two isolates were singletons, by eBURST analysis. Most of the isolates were belong to CC461 of eBURST analysis from the clade 11 and two isolates assigned to CC172 from the clade 15.

In conclusion, Genetic diversity is variable among unrelated strains obtained from different patients at different times and places, and epidemiologically unrelated strains usually are not genetically closely related and classify in different clusters.
Background: The epidemic spread of a special clinical and epidemiological form of pseudotuberculosis, Far East Scarlet-Like Fever (FESLF), was firstly described in Russia (Far East, Vladivostok) in the late 1950s [1].

Objective: To characterize Yersinia pseudotuberculosis strains isolated in Russia (Far East, Siberia, Europe) in 1973-2014 years with the multilocus sequence typing (MLST) method to ascertain a position of the strains in a phylogenetic structure of the species.

Methods. Totally, 80 Y. pseudotuberculosis strains isolated from the stool of patients with clinical signs of FESLF (n=54), vegetables (n=11) and rodents (n=15) were included in the study. The MLST method developed by Achtman et al. (http://www2.warwick.ac.uk/mlst) was used. The MLST scheme is based on 7 housekeeping genes.

Conclusion. The majority of Y. pseudotuberculosis strains isolated from patients belonged to sequence type ST2 (85.2%, serotype 01b). Five and three clinical strains belonged to ST26 (9.3 %, serotype 01b) and ST32 (5.5%, serotype 03), respectively. Y. pseudotuberculosis serotype 01b strains isolated from vegetables belonged to ST2 (81.8%) and ST14 (18.2%). Strains isolated from rodents (01b serotype) belonged to ST2 (60%), ST42 (20%), ST64 (13,3%), ST32 (6,7%). Serotype O3 rodent isolates belonged to ST14 (5.3%). MLST results showed the Y. pseudotuberculosis ST2 serotype 01b clone prevails in Russia among all studied sources including patients, vegetables, and rodents.

PHENOTYPIC AND GENOTYPIC IDENTIFICATION OF SALMONELLA USING ANTISENSE PEPTIDE NUCLEIC ACID PROBE - MULTITYPING.
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Background
Development of novel strategies for rapid and specific identification of bacteria pathogens is urgently needed to aid successful medical and epidemiological interventions in disease outbreaks.

Objectives
In this study we evaluated the feasibility of altering growth phenotypes by silencing specific essential genes with antisense probes as a rapid bacterial diagnostic tool.

Methods
Salmonella Typhimurium and Escherichia coli strains were treated with antisense Peptide Nucleic Acid (PNA) probe targeting Salmonella ftsZ at concentrations 0-3.0µM. Cultures were grown in non-selective Muller Hinton broth (MHB), selective Rappaport-Vassiliadis Soya Peptone (RVS), and modified Rappaport-Vassiliadis Soya Peptone-low salt (mRVSs) and cell morphologies were examined by microscopy.

Results
If ftsZ silencing is successful, cells are expected to elongate, and we here observed that treatment with the Salmonella anti-ftsZ PNA treatment elongated Salmonella grown in MHB and modified RVS low salt (mRVSs), but not in RVS. In contrast, E. coli did not elongate when treated under the same conditions. Likewise, untreated Salmonella cells did not elongate. Also, modified RVS low salt media retained Salmonella selectivity and enrichment qualities.

Conclusions
Anti-ftsZ PNA treatment can provide both phenotypic and genotypic identification of Salmonella, and the method can be combined with selective growth conditions. We are further testing this method using artificially contaminated water and milk.
DEVELOPMENT OF A QPCR TO DETECT PSEUDOMONAS AERUGINOSA IN CULTURE-NEGATIVE SAMPLES FROM CYSTIC FIBROSIS PATIENTS AND MONITOR THE EFFECTIVENESS OF ANTIBIOTIC TREATMENT

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Background

Early detection of *Pseudomonas aeruginosa* in cystic fibrosis (CF) samples is pivotal for disease eradication by antibiotic treatment. However, standard cultural methods are hampered by slow growth and alginate overproduction of mucoid variants, and possibly by viable but non-culturable state induction.

Objectives

This work aimed at developing a qPCR for improved *P. aeruginosa* infection diagnosis in sputum from CF patients and assessment of the effectiveness of antibiotic treatment.

Methods

Thirty-three sputum samples that were negative by culture assays were collected from 28 CF patients. DNA was extracted by the QIAamp DNA kit after centrifugation to remove free DNA. Real-time PCR assays targeting the species-specific gene *ecfX* were performed.

Conclusions

Six/33 samples were positive for *P. aeruginosa* by qualitative Real-time PCR (LOD: 60 cells/ml) and were analyzed by qPCR (LOQ: 3x10² cells/ml). *P. aeruginosa* abundance exceeded the LOQ in three samples, which carried 1x10³, 1.6x10⁴ and 2.8x10⁶ cells/ml, respectively. Three successive samples from a culture-negative, symptomatic patient, showed bacterial counts <LOQ (May 7); 1x10³ (still culture-negative but now receiving inhaled antibiotic therapy; May 11), and <LOQ (at the end...
of antibiotic therapy; May 28). Four months later the patient was positive by cultural assays.

The matching qPCR and clinical results highlight the value of molecular assays in *P. aeruginosa* detection and quantification in CF. Dead cells cannot account for the whole difference found between PCR and culture data. Dormant forms in the pulmonary biofilm, coupled with the greater sensitivity of PCR, are likely to account for a large part of this difference.
AMPEROMETRIC DETECTION OF β-LACTAMASE ACTIVITY AND ITS APPLICATION TO THE IDENTIFICATION OF EXTENDED SPECTRUM β-LACTAMASE PRODUCING STRAINS IN BLOOD SAMPLES

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Background
With the ever-expanding role of extended-spectrum β-lactamase (ESBL) -harboring Enterobacteraceae in causing serious infections, rapid diagnostic assays of such resistant organisms are highly desired to improve patient screening and hospital infection control practices as well as to reduce inappropriate antibiotic use.

Objectives
To reach this goal, we developed an amperometric detection of the β-lactamase activity in blood culture samples using disposable carbon screen-printed sensors in the presence of a well-chosen cephalosporin substrate.

Methods
Using an ESBL enzyme isolated from a clinical strain, we showed that the intensity of a specific anodic peak current resulting from the catalytic hydrolysis of the β-lactam ring was proportional to the amount of ESBL. A novel susceptibility assay for the rapid and specific identification of ESBL-producing bacteria in blood culture samples was then proposed. This assay was based on a two-step protocol performed within 2 hours : (1) subculturing of blood culture samples in the presence or absence of cefotaxime and/or the potassium clavulanate (ESBL inhibitor) for a few hours followed by, (2) incubation of the subculture filtrates with the redox substrate which hydrolysis was monitored by amperometry. This assay allowed the reliable and specific identification of various types of β-lactamases-producing bacterial species (including ESBLs, penicillinases, cephalosporinases) in blood cultures.

Conclusions
Owing to its low cost, portability, simplicity and its ability to perform measurements in turbid media, the electrochemical approach, which did not require prior strain isolation, holds great promise for the rapid screening of β-lactamases in clinical and other complex samples.
RAPID AMPEROMETRIC DETECTION OF ESCHERICHIA COLI IN WASTEWATER BY MEASURING β-D GLUCURONIDASE ACTIVITY WITH DISPOSABLE CARBON SENSORS

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Background
Methods based on the direct measurement of β-D-glucuronidase (GLUase) activity in the presence of the 4-methylumbelliferyl-β-D-glucuronide substrate are well-established to specifically monitor Escherichia coli (E.coli) in water samples without any cultivation step. However, because of the interferences from auto-fluorescence or light-quenching particle in complex samples, the fluorescence detection is often not suitable for the analysis of environmental samples.

Objectives
To overcome this drawback, the amperometric quantification of GLUase activity was investigated and applied to the rapid and specific detection of E. coli in wastewater samples.

Methods
For this purpose, the p-aminophenyl β-D-glucopyranoside (PAPG) was selected as electrochemical substrate for GLUase measurement and the p-aminophenol (PAP) released during the enzymatic hydrolysis was monitored by cyclic voltammetry with disposable carbon screen-printed sensors. The intensity of the measured anodic peak current was proportional to the amount of GLUase, thus providing a measurement of the number of E.coli in the sample (using a calibration curve). Following an optimized protocol, we were able to detect E. coli cells in the range of $5 \times 10^4$ to $10^8$ per filter within 2 h. The amperometric assay was applied to the determination of fecal contamination in raw and treated wastewater samples and it successfully compared with conventional bacterial plating methods and uidA gene detection by quantitative PCR.

Conclusions
Owing to its ability to perform cell density measurements in turbid media, the GLUase amperometric method is a reliable tool for the rapid and decentralized quantification of viable but also non-culturable E.coli in complex environmental samples.
Background

The rapid, cost-effective, and robust detection of pathogens continues to be a major focus for the livestock industry. While PCR has filled the niche for a highly sensitive detection, PCR inherently burdens the end user with cumbersome sample preparation, complex and closed instrumentation with long time–to-result (TTR) (1.5-3h post-enrichment, excluding sample preparation). Other DNA amplification methods have shortened the TTR but have not been able to provide a robust and reliable alternative to PCR. The need for improved testing solutions continues to grow with growing intricacies of production logistics.

Objectives

Here we present an isothermal DNA amplification tool called DNAble for Salmonella spp. detection. This system provides a distinctive advantage over other technologies as it combines high specificity and sensitivity with minimal sample preparation leading to a significantly reduced TTR (30-40 min post-enrichment, including sample preparation).

Methods

Salmonella detection was demonstrated with an analytical sensitivity of <50cp of genomic DNA per reaction or <10 cells post-enrichment in multiple test matrices. Inclusivity and exclusivity data indicate 100% accuracy across 34 Salmonella serotypes and 29 non-Salmonella tested strains.

Conclusions

DNAble requires minimal sample preparation due to its robust chemistry and is compatible with a range of detection equipments. Salmonella spp. detection system also includes an internal amplification control, Salmonella selective media supplement that improves current enrichment, and a user-friendly sample preparation kit. Results are provided by a portable and simple DNAble reader or user provided thermocycler. The inherent robustness, quick TTR, and broad range instrument compatibility support use of DNAble for Salmonella detection.
FABRICATION OF DNA ELECTROCHEMICAL BIOSENSOR BASED ON CARBON NANOTUBES FOR DETECTION OF FECAL CONTAMINATED WATER WITH ENTEROCOCCUS FAECALIS
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Background

Fecal pollution of water can lead to health problems due to presence of infectious microorganisms especially \textit{Enterococcus faecalis} (E. faecalis) which may be derived from human sewage or animal sources. Hence, detection of these microorganisms is of much interest using different methods. Electrochemical DNA biosensors, which employ an immobilized DNA as the biological recognition element, are currently under intense investigation in detection of several microorganisms relying on the conversion of the base-pair recognition event into a useful electrical signal.

Objectives

The aim of this study was to demonstrate a rapid and inexpensive method for accurately detection of \textit{E. faecalis} in contaminated water via fabrication of a specific and selective DNA electrochemical biosensor.

Methods

A modified DNA oligonucleotide with 5’ amino modifier C12 was designed based on a house keeping gene glucose 6-phosphate dehydrogenase of \textit{E. faecalis}. The multi walled carbon nanotubes (MWCNT) were allowed to form amide bond between their carboxylic acid groups and amino groups of oligonucleotides. Hybridization was performed by incubating the DNA/self-assembled MWNTs with complementary DNA oligonucleotides, non-complementary DNA oligonucleotides, genomic DNA and artificial contaminated water under specific conditions for 30 min at room temperature. The hybrid-electrodes were successively transferred into hybridization indicator of methylene blue for 30 min without any potential followed by several washings and subsequently electrochemical measurements.

Conclusions

Such single-use electrochemical biosensors have great promise for decentralized water testing for presence of \textit{E. faecalis} in an accurate and rapid approach.
Background

Acute exacerbations of chronic obstructive pulmonary disease (AE COPD) are predominantly caused by microbial pathogens. Molecular techniques may improve the speed and sensitivity of respiratory infections diagnostics. Currently there is limited knowledge on the best method for DNA extraction from sputum samples.

Objectives

This study is a part of the European JRP HLT08 INFECT-MET. The aim was to evaluate the DNA yield of two different extraction methods, manual and automated, in treated and untreated sputum samples from COPD patients.

Methods

A total of 47 good-quality sputum samples from COPD patients between March 2014 and October 2014 were included in the study. Twenty samples were left untreated and 27 were treated with NALC-NaOH. All samples were split in two aliquotes and DNA was extracted with manual (QIAamp DNA Mini Kit, Qiagen GmbH, Germany) and automated (DNA Extraction Kit, DiaSorin, Italy/NorDiag Arrow) method. Concentration of DNA was quantified (Qubit Fluorometer, Invitrogen, USA).

Conclusions

Out of 47 sputum samples 41 (87,2%) had higher DNA concentration with automated extraction method. Among 20 untreated sputum samples automated method had a higher DNA yield in 14 samples by 68,1%, lower in 5 by 43,1 % and equal in 1 sample. In 27 treated sputum samples automated extraction method had a higher DNA yield in all 27 samples by 60,9%.
Automated extraction method proved to be more efficient in extracting DNA from untreated and treated sputum samples. Automated extraction method is much easier to perform and less time consuming.
DEVELOPMENT OF DUAL-FUNCTION ELISA FOR EFFECTIVE ANTIGEN AND ANTIBODY DETECTION AGAINST H7 AVIAN INFLUENZA VIRUS

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Background

Outbreaks in poultry involving influenza virus from H7 subtype have resulted in human infections, thus causing a major concern for public health, as well as for the poultry industry. Currently, no efficient rapid test is available for large-scale detection of either antigen or antibody of H7 avian influenza viruses.

Objectives

In the present study, a dual function ELISA was developed for the effective detection of antigen and antibody against H7 AIVs.

Methods

The test was established based on antigen-capture-ELISA and epitope blocking ELISA. The two Mabs 62 and 98 which were exploited in the assay were identified to recognize two conformational neutralizing epitopes on H7 HA1. Both of the epitopes exist in all of the human H7 strains, including the recent H7N9 strain from China and > 96.6% of avian H7 strains. The dual ELISA was able to detect all of the five H7 antigens tested without any cross reaction to other influenza subtypes. The antigen detection limit was less than 1 HA unit of H7. For antibody detection, the sensitivity and specificity of the dual ELISA was evaluated and compared to HI and microneutralization using immunized animal sera to different H7 strains and different subtypes of AIVs. Results indicated that antibodies to H7 were readily detected in immunized animal sera by the dual ELISA whereas specimens with antibodies to other AIVs yielded negative results.

Conclusions
This is the first dual-function ELISA reported for either antigen or antibody detection against H7 AIVs. The assay was highly sensitive and 100% specific in both functions rendering it effective for H7 diagnosis.
Background

HIV-1/2 testing algorithms are currently controversial. The most important problem and difficulty about this debate is undeterminate HIV-1 infections (UHIV-1). Studies for an optimized algorithm for UHIV-1 are under research.

Objectives

In this study, we aimed to determine neopterin and sCD-14 levels in cases with UHIV-1 and confirmed HIV-1 (CHIV-1) infections and evaluate the relationship between UHIV-1 and real HIV-1 infection and also aimed to evaluate the diagnostic values of two markers, especially in situations when PCR were not available and western blot were undeterminate after ELISA.

Methods

Eighty-eight cases with UHIV-1, 100 patients with CHIV-1 and 100 healthy control group (HCG) were included. Neopterin and sCD-14 levels were determined by competitive and sandwich ELISA (nmol/mL, µg/mL, quantitatively) methods, respectively. Statistical tests were performed using SPSS 21.0 and p < 0.05 was considered significant.

Conclusions

Results:
Table 1. Neopterin and sCD-14 levels of UHIV-1 and CHIV-1 cases and healthy control group

<table>
<thead>
<tr>
<th>Markers</th>
<th>Undeterminate HIV-1¹ (n:88)</th>
<th>Confirmed-HIV-1² (n:100)</th>
</tr>
</thead>
<tbody>
<tr>
<td>HCG³(n:100)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Neopterin, mean (min-max) 5.73 (1.58-38.71) 15.77 (1.19-127.19) 7.95 (3.16-41.08)
sCD14, mean (min-max) 3.65 (1.86-6.17) 4.20 (1.53-7.59) 3.51 (0.97-3.97)

P value: Neopterin: 1x2; p:0.004, 1x3; p:0.754
sCD14: 1x2; p:0.034, 1x3; p:0.787

Table 2. Diagnostic test performances of neopterin and sCD14 in CHIV-1 infections

<table>
<thead>
<tr>
<th>Markers</th>
<th>Sensitivity</th>
<th>Specificity</th>
<th>PPV</th>
<th>NPV</th>
<th>Kappa coefficient</th>
</tr>
</thead>
<tbody>
<tr>
<td>Neopterin (%)</td>
<td>38</td>
<td>82</td>
<td>76</td>
<td>57</td>
<td>20</td>
</tr>
<tr>
<td>sCD14 (%)</td>
<td>50</td>
<td>100</td>
<td>100</td>
<td>67</td>
<td>50</td>
</tr>
</tbody>
</table>

As conclusion, we couldn’t suggest UHIV-1 pattern as real HIV-1 infection and we concluded that two markers were useless as surrogate markers for real HIV-1 infections.
Background

Isolated Anti-HBc pattern (IAH) positivity causes diagnostic difficulty in laboratory diagnosis of HBV infections. In addition, this pattern is a big problem for clinical diagnosis and blood transfusion.

Objectives

In this study, we aimed to determine neopterin and sCD-14 levels in cases with IAH and chronic active hepatitis (CAH) and to evaluate the relationship between IAH and real HBV infection and also aimed to evaluate the diagnostic values of two less sophisticated and cheaper surrogate markers in situations when the molecular methods were not available.

Methods

IAH (n:102), KAH (n:70) and healthy control group (HCG), (n:100) cases were included in the study. Neopterin and sCD-14 levels were determined by competitive and sandwich ELISA (nmol/mL, µg/mL, quantitatively) methods, respectively. Statistical tests were performed using SPSS 21.0 and p < 0.05 was considered significant.

Conclusions

Table 1. Neopterin and sCD-14 levels in IAH, KAH and HCG cases.

<table>
<thead>
<tr>
<th>Markers</th>
<th>IAH¹</th>
<th>KAH²</th>
<th>HCG³</th>
</tr>
</thead>
</table>

Neopterin, mean (min-max) 8.67 (3.56-67.15) 15.43 (4.75-43.27) 7.95
(s.16-41.08)
sCD14, mean (min-max) 3.61 (1.82-6.17) 4.20 (1.96-6.78) 3.51
(0.97-3.97)

p value: Neopterin: 1x2; p:0.002, 1x3; p:0.915
sCD14: 1x2; p:0.006, 1x3; p:0.821

Table 2. Diagnostic test performances of neopterin and sCD14 in real HBV infections

<table>
<thead>
<tr>
<th>Markers coefficient</th>
<th>Sensitivity</th>
<th>Specificity</th>
<th>PPV</th>
<th>NPV</th>
<th>Kappa</th>
</tr>
</thead>
<tbody>
<tr>
<td>Neopterin (%)</td>
<td>59</td>
<td>82</td>
<td>69</td>
<td>75</td>
<td>42</td>
</tr>
<tr>
<td>sCD14 (%)</td>
<td>51</td>
<td>90</td>
<td>78</td>
<td>73</td>
<td>44</td>
</tr>
</tbody>
</table>

As conclusion, we couldn't suggest IAH pattern as a real HBV infection and we concluded that two markers were useless as surrogate markers for the diagnosis of HBV infections.
FEMS-2559
New diagnostic approaches

DETECTION OF THE OOMYCETE PYTHIUM INSIDIOSUM BY REAL-TIME PCR OF THE EXO-1,3-B-GLUCANASE GENE
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2Research center, Faculty of Medicine Ramathibodi Hospital Mahidol University, Bangkok, Thailand

Background
Pythiosis is a life-threatening infectious disease caused by the pathogenic oomycete Pythium insidiosum. The disease has high rates of morbidity and mortality. Timely diagnosis permits prompt treatment and a better prognosis. PinsEXO1, encoding an exo-1,3-β-glucanase, is a novel and efficient target for identification of P. insidiosum by conventional PCR.

Objectives
In this study, we aimed to develop a real-time PCR approach targeting PinsEXO1 and compare its performance to conventional PCR for the detection of P. insidiosum.

Methods
Genomic DNA samples were prepared from 35 culture-proven P. insidiosum isolates and 58 culture-proven fungi (served as the control), for PCR analyses. Both conventional and real-time PCR assays were positive for all P. insidiosum strains tested, while all control fungi were negative. Turnaround time for conventional PCR was 4 hr, while that of real-time PCR was 1.5 hr. The minimum DNA template required for successful PCR amplification by conventional and real-time PCR were 1 ng and 1 x 10^-4 ng, respectively.

Conclusions
The real-time assay retained high detection sensitivity and specificity. It showed a substantially improved analytical sensitivity and turnaround time that could improve diagnoses of pythiosis.
Background

The proper diagnosis of Malaria disease is essential to provide early treatment and improve the prognosis of patients. Transfusion-transmitted Malaria is rare, but it may produce severe problems in the safety of blood Transfusion and blood related products due to the lack of reliable procedure to evaluate donors potentially exposed to malaria.

Objectives

Microscopy, still considered the gold standard for diagnosing malaria. It is time consuming and requires trained expertise. ELISAs are known to be ideal for high throughput screening with high sensitivity and specificity, but it also requires trained personal and an equipped laboratory. Line Blots are often used as confirmatory tests since they provide high sensitivity and specificity. There is nearly no lab equipment needed to perform this kind of assay. In addition, blots can also be used in automated processes for high throughput screening.

Methods

Here we show an improved diagnostic performance of the new antibody detection Systems (ELISA and Lineblot) utilizing early and late antigens of all 5 human pathogenic Plasmodium species (P. falciparum, P. vivax, P. ovale, P. malariae, P. knowlesi) compared to test systems only relying on antigens derived from one or two Plasmodium species. Assays with a limited number of antigens often fail to detect antibodies from certain regions of the world. For evaluation purpose, we collected samples from all over the world, including samples from newborns.

Conclusions

We evaluated the performance of ELISA and Lineblot directly in endemic countries with samples of patients who presented symptoms akin to malaria infection in local hospitals.
INVESTIGATION OF ORTHOPAEDIC IMPLANT ASSOCIATED INFECTIONS:
ALTERNATIVE APPROACHES TO LABORATORY PROCESSING AND CULTURE
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1 Standards Unit, Public Health England, London, United Kingdom

Background

Since its introduction in the 1960s, joint replacement (arthroplasty) has become a common procedure. Around 15% of revisions are due to ‘septic’ loosening caused by biofilm formation on the implant. Infection rates are now much lower; however, there is still a risk associated with each procedure. UK SMI B44: is a diagnostic tool aimed at practising professionals in the field of microbiology. It describes and recommends methods for the microbiological investigation of orthopaedic implant associated infection.

Objectives

The aim of this poster is raise awareness of the various methods of sample preparation (including the use of glass beads and sonication) and culture. Timely diagnosis and treatment can help reduce the likelihood of systemic dissemination of infection. Therefore, in most cases, this UK SMI recommends up to 5 days culture using either cooked meat broth or continuous monitoring blood culture system.
Methods

Produced by the Standards Unit, UK SMIs are developed, reviewed and updated through a wide consultation process with users and other stakeholders. The process follows the AGREE tool and resulting documents reflect best evidence based practice. Where evidence is not available, the documents are based on national working group consensus decisions.

Conclusions

B44 provides guidance on the best minimum practice when investigating orthopaedic implant associated infection. It is a useful resource for laboratories which aims to help drive pathology modernization by recommending alternative technologies for timely laboratory diagnosis of infection.
New diagnostic approaches

MASS SPECTROMETRY PROTEOTYPING FOR THE DETECTION, CHARACTERISATION AND IDENTIFICATION OF INFECTIOUS BACTERIA
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\textsuperscript{5}Department of Analytical Chemistry, Nanoxis Consulting AB, Gothenburg, Sweden

Background

Diagnosing infectious diseases requires a range of methodologies for the comprehensive detection, characterisation and identification of infectious bacteria. Proteomics-based approaches represent an alternative to traditional methods of characterising microorganisms, elucidating expressed biomarkers of genome information, applied to ‘proteotyping’ microorganisms at all taxonomic levels.

Objectives

Apply state-of-the-art and novel techniques of tandem mass spectrometry (MS) analyses of expressed proteins for species- and strain-level identification and detection of antibiotic resistance and virulence, with the focus on optimizing MS-based proteomics analyses for ‘proteotyping’ infectious bacteria.

Methods

Intact bacteria or cell fractions were immobilised, via membrane-gold interactions within a flow-cell. Within the flow-cell, proteins were subjected to enzymatic digestion to generate peptides, which were identified, using LC-MS/MS. Following database matching, peptides were used for bacterial species-level identification, and for detection of antibiotic resistance and sub-species typing. To demonstrate proteotyping capability and differentiate closely related species, mixtures of different \textit{Streptococcus} spp. were analysed. Results of \textit{Streptococcus} spp. identifications from clinical samples were confirmed by standard microbiology, including cultivation of bacteria in selective media, PCR, DNA sequencing and MALDI-TOF MS analyses.

Conclusions

Proteotyping of infectious bacteria, using LC-MS/MS enabled the differentiation and identification of \textit{Streptococcus} spp. by ranking identified expressed proteins, according to the number of peptide matches to genome sequence information available in databases, and was applied to detection in clinical samples. The methods
have been shown to enable reproducible, sensitive, rapid and cost-effective typing of bacteria for diagnostics of infectious diseases.
APPLICATION OF MOLECULAR TECHNIQUES TO THE RAPID DETECTION AND CONFIRMATION OF HIV

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DIRECCION GENERAL, CONSEJO ESTATAL PARA LA PREVENCIÓN Y CONTROL DEL SIDA (COESIDA-CAPASITS OAXACA), SAN BARTOLO COYOTEPEC OAXACA, Mexico

COORDINACION, CENTRO AMBULATORIO PARA LA PREVENCIÓN Y CONTROL DEL SIDA E ITS (CAPASITS OAXACA), SAN BARTOLO COYOTEPEC OAXACA, Mexico

HIV LABORATORY, INSTITUTO DE DIAGNOSTICO Y REFERENCIA EPIDEMIOLOGICOS, MEXICO D.F., Mexico

Background
Western Blot (WB) is the gold standard as HIV-1 confirmatory test, which has high cost and complexity in processing, our setting send the reactive samples to a national reference center for confirmation and reporting of results takes at least 15 days.

Objectives
The aim of this work was to standardize a technique multiplex PCR as a complementary test for the detection and confirmation of HIV-1 infection in Oaxaca, Mexico.

Methods
Standardization of the multiplex PCR was perform using primers designed to amplify the gag, pol and env genes of M, N, O groups and other specific to Subtype B. For the standardization, we use DNA of three patients that was previously characterized as HIV positive and samples sequenced for HIV-1 (pol, gag, env). We used this multiplex PCR in DNA samples from PMBC of 600 patients. All patients were screening by HIV rapid test. To confirm HIV infection, both WB and multiplex PCR were perform.

Conclusions
We obtain concordant results with multiplex PCR and those of WB. The implementation of a multiplex PCR as a supplemental test for confirmation of HIV-1 infection is reliable and safe. Its implementation has the possibility of decreasing the time of detection and reporting of confirmatory results to a day, which is essential, for example, in cases of pregnant women the confirmation or discard of HIV must be timely, with the aim to start prophylaxis on time.

Acknowledgements: This work is found by Fondo Mixto CONACYT-Gobierno del Estado de Oaxaca, FOMIX 193298
INFLUENCE OF STORAGE CONDITIONS ON THE SPECTRAL STABILITY OF BACTERIAL SUSPENSIONS IDENTIFIED BY MALDI BIOTYPER

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²Centro VISAVET, Universidad Complutense de Madrid, Madrid, Spain

Background

Although direct smear technique is an excellent approach for the preparation of samples for MALDI-TOF MS identification, the usefulness of this approach is limited to BSL-1/BSL-2 bacteria, it is not recommended for MSP creation and it requires fresh material for an optimal performance. Formic acid extraction is proposed as a more precise method for MALDI Biotype identification that includes a step in which bacteria are suspended in 70% ethanol which could potentially be used for the storage of bacterial suspensions in their optimal growth phase. However, there is a lack of information regarding the impact of storage of bacterial suspensions in ethanol on the reliability of the MALDI-Biotype identification.

Objectives

A study was carried out aiming to assess the influence of time (90 days) and temperature (room temperature and 4°C) on the identification by MALDI Biotype.

Methods

Two bacterial suspensions (E. coli and S. aureus) stored in ethanol 70% were included. Half of the aliquots (n=27) were conserved at room temperature and the other half at refrigeration until the day of analysis (0, 5, 8, 12, 19, 26, 42, 71 and 90 days).

Conclusions

Our data suggest that E. coli and S. aureus ethanol suspensions can be stored up to 90 days for MALDI Biotype identification in laboratory conditions. Although it has to be validated, it may apply to other bacteria and could greatly facilitate laboratory work-flow and shipment of bacterial suspensions.
IDENTIFICATION OF H. PYLORI EPITOPES RESPONSIBLE FOR HOST IMMUNO-RESPONSE MODULATION THROUGH ORF-FILTERED PHAGE DISPLAY LIBRARIES AND INTERACTOME-SEQUENCING

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²Department of Health Sciences, “A. Avogadro” University of Eastern Piedmont, Novara, Italy
³Unity of Helicobacter Pathogenicity Department of Microbiology, Institute Pasteur, Paris, France
⁴Department of Experimental and Clinical Medicine, University of Florence, Florence, Italy

Background

To elucidate the molecular mechanisms involved in persistency/latency of the H. pylori infection or in its progression towards serious diseases, it is necessary to analyze the host pathogen interaction in vivo. The circulating antibody repertoire represents an important source of diagnostic information, serving as biomarker to provide a 'disease signature'.

Objectives

The aim of this work is the identification of H. pylori epitopes responsible for host immunoresponse modulation though a discovery-driven approach that couples 'phage display' and deep sequencing.

Methods

We used an approach for identifying novel antigens by screening gDNA libraries created from the pathogen genome, directly with sera from infected patients. Three phage display libraries from three H. pylori strains (HP26695, HPB128, HPSS1) have been constructed by using βlactamase ORF selection vectors (Di Niro et al., 2010). Genomic DNA was sonicated, fragments cloned into the filtering vector, after transformation libraries of 1x10⁶ clones were obtained and sequenced by 454 technology.

Conclusions
More than 93% of HP CDSs were represented in the phage genomic library therefore being representative of the whole *H. pylori* antigenic ORFome. Putative antigens were selected from libraries using sera from patients affected by *H. pylori* presenting increasing degrees of infection: i) autoimmune gastritis and pernicious anemia; ii) gastric adenocarcinoma; iii) MALT lymphoma. The results show that the diversity of the libraries obtained after selection is significantly reduced. Furthermore, individual ranks, for each infection condition, have been compared highlighting the pattern of putative antigens, shared by all the conditions, and some that can distinguish the different stages of infection.
DETECTION OF CRYPTOSPORIDIUM OOCYST RNA BY SURfactANT extraction treatment AND RT-PCR: inhibition OF reverse transcription BY SDS AND ITS suppression using nonionIC surfactants

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Background
Cryptosporidium causes the symptoms of cryptosporidiosis including watery diarrhea, vomiting and fever. Cryptosporidium oocysts possess a robust wall, which is extremely resistant to chlorine used for potable water disinfection. Successful DNA detection from the robust oocyst usually requires complicated procedures including DNA extraction such as freeze-thaw cycling and enzyme treatment. Therefore, we previously developed a surfactant extraction treatment (SET) method for extracting DNA from the oocysts with only sodium dodecyl sulfate (SDS).

Objectives
We examined the inhibition of reverse transcription-PCR (RT-PCR) by SDS and its suppression using four kinds of nonionic surfactants in order to develop a new method for detecting 18S rRNA from the oocyst by SET and RT-PCR.

Methods
The template DNA/RNA was amplified in the presence of SDS and/or the nonionic surfactants; Tween 20, Tween 80, Triton X-100, Triton X-114 by RT-PCR to evaluate the inhibition of reverse transcriptase and DNA polymerase. The oocysts were incubated in a 0.1% SDS solution to extract DNA/RNA.

Conclusions
DNA/RNA amplification was inhibited at 0.01% SDS but its inhibition was suppressed by adding any of the nonionic surfactants at 5%. There was a difference in the suppression efficacy of the nonionic surfactants in tests that used 0.1% SDS. 18S rRNA molecules are present in high copy numbers in viable cells, which increases detection sensitivity. Therefore, we succeeded to detect 18S rRNA equivalent to $10^{-4}$ oocysts by SET and RT-PCR. This work was supported in part by JSPS KAKENHI Grant Number 25420559 and the Kurita Water and Environment Foundation.
NEW DIAGNOSTIC APPROACHES IN INFECTION DETECTION BASED ON THE IMMUNE SYSTEM DERIVED ENZYMES IN WOUND FLUID

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²Environmental Biotechnology, University of Natural Resources and Life Sciences, Tulln, Austria
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⁴Health, Joanneum Research Forschungsgesellschaft, Graz, Austria
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⁶Environmental Biotechnology, Austrian Centre of Industrial Biotechnology University of Natural Resources and Life Sciences, Tulln, Austria

Background

Quick and proper wound diagnostics could avoid wound infections of currently 5-10% of post-surgical - and 25% of chronic wound patients. Apart from standard methods, this study focusses on the wound fluid, harboring enzymes of the human immune system and reflecting a critical wound status upon increasing activity.

Objectives

The determination of the enzyme levels of the neutrophil derived enzymes human neutrophil elastase (HNE), myeloperoxidase (MPO) and lysozyme (LYS) was assessed and correlated to the bacterial burden of the wounds [1]. New diagnostic approaches were based on these enzymes and include electrochemical and optical sensor systems for fast diagnosis of infections in chronic wounds [2].

Methods

The enzyme activities of HNE, LYS and MPO were directly monitored in wound fluid of affected patients via biochemical investigations. Both electrochemical sensors such as measuring the hydrogen peroxide consumption by MPO as current decrease and lateral flow devices based on release of dyes were developed.

Conclusions
Determination of the enzyme activities showed more than 6 to 10 fold higher substrate conversion in infected wound fluids when compared to non-infected wound fluids and clearly correlated with the elevated bacterial burden (Fig1). Consequently, both the electrochemical sensor as well as lateral flow devices were able to differentiate between infected and non-infected wounds.


MOLECULAR SURVEILLANCE ON NASOPHARYNGEAL CARRIAGE OF
STREPTOCOCCUS PNEUMONIAE IN CHILDREN VACCINATED WITH
CONJUGATED POLYSACCHARIDE PNEUMOCOCCAL VACCINES

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Background
As a commensal of the upper respiratory tract, Streptococcus pneumoniae is a
potential pathogen causing respiratory and invasive diseases. Following
implementation of pneumococcal conjugate vaccination (PCV) for infants,
surveillance studies have proven essential for monitoring direct (carriage of serotypes
targeted by vaccine, VTs) and indirect effects (changes in carriage of non-vaccine
serotypes, NVTs).

Objectives
To compare the detection of pneumococcal carriage and serotypes in a unique study
setting using both conventional culture and molecular methods, in nasopharyngeal
samples from healthy PCV-vaccinated infants in two large, cross-sectional
surveillance studies on PCV-effects in the Netherlands.

Methods
Nasopharyngeal samples were collected from 1182 11- and 24-month old children
(n=591 each) during autumn/winter 2010/11 (n=584) and 2012/2013 (n=598).
Following conventional culture on plates selective for S. pneumoniae, DNA extracted
from all bacterial growth was tested by quantitative-PCR (qPCR) for the presence of
pneumococci and a panel of serotypes, including serotypes targeted by the thirteen-valent PCV (PCV13).

Conclusions
There was a correlation (Spearman’s rho=0.980; p<0.001) between the frequency of
serotypes detected using qPCR and prevalence according to conventional culture.
Moreover, we observed a trend (p=0.056) for an underestimation of carriage
detection for serotypes not targeted by PCV7 or PCV10 (in use in the Netherlands)
compared to PCV10-serotypes when detected using the culture-method alone. We
found no evidence of a hidden circulation of serotypes rarely detected by culture or
those targeted by vaccination. This suggests that surveillances based on the culture
method alone do not underestimate carriage of VTs in immunised children.
Background

In the field of natural product research, finding new sources of bioactive compounds is of primary importance. In this respect, microorganisms have provided a large number of biologically active molecules.

Objectives

Recently, the use of fungal co-cultures for the induction of new natural products has emerged as a promising field in drug discovery. For the success of such studies, a key element is the development of a co-culture methodology that provides high reproducibility of metabolite induction patterns and that is compatible with high throughput analytical procedures.

Methods

To tackle this issue, a method based on 12-well-plate miniaturized Petri dishes compatible with high throughput UHPLC-TOF-MS metabolomics [3] has been developed. This strategy was used to screen for metabolite induction in co-cultures of various fungal species. This approach provided a satisfactory reproducibility and was used for the identification of induced biomarkers.

Conclusions
This study demonstrates the consistent induction of new metabolites through co-culture. Moreover, the developed strategy is generic and can be applied to other types of microorganisms that can grow on solid media and that are part of the myco- or microbiome. This 12 well-plate approach and the adapted data mining strategy were validated by the untargeted metabolomic study of a model co-culture (Eutypa lata versus Botryosphaeria obtusa responsible for confrontation zone lines in Vitis wood). This procedure is currently used for screening novel metabolite induction in various fungal co-cultures.
EXPANDING THE DIVERSITY OF THE CYCLODipeptide SYNTHASE FAMILY

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Background
Cyclodipeptide synthases (CDPSs) constitute a novel family of enzymes that use charged tRNAs to synthesize cyclodipeptides in biosynthetic pathways dedicated to the synthesis of diketopiperazines, a large class of secondary metabolites with noteworthy biological activities. To date, 11 CDPSs have been biochemically characterized, showing the incorporation of hydrophobic amino acids in cyclodipeptides (essentially Phe, Leu, Tyr and Trp). Biochemical and structural characterizations of three CDPSs identified catalytic residues and allowed to propose a ping-pong mechanism for cyclodipeptide formation. However, bioinformatic searches in databases identified in microbial genomes numerous putative CDPSs whose activities are not predictable. Furthermore, some of these CDPSs showed differences in predicted catalytic residues.

Objectives
Our objective was to provide a better characterization of the CDPS family of enzymes notably by determining the cyclodipeptide-synthesizing activity of a large set of unrelated putative CDPSs identified in databases.

Methods
We used bioinformatic tools to select the CDPS candidates for biochemical characterization and to analyze their amino acid sequences. As previous work showed the recovery of cyclodipeptides in culture supernatants upon CDPS expression in \textit{Escherichia coli}, CDPSs were produced in this host and cyclodipeptides were searched in culture supernatants by HPLC coupled to mass spectrometry.

Conclusions
The determination of the activities of 41 novel CDPSs enlarged the chemical diversity synthesized by these enzymes, as they were shown to incorporate 17 of the 20 proteinogenic amino acids. CDPSs can be classified into several phylogenetically distinct subfamilies characterized by specific functional subsequence signatures, suggesting differences in catalysis by members of the two subfamilies.
FEMS-0908
Secondary metabolites, metabolomics

IDENTIFICATION OF ANTIMICROBIAL LIPOPEPTIDES OF BACILLUS STRAINS OBTAINED BY DIFFERENT WAYS OF EXTRACTION USING MALDI-TOF MASS SPECTROMETRY
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3Biochemistry and Molecular Biology, Faculty of Biology University of Belgrade, Belgrade, Serbia

Background
Bacillus cyclic lipopeptides of surfactin, iturin, fengycin, and recently discovered kurstakin families have well-recognized potential in biotechnology and biopharmacies. Analytical method such as matrix-assisted laser desorption ionization-time of flight mass (MALDI-TOF) spectrometry proved very effective for detection and identification of various antimicrobial substances.

Objectives
The present study was aimed at investigating how different mode of extraction of crude lipopeptides produced by different Bacillus strains influence the amount of crude lipopeptide and highlights the best extraction mode for further use.

Methods
Five proven lipopeptide producers were grown under previously determined conditions and consequently extracts were obtained by ethyl acetate extraction and by combination of acid precipitation and methanol extraction. Also, cell-free supernatants in the form of aqueous extracts were examined. Extracts were subjected to MALDI-TOF-MS analysis in the acquisition mass range 800-1700 Da.

Conclusions
Mass spectra obtained from all extracts tested, showed clearly three peak clusters corresponding to different lipopeptide families. Cluster of peaks, corresponding to iturin and surfactin families, with similar intensities and points to the dominant presence of these compounds in all strains and extracts, was observed. However, for peaks within the mass range of 1450-1540, corresponding to fengycin family, the best detection was achieved for ethyl acetate extracts. The peaks in the mass range of kurstakin family, for most cases, were more pronounced in the aqueous extract, while the lowest detection was obtained for methanolic extraction. In conclusion, the most efficient was ethyl acetate extraction, which gives the most prominent peaks together with the aqueous extract as a positive control.
Background

Antibiotic-associated hemorrhagic colitis (AAHC) develops during antibiotic-driven intestinal dysbiosis and is caused by production of the bacterial pyrrolobenzodiazepine tilivalline.

Objectives

To understand the function of this secondary metabolite during health and disease we study the regulation of tilivalline biosynthesis and degradation.

Methods

Genes involved in tilivalline biosynthesis were identified via transposon mutagenesis of the clinical isolate AHC-6. Genes encoding a 3-deoxy-D-arabino-heptulosonate-7-phosphate (DAHP) synthase (aroX), a dehydroquinate-synthase (aroB) and two non-ribosomal peptide synthases (npsA and npsB) were identified as essential genes for tilivalline production. Adjacent genes npsA, npsB and aroX are located on a pathogenicity island present in all toxin positive Klebsiella isolates.

Under laboratory conditions tilivalline is detected in conditioned medium of bacterial cultures. Cytotoxicity towards human epithelial HeLa cells appears at the end of the exponential phase (8 h), reaches a maximum during stationary phase and decreases after 40 h to undetectable levels at 48 h. The loss of cytotoxicity can indicate a growth-dependent regulation of biosynthesis and/or inactivation of tilivalline. Emergence of degradation products in tilivalline-depleted supernatant was analyzed by HPLC-MS and NMR. Regulation of tilivalline biosynthesis genes in vitro was monitored via qRT-PCR.

Conclusions

We find that transcription of npsA and npsB is regulated in a growth dependent
manner. Current studies focus on effects of host and microbial factors on tilivalline biosynthesis and cytotoxicity.
Background

Many of natural compounds produced by microorganisms, are synthesized by non ribosomal peptides synthetases (NRPS), working as assembly lines. Among them, lipopeptides as those produced by *Bacillus* and *Pseudomonas*, may play an important role in sustainable agriculture, used as biopesticides to kill plant pathogens.

Objectives

As lipopeptides are synthesized by NRPS, we have decided to screen and identify *in silico* all NRPS gene clusters of *Burkholderia*, especially those producing siderophores and lipopeptides, potentially implicated in biocontrol of phytopathogens.

Methods

We performed *in silico* detection and analyses of NRPS genes present in 48 gapless complete genomes of *Burkholderia* available in the NCBI, by following Florine, a workflow we have developed especially with this aim.

Conclusions

Genome analyses revealed 161 clusters containing NRPSs, with the potential to synthesize at least 11 novel products. Although most strains produce the main siderophore ornibactin or malleobactin, a cluster corresponding to a new siderophore, called phymabactin, was identified *in Burkholderia phymatum* STM815 and the cluster for cepaciachelin biosynthesis was, for the first time, identified in *Burkholderia ambifaria* AMMD. Elsewhere, the cluster for the antifungal burkholdin was detected in both the genomes of *B. ambifaria* AMMD and *Burkholderia* sp. KJ006. We also
identified a novel lipopeptide called burkhomycin specifically produced by *Burkholderia pseudomallei* strains. This study revealed the significant of the genus *Burkholderia* as a promising source of bioactive compounds. It also gave new insights on the non-ribosomal synthesis exemplified by the identification of dual C/E domains in lipopeptide NRPSs, as frequently found in *Pseudomonas* strains.
Background

Trigonelline (N-methyl nicotinate), a pyridine alkaloid, is produced by plants to resist draught stress. Its catabolism by soil bacteria is presumed to proceed through methylamine and succinate but no pathway is described until now.

Objectives

Our model organism is Acinetobacter baylyi ADP1, a strictly aerobic, nutritionally versatile soil bacterium which is easily amenable to genetic manipulations. Biological tools and resources have been developed in the laboratory to enable the integrative analysis of its metabolism. Since it grows well on trigonelline, we wanted to elucidate this pathway at the finest level.

Methods

By comparative genomics, we could identify a conserved cluster of 10 genes involved in trigonelline breakdown. We dissected the pathway by combining bacterial physiology with mutant strains, classical enzymology with recombinant enzymes and untargeted LC/MS based metabolomic approaches.

Conclusions

In reaction mixtures with trigonelline, we could detect succinate semialdehyde and succinate along with three new metabolites, which were produced on a preparative scale, purified and structurally characterized by 1D- and 2-D NMR. Unexpectedly, the first intermediate exhibited a rare 5-hydroxy-butyrolactone structure in equilibrium with an open form, which resulted from a successive reduction-oxidation and cleavage of the pyridine ring followed by a recyclization. This reaction scheme remains unprecedented for a natural pyridine compound and may have valuable spin-off effects in the field of bioremediation since pesticide derivatives or pyridine itself may also be catabolized by this way.
In addition, we showed that trigonelline metabolism is connected with the metabolism of compatible solutes in the cell, extending its physiological function in bacteria beyond nutrition.
Background

Soil microorganisms produce a range of secondary metabolites like antibiotics, toxins, biosurfactants, siderophores. Beside these metabolites soil microorganisms are capable of emitting another class of secondary metabolites so called volatile organic compounds (VOCs). Volatiles are low molecular mass compounds (100-300 Da) with high vapor pressures, low boiling points and lipophilic character. These properties facilitate evaporation and diffusion through both water- and gas-filled pores in soil and rhizosphere environments. As compare to other microbial secondary metabolites volatiles are relatively less studied.

Objectives

The major aim of our study was to obtain more insight in the role of volatiles in microbial interactions.

Methods

In order to obtain insight in the importance of volatiles in the inter-specific interactions between soil microorganisms we performed several experiments in glass Petri dishes plates, which were designed as such that growth of different microorganisms occurred in physically separated areas within a common atmosphere. In this way we studied the role of volatiles in fungal-bacterial and bacterial-bacterial interactions. The volatiles were analysed by using thermal desorption GSMS.

Conclusions

The obtained results revealed that volatiles play important role in the interaction between soil microorganisms. Here we will report on bacterial volatiles with (1) antimicrobial activity and (2) on volatiles acting as infochemicals affecting the behavior, growth and gene expression in responding bacteria. Furthermore we will report on the effect of fungal volatiles on bacteria and on the importance of interspecific interactions for the production of volatile.
ANALYSIS OF THE DRAFT WHOLE GENOME SEQUENCE OF THE HUMIDIMYCIN PRODUCER STREPTOMYCES HUMIDUS F-100.629

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Background

Characterization of new secondary metabolite biosynthetic clusters is gaining increased attention because of their potential to produce new molecules with medical and biotechnological application. Most biosynthetic pathways in actinobacteria are cryptic or silenced in standard laboratory conditions, requiring alternative approaches to identify and these new clusters.

Humidimycin (MDN-0010) is a peptide structurally related to the antiviral siamycins that potentiates the antifungal activity of caspofungin against Aspergillus fumigatus and Candida albicans (1). Humidimycin is produced by the strain Streptomyces humidus F-100.629.

Objectives

This study has been focused on a gene mining of S. humidus F-100.629 to identify the humidimycin biosynthetic pathway and other potential cryptic biosynthetic gene clusters.

Methods

Whole genome sequence was obtained with Illumina. Assembly and annotation were performed using standard bioinformatics tools.

Conclusions

The draft genome size of S. humidus F-100.629 was estimated in 8.4 Mb. The biosynthetic cluster of humidimycin was identified and 50 putative secondary metabolite clusters were predicted using the antiSMASH application (2), including PKS-I, PKS-II, NRPS, terpenes, siderophores and bacteriocin, suggesting the genomic potential of the strain as producer of diverse natural products.

MICROEVOLUTION OF THE BIOACTIVE PEPTIDE ANABAENOPEPTIN IN CYANOBACTERIA

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Background

Bloom-forming cyanobacteria such as the genus *Planktothrix* sp. form a large number of bioactive peptides, e.g. the family of the Anabaenopeptins (AP) through nonribosomal peptide synthesis (NRPS).

Objectives

In total 140 *Planktothrix* strains, isolated from various climatic zones, were phylogenetically assigned into three major lineages that differed in ecosystem type colonization. We analyzed the distribution and recombinations of the AP synthesis gene cluster (*apn*).

Methods

The presence or absence of the *apn* gene cluster was determined by polymerase chain reaction (PCR) and sequencing of PCR products.

Conclusions

We found that the majority of strains of lineage 1, occurring in shallow lakes, carried specific *apn* remnants in parallel to the full gene cluster. The recombinations occurred within adenylation domains resulting in rare structural variants as well as a fusion of part of the ABC transporter domain from another NRPS gene cluster. In contrast, strains of lineage 2, isolated from deep lakes, did not contain *apn* remnants but always contained the full gene cluster. Strains of lineage 3, isolated from tropical regions, did not carry any sequence related to *apn*. Overall evolutionary diversification of *apn* genes was congruent with ecological diversification. Because of the occurrence of *apn* remnants and recombination events within the *apn* in lineage 1 we hypothesize that its ancestor lost the *apn* genes but some of the genotypes regained it through horizontal transfer.
FEMS-0924
Secondary metabolites, metabolomics

A NEW GENE CLUSTER FOR MYCOSPORINE-LIKE AMINO ACID BIOSYNTHESIS IN CYANOBACTERIUM: HETEROLOGOUS EXPRESSION, PURIFICATION, AND CHARACTERIZATION

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Background

Mycosporine-like Amino Acids (MAAs) are an important class of secondary metabolites known for their protection against ultra violet (UV) radiation and other cellular functions, such as scavenging of free oxygen radicals, regulation of osmotic balance, and resistance to thermal and salt stress and desiccation. Cyanobacteria are microorganisms that colonize diverse ecological niches exposed to the UV light and produce MAAs. Because of its efficient sunscreen role, the MAA shinorine from cyanobacteria is utilized commercially in sunscreen creams.

Objectives

The biosynthesis of MAA was recently solved and showed that the structural diversity is directly encoded by the gene cluster. Bioinformatic analysis of the genome of the soil strain Cylindrospermum stagnale revealed a new gene cluster homolog to the MAAs biosynthetic gene cluster. Analysis of this gene cluster presents five genes instead of three/four common biosynthetic genes in other cyanobacteria.

Methods

The gene cluster was successfully cloned and heterologously expressed in E. coli. The MAA was extracted from recombinant E. coli cultures and identified through HPLC and a UV absorption maxima of 310 nm. Structural characterization is underway, combining the data from mass spectrometry and NMR.

Conclusions

The characterization of MAA gene cluster from Cylindrospermum stagnale shows a new gene organization suggesting that it codes for a novel biosynthesis pathway. The new MAA, potentially produced by this terrestrial strain, might play a role for combating the terrestrial environmental stress(s).
Background

Phycocyanin (PC) is the major pigment of the phycobiliproteins in cyanobacteria. The commercial applications of PC could be divided into two sections; the first is an ingredient for nutrient elements, natural dyes for food and cosmetics, and the second is a potential remedy in oxidative stress-induced diseases.

Objectives

The aim of this study was to increase the PC content in the isolated cyanobacterium (Nostoc sp.) with nitrogen-free medium and chromatic acclimation.

Methods

Nostoc sp. was cultured for 10 days in 1-L cylinder flasks containing 800 ml nitrogen-free BG11 medium. For chromatic acclimation, two types of light sources were compared, i.e. plant-fluorescent lamp (only blue and red wavelengths are provided) and white fluorescent lamp.

Conclusions

Under fluorescent lamp condition, the maximum biomass production was 2.24 g DCW/L (dry cell weight g/L). High biomass production could be achieved by nitrogen fixation of Nostoc sp., even without nitrogen supply. The maximum biomass concentration was higher at 2.65 g DCW/L under plant lamp condition. It means that blue and red lights in plant lamp are more critical in promoting cell growth. Cell color also changed from brown to green through chromatic acclimation by plant lamp. Higher PC content (15%) in cells was obtained and maintained throughout the cultivation. By changing light source from white to plant lamp, biomass production and PC content have been increased. Cost-effective PC production was possible,
due to its nitrogen fixation ability. More simplified PC extraction process was also devised for more efficient PC production.
ENDOPHYTIC MICROBIOTA OF THE AMAZON: ANTIBIOTIC ACTION OF METABOLITES IN PATHOGENS PRESENT IN WOUND

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⁴Faculty of Agricultural Sciences, Amazonas Federal University, Manaus, Brazil

Background
The endophytic microorganisms (EM) of the Amazon are potential producers of secondary metabolites with biological activity that can be used on infected wounds.

Objectives
This research investigated the antibiotic action of ME from the Amazon front of pathogenic microorganisms present in the in wound healing process.

FIG 01.
Methods
The antibiotic activity of 171 secondary metabolites of endophytic microorganisms (MSME) were analyzed against *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Escherichia coli* and *Candida albicans* by diffusion in agar. In the test were used 50µL of each MSME extract; ampicillin, tetracycline, ciprofloxacin and fluconazole, all in 2% as positive control according to the test microorganism and DMSO as a negative control. The readings of the zones inhibition were carried out in 24 and 48 hours. The mean, standard deviation, ANOVA and Tukey's test (p <0.05) were performed for analysis.

Conclusions
The largest zone of inhibition with significant differences for *C. albicans* were MSME DfGa2 1.2 G4 (2.88 ± 0,71cm) and DfGa2 1.2 G5 (2.72 ± 0,69cm), for *S. aureus* were MSME DfGa2 1.2 G4 (2,52±0,83cm) and DfGa2 1.2 G5(2,32±0,45cm), for *P. aeruginosa* were MSME DfGa2 1.2 G5 (2,24±0,30cm). For *E.coli* there was no significant difference between the means of the zones of inhibition. The authors concluded that the samples DfGa 2 1.2 G4 and DfGa 2 1.2 G5 had better antibiotic action for the tested pathogens, suggesting a formulation of a gel and, subsequently, its evaluation in vivo of the activity of healing wounds in rats.
ANTIFungal activity of metabolites from streptomycetes diastatochromogenes (No.1628) and its active principle
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Background
The fermentation broth by Streptomyces diastatochromogenes (No.1628), isolated from a soil sample collected in Mountain Tianmu, Hangzhou, China, had strong antifungal activity against Alternaria solani, Botrytis cinerea, Fusarium oxysporum and Rhizoctonia solani.

Objectives
The aim of this research is to isolation and identification of active compounds from S. diastatochromogenes 1628.

Methods
A bioactivity-guided approach was employed to isolate and determine the chemical identity of bioactive constituents with antifungal activity from S. diastatochromogenes 1628.

Conclusions
By the mycelium growth rate assay, it was showed that the n-butanol extract dosedependently inhibited growth of above tested phytopathogenic fungi with a potency at the concentration of 175 mg/L that was equal to that of commonly used antifungal agents at the concentrations tested. Furthermore, n-butanol extract of S. diastatochromogenes (No.1628) could effectively suppress and control cucumber rhizoctonia rot caused by R. solani. Through bioassay-guided fractionation, the n-butanol extract from S. diastatochromogenes (No.1628) afforded a new tetraene macrolide, (7E, 12Z,13E,15E,17E,19E)-21-((4-amino-3,5-dihydroxy -6-methyltetrahydro -2H -pyran-2-yl)oxy) -12-ethylidene- 1,5,6,25 -tetrahydroxy -11 -methyl-9-oxo-10,27-dioxabi-cyclo[21.3.1]heptacosa-7,13,15,17,19–pentaene-24-carboxylic acid (1), together with tetrin B (2), tetramycin A (3), toyocamycin(4) and anisomycin (5). The structures of compounds were established on the basis of spectroscopic analyses. This new compound strongly inhibited hyphal growth of R. solani and B. cinerea with IC₅₀ of 0.20 and 1.53 μg/mL, respectively. Our study demonstrated that S. diastatochromogenes (No.1628) is a promising source of natural bioactive and novel metabolites and has wide application prospect in biocontrol field.
Background

Obesity is a disease resulting from improper balance between energy intake and expenditure and is increasingly becoming a major cause of preventable mortality. Pancreatic lipase (PL) is considered as one of the safest target for diet-induced anti-obesity drug development.

Objectives

In the process of exploration of new PL inhibitors, we have screened culture filtrates of 200 endophytic fungi from different medicinal plants using qualitative and quantitative assays. The organism was identified and partial purification of the bioactive moiety was carried out using analytical techniques.

Methods

Rhodamine olive oil plate assay and PNPL (p-nitrophenyl laurate) as substrate was used for quantitative assessment of PL activity inhibition.

Conclusions

The qualitative assays indicated potential PL inhibition in 27 isolates. Further, only organic extract of these culture filtrates exhibited PL inhibition in range between 30-100%. #6 AMLWLS exhibited complete inhibition of PL with an IC$_{50}$ of 2.12 μg/ml which was comparable to the Orlistat exhibiting an IC$_{50}$ value of 2.73 μg/ml. Purification of the extract by column chromatography led to isolation of the pure compound which was identified as a tetra peptide molecule on the basis of C$^{13}$ NMR, HR/MS and biochemical analysis. Further molecular phylogenetic tools and morphological studies were used to identify the isolate #6 AMLWLS as Fusarium incarnatum species.

Hence endophytic fungal isolate #6 AMLWLS stands out as a potential candidate for anti-obesity therapy.
FEMS-3086
Secondary metabolites, metabolomics

SCREENING CYTOTOXICITY AND LEISHMANICIDE ASSESSMENT
ENDOPHYTIC FUNGI FROM HYPTIS SUAVEOLENS (L) POIT AND COMBRETUM LANCEOLATUM POHL

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Background
Bioprospecting using endophytic fungi is a promising source for the detection of novel compounds with biological activities of interest in health care, especially for diseases such as leishmaniasis (neglected tropical diseases) and cancer.

Objectives
The objective was to evaluate the cytotoxicity and the leishmanicide activity of endophytic fungi extracts isolated from H. suaveolens (L) Poit and C. lanceolatum Pohl against tumor cell lines MCF-7 and MDA-MB-231 and promastigotes forms of L. amazonensis.

Methods
We have evaluated 25 fungal culture extracts obtained by maceration of the mycelium in ethyl acetate (EtOAc). The fungi have isolated from H. suaveolens and C. lanceolatum. The extracts were tested against tumor cell lines MCF-7 and MDA-MB-231 and promastigotes forms of L. amazonensis. The test used was a colorimetric method with tetrazolium salt 3- [4,5-dimethylthiazol-2-yl] -2,5-diphenyltetrazolium bromide (MTT). The concentration of the extracts ranged from 100 to 12.50 µg/mL.

Conclusions
Extracts of 5 fungal isolates (Taifanglania curticatenata-F27; Marasmius sp.-21C, Fusarium oxysporum-33C; Macrophomina phaseolina-46C and Trichoderma spirale-66C) inhibited the proliferation of promastigotes at a low IC(50) of between 25.61 and 52.60 µg/ml. Six extracts (M. phaseolina- F1/46C; T. curticatenata-F27; Cladosporium flabelliforme- F29; Neosartorya pseudofischeri-F36, and Corynespora cassiicola-72C) were cytotoxic to both cell lines at a low IC(50) of between 23 and 48.72 µg/ml. Our results indicate that the medicinal plants living in wetlands Brazilian shelter an interesting bioactive fungal community that is able to produce leishmanicidal and antitumoral molecules. These molecules may be used to develop new leishmanicidal and anticancer drugs.

Acknowledgements: CNPq and FAPEMAT
Background

Filamentous fungi are producers of a large number of valuable secondary metabolites due to their promising biological effects. Identification of these compounds and characterization of their biological activities are the important goals of research working at the interface of chemical and biological sciences.

Objectives

We aimed to verify the peptaibol production of a *Trichoderma* strain selected from the Szeged Microbiological Collection by a *Micrococcus luteus*-based agar plate screening method.

Methods

Mass spectrometric analysis of putative peptaibols produced by a *T. pleuroticola* strain have been carried out via on-line reversed-phase high performance liquid chromatography (HPLC) coupled to electrospray ionization ion trap mass spectrometry (ESI-IT-MS) after the solid phase clean-up of culture extracts. Initially, the separation and the mass spectrometric parameters were optimized using alamethicin standard peptaibol mixture and the unknown components produced by the selected isolate were investigated and determined.

Conclusions

Our mass spectrometric measurements confirmed the results of the biotest selection method used for the detection of peptaibols. Some of the produced peptaibols showed the characteristic mass spectral components of trichorzianins, including TA IIIb, TB IVb, TAP-14a and TA VII. However, besides the known molecules, seven new trichorzianin-related compounds were also detected; these contained some
amino acid changes in the core trichorzianin sequence. Comparing the amount of the different components, compounds with a molecular mass of 1909 amu were present in the highest amount. This research was supported by OTKA grant K-105972 from the Hungarian Scientific Research Fund and a bilateral grant from “Stiftung Aktion Österreich-Ungarn”.
Background

An outstanding group of secondary metabolites are peptaibols, produced mainly by fungal species from the genus Trichoderma. These compounds show a wide spectrum of biological activities including antibacterial, antifungal and antiviral effects.

Objectives

We aimed to develop a simple and easy-to-follow peptaibol characterization strategy including purification and structural elucidation steps, which can help to analyze the peptaibol-producing abilities of various isolates within the genus.

Methods

Our characterization strategy includes the mass spectrometric analysis of the culture extracts of possible peptaibol-producing Trichoderma strains, the semi-preparative purification of the selected compounds, identification of amino acids in their sequences, and chiral analysis of the identified building blocks. These tasks require the use of microbiological methods for strain identification and for different cultivations and the application of separation techniques such as solid phase extraction, normal and reversed-phase HPLC and mass spectrometry.

Conclusions

During the study, the different methodological steps were optimized and their application served the detailed characterization of some Trichoderma strains from the point-of-view of their peptaibol profiles. The examined isolates are belonging to the species T. aggressivum, T. atroviride, T. longibrachiatum, T. orientale, T. pleuroticola, T. pleurotum and T. virens. The peptaibol compositions showed high level of diversity and their amounts presented in a wide range. A number of already described compounds have been identified from species other than the original producer and novel peptaibols were also defined. This research was supported by OTKA grant K-105972 from the Hungarian Scientific Research Fund and a bilateral grant from “Stiftung Aktion Österreich-Ungarn”.

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PURIFICATION AND IDENTIFICATION OF FIVE OPHIOBOLIN ANALOGUES FROM BIPOLARIS ORYZAE

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³Botany and Microbiology Department, King Saud University, Riyadh, Saudi Arabia

Background

Ophiobolins are a remarkable family of secondary metabolites with various biological activities such as calmodulin antagonism, cytotoxic, antimicrobial and nematocid effects.

Objectives

Our aim was to develop a rapid and efficient method for purification of different bioactive sesterterpene-type fungal secondary metabolites belonging to the ophiobolin family.

Methods

Based on our preliminary results, a Bipolaris oryzae strain was selected and cultivated in PDB. The ferment broth was extracted with ethyl-acetate and concentrated. The purification of the crude extract was carried out using subsequential semi-preparative liquid chromatographic techniques. Initially, normal phase column chromatography was carried out using the mixture of ethyl-acetate and n-hexane, and the fractions containing large amount of potential ophiobolin compounds were further purified on a semi-preparative reverse phase HPLC system with the mobile phases of acetonitrile and water. After each purification step, the collected fractions were analyzed on an analytical HPLC system to determine their purity. The purified compounds were identified using mass spectrometric and NMR experiments.

Conclusions

The selected B. oryzae strain produced several ophiobolin analogues. The purified compounds synthetised in the highest amount proved to be ophiobolin A; its identity was confirmed with HPLC-MS and NMR experiments. Besides ophiobolin A, four other ophiobolin analogues were also purified including 3-anhydro-ophiobolin A,
ophiobolin I, 6-epi-ophiobolin A and 3-anhydro-6-epi-ophiobolin A with the purities over the 95%. This research was supported by grant TÁMOP-4.1.1.C-12/1/KONV-2012-0012. Csaba Vágvölgyi thanks the visiting professor program, Deanship of Scientific Research, King Saud University, Riyadh.
Background

Rhamnolipids are potent biosurfactants with high potential for industrial applications. They are currently produced with the opportunistic pathogen *Pseudomonas aeruginosa* during growth on hydrophobic substrates such as plant oils. The heterologous production of rhamnolipids enables the use of a non-pathogenic host and cheaper substrates such as glucose. Glucose also has the advantage of simpler purification, e.g., via coupled foam fractionation and adsorption [1].

Objectives
To engineer a non-pathogenic rhamnolipid producer converting glucose or other renewable resources efficiently into the target product.

Methods

Introduction of the biosynthesis pathway for rhamnolipids from *P. aeruginosa* in the non-pathogenic *P. putida*. Metabolic engineering of secondary metabolite producers implicitly relies on high flux through central carbon metabolism. Our strategy relies on the ability of *P. putida* to reroute metabolic resources into a peripheral pathway when a high demand exists, e.g., initiated by high transcriptional activity.

Conclusions

We previously presented a recombinant *P. putida* able to produce 0.2 g/L rhamnolipids [2]. For optimization, several techniques were used. A synthetic promoter library was developed using an approach based on degenerated primers [3]. This technique yields an array of transcriptional activity, potentially allowing the identification of optimal enzyme activity for high flux towards rhamnolipid synthesis. The second step was eliminating a pathway competing for a common precursor. The best producing strain was able to reach a titer of 3 g/L with a yield of 40% and a very high specific rhamnolipid synthesis rate, which is possible because the flux through the precursor providing pathways is significantly increased in the engineered strain.
FUNCTIONAL CHARACTERIZATION OF A GENE INVOLVED IN THE BIOSYNTHESIS OF MYCOPHENOLIC ACID IN THE FUNGUS PENICILLIUM ROQUEFORTI

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Background

Penicillium roqueforti is a filamentous fungus used in the ripening of blue-veined cheeses. Previous studies have shown that this organism produces several secondary metabolites, including mycophenolic acid, a meroterpenoid with immunosuppressive activity of great pharmaceutical interest. However, the genes involved in the synthesis of this metabolite in P. roqueforti have not been described or characterized so far. In other organisms such as P. brevicompactum, it has been described that the mycophenolic acid biosynthetic cluster consists of seven genes, among them mpaDE, which encodes for a natural fusion enzyme consisting of a cytochrome P450 and a hydrolase.

Objectives

To evaluate the role of the mpaDE gene in the biosynthesis of mycophenolic acid in P. roqueforti.

Methods

The genome of P. roqueforti was analyzed using bioinformatic tools and an ORF that corresponds to the hypothetical orthologue of mpaDE was identified. The expression of this gene was down regulated using RNAi-silencing technology. For this purpose, P. roqueforti was transformed with an appropriate genetic construct, and several transformants strains showing dramatic reductions in the levels of mpaDE transcript were selected. Finally, extracts from these transformants were analyzed by HPLC.

Conclusions

P. roqueforti wild-type showed high levels of mycophenolic acid. On the contrary, the transformants showed barely detectable levels of this metabolite. These results indicate that mpaDE is involved in the biosynthesis of mycophenolic acid in P. roqueforti.

This work was supported by FONDECYT 1120833 y DICYT-USACH.
A UNIQUE GLUCOSE SENSOR GCR1 OF THE METHYLOTROPHIC YEAST HANSENULA POLYMORPHA CAN TRANSPORT MONOSACCHARIDES

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Background
Several proteins involved in glucose sensing, signalling and transport have been described for methylotrophic yeast Hansenula polymorpha. Among them Gcr1, a peculiar C-tale-less glucose sensor, required for glucose repression and regulation of hexose transport.

Objectives
The aim of this work was to further elucidate the H. polymorpha Gcr1 protein function in glucose transport.

Methods
Wild type NCYC495 (WT) and mutant strains of H. polymorpha, classical methods of molecular genetics and microbiology were used.

Conclusions
The closest homologue for glucose sensor Gcr1 of H. polymorpha is the functional high-affinity monosaccharide transporter involved in H⁺-dependent glucose symport – MstA from Aspergillus niger. Regulated HpGcr1 production in hexose transporter-less Saccharomyces cerevisiae mutant did not restore growth of this strain on different glucose concentrations. Similarly to Gcr1, functional high-affinity glucose transporters of Trichoderma harzianum Gtt1 and Aspergillus nidulans HxtA did not complement growth deficiency of S. cerevisiae hxt null on different carbohydrate substrates. Grown on methanol-containing medium WT-derivative strains with constitutive expression of GCR1 and HXT1 encoding low-affinity hexose transporter were more sensitive to exogenous glucose antimetabolite 2-deoxy-D-glucose (2-DOG) (concentration 0.3 mM) compared to the WT strain. The toxic effect of 2-DOG was more acute on medium with lower pH. Therefore, Gcr1 can possibly transport glucose toxic analogue into cells, thus increasing its harmful effects. H. polymorpha protein Gcr1 that most probably results from a horizontal transfer from Aspergillus fungi, is essential for glucose catabolite repression of genes involved in metabolism of alternative carbon substrates and "very-high affinity" glucose transport.
Background

The virulence of Bordetella pertussis, the whooping cough agent, is regulated by the two-component system BvgAS. BvgA is a classical response regulator while BvgS in an unorthodox sensor-kinase harboring receiver and Histidine phosphotransfer domains. BvgS contains three putative perception domains, two periplasmic Venus Flytrap domains linked by a transmembrane segment to a cytoplasmic PAS domain. The kinase of BvgS is active by default and is turned off by negative modulators such as nicotinate or sulfate ions.

Objectives

BvgS functions as a homodimer, and its phosphorylation cascade works in trans. We took advantage of this property to construct a merodiploid strain in which only heterodimers, but not homodimers, are functional to decipher the signal transduction mechanism involved in BvgS.

Methods

We introduced point mutations in the various sensor domains to test their effect in heterodimers. The same substitutions in the context of a wild type phosphorylation cascade affect either the basal kinase activity of BvgS or its ability to respond to modulation, as seen using a lacZ reporter gene under the control of a virulence gene promoter.

Conclusions

When substitutions affecting the response to modulation are introduced in the periplasmic VFT domains of BvgS, the WT monomer is able to partly compensate for the defect of the other monomer, yielding intermediate phenotypes. In contrast, the substitutions introduced in the PAS domain appear to be dominant over a wild type PAS domain. This suggests that signal transduction is achieved through different mechanisms in the periplasmic and cytoplasmic moieties of BvgS.
Background

All cell types maintain the integrity of their membrane. One widely distributed plasma membrane stress response system in bacteria is the phage shock protein (Psp) system. Its central component PspA has a counterpart Vipp1. Both proteins are directly responsible for membrane maintenance under stress conditions while PspA also negatively regulates its own expression via interaction with the AAA+ ATPase PspF.

Objectives

The PspA and Vipp1 N-terminal amphipathic helix A (ahA) is implicated in their membrane binding, and ahB in PspA is required for negative regulation. A direct interaction between ahA and the lipid bilayer for effector function and ahB and PspF for regulatory function has yet to be determined.

Methods

Purified short peptides derived from the amphipathic helixes were used to directly probe membrane binding and regulatory function. Defined membrane vesicle in vitro assays and CD spectroscopy characterised the functionalities of the ahss and the downstream effects on membrane stability and transcription regulation.

Conclusions

We observed direct membrane-binding of ahA derived peptides and in the case of Vipp1 an accompanying change in secondary structure from random coil to alpha
helical upon membrane association. Binding specificity by varying the membrane
anionic lipid content and stored curvature elastic stress linked the functionalities of
the peptides with those of full length proteins. ahB of PspA inhibited the ATPase of
PspF proving its direct regulatory role. These findings establish synthetic peptides
can probe PspA structure-function and target regulation of the Psp response, which
could be of interest for controlling pathogens where Psp response is important and
conserved.
Background

Cell-surface signaling (CSS) is a regulatory mechanism used by gram-negative bacteria to modify gene expression in response to environmental signals. CSS regulation is extensively present in the human opportunistic pathogen *Pseudomonas aeruginosa*. Most CSS systems of this bacterium regulate iron uptake. However, the PUMA3 CSS system regulates virulence. *P. aeruginosa* PUMA3 is constituted by the VreA receptor, the VreR anti-sigma factor and the $\sigma^{Vrel}$ extracytoplasmic function (ECF) sigma factor. VreR binds to and keeps inactive $\sigma^{Vrel}$ in absence of the PUMA3 inducing signal. The role of VreA is to sense the presence of the signal and to transduce it to the VreR anti-sigma factor allowing the release and activation of $\sigma^{Vrel}$ and the expression of the PUMA3 regulated genes.

Objectives

The PUMA3 inducing signal is still unknown. However it has been recently shown that expression of the *vreAIR* operon is induced in low phosphate (Pi). In this condition, some degree of $\sigma^{Vrel}$ activity is also observed despite the fact that the VreR anti-sigma factor is also produced. In this work, we aimed at analyse the role of VreA and VreR in both the expression and the activation of $\sigma^{Vrel}$ in low Pi.

Methods

*P. aeruginosa* vreR and vreA deletion mutants has been constructed by allelic exchange. Gene expression was monitored using *lacZ* transcriptional fusions. $\sigma^{Vrel}$ stability has been assayed by Western-blot.

Conclusions

Full activation and stability of $\sigma^{Vrel}$ in low Pi requires the removal of VreR and the presence of an additional inducing signal that targets this process.
IMPACT OF POTENTIAL IN VIVO DIVK DIMERIZATION ON CAULOBACTER CRESCENTUS CELL CYCLE

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Background

Response regulators are crucial actors of two-component systems and have often been shown to dimerize. We focus on DivK, an essential single domain response regulator of the aquatic \(\alpha\)-proteobacterium \textit{C. crescentus}. DivK is part of a phosphorelay controlling developmental events and belongs to the same protein subfamily as CheY and Spo0F, two response regulators known to dimerize. A previous study has highlighted DivK dimerization \textit{in vitro} (Guillet et al., The Journal of Biological Chemistry, 2002) but the relevance of this dimerization \textit{in vivo} is still a mystery.

Objectives

The goal of our research is (i) to assess whether DivK is able to dimerize \textit{in vivo} and (ii) to determine whether this dimerization mediates DivK subcellular localization and/or the interaction with potential partners. We are also interested in investigating the importance of DivK phosphorylation in its dimerization.

Methods

Under native conditions, DivK appears as a doublet which is not visible under reducing conditions. Interestingly, the relative intensity of the DivK bands varies when the PleC phosphatase or the DivJ kinase, both acting on DivK, are knocked-out. In addition, the doublet is upshifted in the DivK\textsubscript{D90G} mutant, whose subcellular localization is affected. The nature of this doublet is currently under investigation. We are also trying to identify DivK partners by MS-MS analysis of DivK co-immunoprecipitates obtained from various genetic backgrounds.

Conclusions

Preliminary results suggest that DivK is indeed able to dimerize \textit{in vivo} and to bind different partners. This dimerization could be triggered by phosphorylation.
USE OF ANTISENSE RNA TO MODULATE HSP GENE EXPRESSION IN OENOCCUS OENI

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Background

Oenococcus oeni is a wine-associated lactic acid bacterium responsible for wine malolactic fermentation. Wine exhibits harsh and challenging conditions: low pH, low temperature, nutrient-poor and presence of ethanol leading to O. oeni stress response. Understanding of the mechanisms involved in stress tolerance is essential to improve O. oeni development in wine.

Stress adaptation of O. oeni has been studied and many hsp genes, induced in oenological conditions, have been identified. The hsp18 gene, encoded the small Hsp Lo18, have been particularly characterized. In addition to its chaperon activity, Lo18 could have a lipochaperon-function and maintains membrane integrity in stress conditions.

Objectives

Up to now, characterization of O. oeni genes was restricted due to lack of genetic tools for gene replacement. Accordingly, we focused our work on gene inactivation by using antisense RNA approach to modulate hsp gene expression in O. oeni. With the goal of understanding the function of O. oeni hsp genes in vivo, we have developed an efficient expression vector to produce antisense RNA targeting hsp18 mRNA and reduce sense RNA transcript.

Methods

After transformation into O. oeni, transformants were exposed to multiple stresses inducing hsp18 gene expression: heat shock, acid shock and presence of ethanol.

Conclusions

We highlighted that in vivo antisense inhibition of hsp18 expression strongly affects the survival of O. oeni in stress conditions. This study presents a new efficient genetic tool for O. oeni and demonstrates for the first time the use of antisense technology for modulating gene expression in O. oeni.
DIFFERENTIAL EXPRESSION OF SMALL RNAs IN RESPONSE TO CHEMICAL STRESS

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Background
Bacterial sRNAs are often expressed in response to changing environmental conditions and function to modulate gene expression. There are numerous documented connections between sRNAs and stress, where sRNAs regulate important processes in response to metabolite, envelope, oxidative, iron deficiency, anaerobic and pH stress.

Objectives
Although chemical stress is routinely encountered in microbial processing applications due to the toxicity of substrate, intermediate or product compounds, the cellular response and the involvement of sRNAs in this process is poorly understood. We have used an RNA sequencing approach to map the E. coli sRNome during chemical stress and high cell density fermentations with the aim of gaining insights into the chemical stress response and identifying sRNAs with roles in stress tolerance that have potential applications in the design and optimization of future production strains.

Methods
RNA sequencing libraries were prepared from RNA isolated from E. coli MG1655 cells subjected to chemical stress with twelve compounds including organic acids, amino acids, and organic solvent-like compounds. The E. coli MG1655 strain was also grown under high cell density fermentation conditions, where cells were harvested in exponential batch, glucose-limited exponential fed-batch, transition and stationary growth phases.

Conclusions
Over 250 novel intergenic sRNAs have been identified with this approach, adding to the roughly 200 previously reported sRNAs in E. coli. A significant fraction of the sRNAs exhibit specific expression patterns during high cell density fermentation and
a group of them are differentially expressed in the presence of multiple chemicals, suggesting they may play regulatory roles during these stress conditions.
**FEMS-1568**  
**Small regulatory RNAs**

**HFQ BINDING PROFILES OF SRNAS AND THEIR TARGET MRNAS**  
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**Background**  
Bacterial sRNAs are 50-400 nucleotide-long RNA molecules, most of which exert their regulation by base-pairing with their target mRNAs. In many bacteria, including *Escherichia coli*, the interaction between an sRNA and its targets is mediated by the Hfq protein, resembling the situation in eukaryotic cells where the interaction of a microRNA and its targets involves Argonaute. While it is widely accepted that miRNAs guide Argonaute proteins to their specific targets through sequence complementarity, the dynamics of sRNA-Hfq-target interaction, to the best of our knowledge, has not been yet fully determined.

**Objectives**  
As a first step towards this question, we study the relationship between the levels of Hfq-bound sRNA and its target mRNAs. More specifically, we aim to measure the change in Hfq-bound target mRNA levels following a change in the expression level of their sRNA regulator.

**Methods**  
To this end we applied RNA-seq to measure the levels of total RNA and RNA co-IPed with Hfq from three *E. coli* strains (MG1655 Hfq-FLAG, MG1655 hfq::kan and MG1655 Hfq-FLAG rnc14::Tn10) with and without overexpression of the sRNA MicA.

**Conclusions**  
Our results indicate that most MicA target mRNAs, as well as MicA itself, were enriched in Hfq when MicA was overexpressed. In contrast, most target mRNAs of other sRNAs were depleted. In conclusion, our initial results indicate that there is a relationship between sRNA and target mRNA levels on Hfq, offering a strategy to identify putative novel targets.
Background

RNA sequencing has recently revolutionized transcriptome analyses. As a result, novel non-coding RNAs have been discovered in several organisms and subsequent studies have revealed an increasing number of new regulatory mechanisms at the level of RNA. The lactic acid bacterium *Lactococcus lactis* is of high importance for biotechnological applications, specifically in the dairy industry. The stress response systems are very well studied for this bacterium. In addition, global regulators of carbon and nitrogen metabolism have also been studied in detail. However, the presence and roles of non-coding RNAs with regulatory functions has not been assessed until now.

Objectives

We aimed to identify novel RNA elements, such as small regulatory RNAs, antisense RNAs and sORFs. In addition, expression of these novel RNAs under various stress conditions was assessed, and for one novel sRNA, the function was studied in depth.

Methods

By using differential RNA sequencing (dRNA-seq) data, a transcriptome landscape of *L. lactis* was constructed that was used for manual and automated mining using TSSer. Northern hybridization was used to confirm 12 putative regulatory RNAs.

Conclusions

This study used both manual and automated approaches to identify hundreds of novel RNA species. Experimental validation was performed for a selection of these potential RNA regulators. The role of one non-coding RNA located in the 3'UTR of *argR*, the regulator of *e.g.* the *arc*-operon in *L. lactis*, is currently being examined. This small RNA, called ArgX, appears to stabilize the *arc*-operon. Results of this sRNA ArgX and its role in regulating *arc* will be presented.
A GROUP B STREPTOCOCCUS SMALL RNA PRESERVES CELL INTEGRITY AND CONFRS RESISTANCE TO A CATIONIC ANTIBACTERIAL PEPTIDE


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Background

Gram-positive bacteria reduces the effectiveness of cationic antimicrobial peptides (CAMPs), such as colistin, and preserves cell integrity by the addition of D-alanine to lipoteichoic acids (LTAs). Thus far, no small RNA (sRNA) has been described to be involved in the regulation of the genes involved in the D-alanylation of LTAs in the human neonatal pathogen Group B Streptococcus (GBS).

Objectives

Here, we present the first study describing the functional characterization of a GBS sRNA. We highlight its pleiotropic role in maintaining cell integrity and colistin resistance.

Methods

The GBS sRNA was identified by RNAseq and its expression was confirmed by northern blot analyses. The biological role of the sRNA was investigated by analyzing a deletion mutant (Δsrna). The interaction region between the sRNA and its mRNA target was predicted in silico and confirmed by in vivo mutagenesis.
Conclusions

The northern blot analyses showed that the sRNA is mainly expressed at the onset of the stationary phase until late stationary phase. Microscopic observations showed that the Δsrna strain forms significantly shorter chains, and is more sensitive to colistin (MIC = 16 µg/mL) when compared to the wild-type (MIC > 256 µg/mL). Interestingly, the in silico predicted mRNA target is downregulated in the mutant strain. Mutations in the sRNA sequence likely involved in RNA-RNA pairing are sufficient to provoke similar chains alterations than the Δsma strain. All together, these results suggest that the sRNA is required for colistin resistance and cell integrity due to a direct regulation of the D-alanylation pathway.
Background

Papillomaviruses are a diverse group of small, non-enveloped, double stranded DNA viruses that cause proliferations of the stratified squamous epithelium of the skin and mucosa in a wide variety of vertebrate hosts. Papillomaviruses play an important role in human cancer development, and have been isolated from a number of animal malignancies. Recently we identified a novel ovine papillomavirus, OaPV3, associated with squamous cell carcinoma. Like other papillomaviruses, OaPV3 is not able to productively infect cells in culture. The lack of in vitro factories for viral production hampers the investigation of pathogenetic mechanisms and interaction with host immune response.

Objectives

Main goal of this study is the production of non-infectious OaPV3 viral-like particles (VLPs) for evaluating humoral immune response in naturally infected sheep.

Methods

Non-infectious VLPs were obtained by expressing the L1 major capsid protein in insect cells using recombinant baculoviruses. Two sheep were immunized with OaPV3 VLPs. Sera were collected after each of the 3 immunisations. VLPs were used in western blotting and ELISA to test reactivity of 90 sera collected from sheep sampled in flocks naturally infected by OaPV3.

Conclusions

Data indicate a specific immune response of immunised sheep. In naturally infected flocks, only sheep with clinical lesions showed detectable antibodies. OaPV3, like human cutaneous papillomavirus seems to escape humoral response by playing “hide and seek” with host immune system.
Background

Papillomaviruses play an important role in human cancer development, and have been isolated from benign and malignant proliferative skin lesions. However, the association of papillomaviruses with cutaneous lesions has been poorly investigated in sheep. To date, three ovine papillomavirus types have been classified. OaPV1, OaPV2 belong to Deltapapillomaviruses genus and were found in cutaneous fibropapillomas of australian merinos. OaPV3 belongs to a new genus, Dyokappa, and has been recovered from cutaneous squamous cell carcinomas of sarda breed sheep.

Objectives

In this study we investigated unknown ovine papillomaviruses in cutaneous fibropapillomas of sarda breed sheep.

Methods

Rolling circle amplification was performed on total DNA extracted from skin lesions biopsies, and resulted in the presence of a novel ovine Papillomavirus (OaPV4). The entire OaPV4 genome was cloned and sequenced. IHC with L1 antibodies and ISH with E6 dig-probe were carried out in order to establish viral cellular tropism.

Conclusions

A novel papillomavirus, OaPV4, was identified. Viral L1 shares 82.5% nucleotide homology with the closest relative (OaPV1). Maximum likelihood phylogenetic tree placed OaPV4 in a monophyletic clade including all Deltapapillomaviruses so far described.

OaPV4 infects both epithelial cells and fibroblasts and has a role only in benign lesion development. This is the first report of a Deltapapillomavirus 3 species in European sarda breed, and this support the existence of an OaPV1/OaPV4 common ancestor infecting sheep before radiation and evolution of modern sheep breeds.
Background

The Papillomaviridae family consists of a large and diverse group of viruses characterised by a double strand, covalently linked circular genome typically ranging from 7 to 8 kb in size, and causing proliferative lesions in animals and human.

Objectives

Aim of this study was to investigate papillomavirus diversity in Equidae and to establish the presence of PV in species related to horse. We detected a novel papillomavirus (EaPV1) from healthy skin and from sun associated cutaneous lesions of an Asinara (Sardinia, Italy) white donkey reared in captivity in a wildlife recovery centre. EaPV1 genome is 7467 bp long, and shows some characteristic elements of horse papillomaviruses, including a small untranslated region between the early and late regions. A typical E6 ORF is missing. EaPV1 DNA was detected in low copies in normal skin of white and grey donkeys of the Asinara Island, and does not transform rodent fibroblasts in standard transformation assays. Pairwise nucleotide alignments and phylogenetic analyses based on concatenated E1-E2-L1 amino acid sequences revealed the highest similarity with the Equine papillomavirus type 1.

Methods

The entire genome of EaPV1 was cloned, sequenced, and characterised. Methods included: DNA extraction, traditional and real-time PCR, RCA, cloning, sequencing and phylogenetic analyses.

Conclusions

The discovery of EaPV1, the prototype of a novel genus and the first papillomavirus isolated in donkeys, confirms a broad diversity in Equidae papillomaviruses. Taken together, data suggest that EaPV1 is a non-malignant papillomavirus adapted to healthy skin of donkeys.
Background
The spread of multidrug-resistant Enterobacteriaceae is complicating the treatment of diarrhea in neonatal calves. Growing resistance of microorganisms to antibiotics requires to find novel effective antibacterial drugs.

Objectives
To evaluate the bacteriostatic effect of human lactoferrin (hLf), silver nanoparticles (AgNPs), zinc nanoparticles (ZnNPs) and their combinations on pathogenic enteric bacteria isolated from diarrheal calves.

Methods
Recombinant hLf was isolated from milk of transgenic goats generated by microinjecting sequence encoding hLf cDNA to the pronuclear. AgNPs and ZnNPs were prepared by chemical methods. The radii of particles were between 20 and 25 nm. E.coli, Pr. mirabilis and Kl. pneumoniae used in these experiments were isolated from diarrheal calves. Isolated bacteria were resistant to ciprofloxacin and erythromycin. To evaluate the bacteriostatic effect of hLf, nanoparticles and their combinations the broth microdilution minimal inhibitory concentration (MIC) testing was applied.

Conclusions
The results suggested that the MIC value of AgNPs against E.coli, Pr. mirabilis and Kl. pneumoniae was about 2-4 μg/ml; ZnNPs activity was lower (about 8-16 μg/ml). The synergistic antimicrobial effect between hLf and NPs was also evident. The MICs of hLf decreased when used together with NPs. So the MIC of hLf against all of the tested bacterial strains was reduced from 4-8 mg/ml when used alone to 0.5-2 mg/ml when used in conjunction with 1 μg/ml of AgNPs. The synergistic activity of hLf and ZnNPs combination was less pronounced. The best synergistic combination was AgNPs and hLf that allows to recommend it for the treatment of diarrhea in neonatal calves.
EFFECTS OF COLD STRESS ON BACTERIAL ISOLATION AND IMMUNE CELLS IN CAMPYLOBACTER JEJUNI INFECTED SPF CHICKENS.
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Background

Stress activate the hypothalamic-pituitary-adrenal axis and the sympathetic nervous system resulting in a series of neural and endocrine adaptations. In industrial production the cold stress are one of the most important problem in the first week of poultry production. Many microorganisms are present in the livestock and poultry products are considered main source of Campylobacter jejuni to humans.

Objectives

Our aims were challenge chickens with cold stress and Campylobacter jejuni (ATCC33291) infection to assess the role of stress in the interaction host-pathogen

Methods

Thirty-two Leghorn SPF birds were housed in isolators divided into three groups: C (negative control not shown), CI (Campylobacter jejuni infected - 10^4 ufc) kept for 21 days in thermal comfort and the group CI+CS (Campylobacter jejuni infected - 10^4 ufc + cold stress) was subjected to cold stress for 6 h per day during 7 days at a 19°C. At 7 and 21 days old, bacterial isolation from liver and cecum, blood flow cytometry to CD45, CD3, CD4 and CD8 antibodies, plasma corticosterone (CS) (Fig.1) and blood glucose (BG) levels were performed in the birds.

Conclusions

We observed high BG and CS (p<0,005) during entire experiment in CI+CS group. Also, the cold stress increased bacterial isolation at 21days in the liver and cecum (Fig. 2). At 21days of age, we observed a increase of CD3+ (p<0,05) CD4+ and CD8+ peripheral cells in CI+CS group (Fig.3). We may conclude that high CS and BG in birds subjected to cold stress affect negatively the bacterial clearance and immune cells distribution.
CD4+ and CD8+ cells from SPF Chicken blood at 21 days old.
Background

Mycoplasmas are the smallest self-replicating bacteria that evolved a parasitic lifestyle through regressive evolution. The ability of mycoplasmas to modulate immune responses allows the establishment of persistent infection resulting in chronic inflammatory diseases. Mycoplasmas evolved different strategies to escape host immune system. Despite mechanisms of humoral response evasion are known, resistance to innate response is still insufficiently investigated.

To date, studies of interaction between mycoplasmas and granulocytic neutrophils are still lacking. NETs are a mixture of antimicrobial peptides and proteins "caught" in a backbone of DNA filaments that trap bacteria, which are cleared by macrophage through phagocytosis. It has been demonstrated that *Staphylococcus aureus* (S. aureus) breaks down NETs thorugh its SNase and induces macrophages apoptosis. Recently, we demonstrated the *in vivo* expression of MAG_5040, the SNase homologue of *M. agalactiae*. We speculate that MAG_5040 might also be an important virulence factor of mycoplasmas.

Objectives

Main goal of this study is to investigate the interaction of *M. agalactiae* with NETs and to verify if MAG_5040, similarly to its *S. aureus* homologue, allows *M. agalactiae* to avoid NETs-mediated neutrophils extracellular killing.

Methods

Sheep neutrophils, either untreated or activated with PMA and/or MAG_5040, were incubated with *M. agalactiae*. After 3 hours, extracellular DNA was stained with SYTOX Orange and images were detected with a confocal microscope. Differences in fluorescence intensity were also calculated.

Conclusions

Data suggest that *M. agalactiae* is able to induce NETs formation and their digestion *ex vivo*, suggesting a novel mechanism for escaping sheep innate immune response.
Background

*Clostridium perfringens* is a cause for increasing concern due to its involvement in severe infections in both humans and animals, especially poultry. Although *C. perfringens* is part of the natural microbiota of the intestinal tract in poultry, it is an opportunistic bacterium which can be pathogenic resulting in the possibly severe disease - necrotic enteritis.

Objectives

The aim of the present study was to determine the occurrence of *C. perfringens* on one single sampling occasion from poultry enterprises widely distributed across the United Kingdom. The samples were toxin genotyped by PCR and analysed by PFGE and antimicrobial testing.

Methods

172 samples were collected from 50 poultry establishments described as 'free range' operations. None of the enterprises either prior or during the study reported necrotic enteritis in their poultry. Samples for bacterial isolation were grown on tryptose sulphite cycloserine (TSC) agar and quantified; typical colonies of *C. perfringens* putatively identified and confirmed biochemically. The samples were subjected to toxin production identification, ribosome typing, PFGE and antibiotic susceptibility testing.

Conclusions

The presence of *cpa, cpb, etx, iA, cpb2, cpe*, and *NetB* toxin genes were determined by PCR. 89.7% *C. perfringens* isolates were classified as type A and the remaining 10.3% type C; no other toxin genes were identified. Quantitative estimates of numbers of isolates was higher than expected (from $10^4$ to $10^9$ CFU/g) from poultry
farm sources, regardless of the cleaning protocol used to sanitise the housing environment. Attempts to correlate specific clusters of *C. perfringens* with specific locations will be presented.
MSSA AND MRSA CO-COLONIZATION DYNAMICS AND CLONAL DIVERSITY IN PIGS

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Background
Methicillin-susceptible Staphylococcus aureus (MSSA) and methicillin-resistant Staphylococcus aureus (MRSA) are colonizers of skin and mucosa. Studies about MSSA and MRSA in human patients showed a competition of these two microorganisms for colonization space in the anterior nares. Moreover, in humans one clone can be found rather than differing types of MSSA and MRSA. At present, it is unknown whether this is also true for animals.

Objectives
The aim of this study was to investigate the colonization dynamics of both MSSA and MRSA in pigs over a longer time period and investigate their clonal diversity. The results might be of interest for the development of control strategies. This is important as in farm animals, and particularly in pigs common MRSA are found that are transmissible to humans and the environment.

Methods
Eighteen pigs were sampled three times every ten weeks with a nasal swab. Additionally, environmental samples were taken. All samples were investigated for MSSA and MRSA, respectively. Spa-typing was done with up to five MRSA and MSSA isolates, respectively, found per sample and time point. Of almost 400 MSSA and MRSA isolated, 62 isolates were further investigated by microarray.

Conclusions
The results do not support the hypothesis of a competitive colonization of MSSA and MRSA in the anterior nares of pigs. Rather we found a changing status. Hence, highly identical clones of MSSA and MRSA are present in the anterior nares of pigs and their environment. CC398 and CC9 associated spa-types were the predominating clonal lineages found among MRSA and MSSA isolates, respectively.
Background

Induction of parturition can result in some infective postpartum vaginal complications in ewes due to their side effects on parturition. Therefore, it is important to reveal whether induction methods responsible to the vaginal bacterial changing in ewes.

Objectives

This study aims to reveal vaginal bacteriology and antibiogram results of the ewes induced with different parturition induction methods.

Methods

Twenty-four curly-fleeced ewes (n=24) at 3rd pregnancy period on 138th day were devided as group I (n=6): control; group II (n=6): dexamethasone (16 mg, im); group III (n=6): aglepristone (5mg/kg, im) and group IV (n=6): dexamethasone (8mg, im) + aglepristone (2.5 mg/kg, im). For bacteriological examination, the vaginal swaps were taken from ewes at 138th pregnancy day and then they were induced as below. Parturitions had not any clinical complications. Vaginal swaps were repeated on days of postpartum 15 and 30. Bacterial identifications revealed that *Escherichia coli* was the dominant pathogen bacteria both pre-induction and postpartum days in all groups. In postpartum 15th day, *Acinetobacter* spp. was the other pathogens for only in group III. And in postpartum 30th day, *Acinetobacter baumannii* was the other pathogen in group III. Antibiotic susceptibility of these pathogens was performed by Kirby Bauer disc diffusion methods. *Escherichia coli* and *Acinetobacter baumannii* were 100.0% sensitive to enrofloxacin, *Acinetobacter* spp. was 100.0% sensitive to trimethoprim / sulfamethoxazole.

Conclusions

It has been considered that induction of parturition can affect the vaginal flora and can be responsible for the postpartum vaginal complications to be encountered.
CHARACTERISTICS AND ANTIMICROBIAL SUSCEPTIBILITY OF TRUEPERELLA PYOGENES ISOLATED FROM BOVINE MASTITIS CASES IN CHINA

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Background
Trueperella (T) pyogenes is an opportunistic pathogen causes suppurative diseases in dairy cows. However, the pathogenesis and antibiotics resistance of T. pyogenes are still unclear.

Objectives
T. pyogenes isolates morphological characteristics, the presence of genes (plo, cbpA, fimA, fimC, fimE, fimG, nanH, nanP, tetW, erm/X, erm/B), biofilm formation, the cytopathological effects in intracellular assay and antimicrobial resistance were investigated.

Methods
T. pyogenes was isolated from 50 out of 275 clinical and subclinical bovine mastitis cases in China. Both pyolysin (plo) and collagen-binding protein (cbpA) virulence factor genes were detected by conventional PCR in all T. pyogenes isolates. The tissue culture plate method was used to assay the capacity of T. pyogenes for biofilm formation and showed that 90% of T. pyogenes isolates were able to form biofilms with different production amount. Minimum inhibitory concentrations (MICs) of 14 antimicrobial agents were determined and observed high susceptibility to rifampin (96%), while high resistance to trimethoprim–sulfamethoxazole (90%) and bacitracin (98%). Intracellular assay revealed that 4 different T. pyogenes isolates had different cytopathological effects on infected bovine mammary gland epithelial cells.

Conclusions
18% T. pyogenes isolates indicates that T. pyogenes is important contributors to bovine mastitis. Moreover, the within-host quantitative, spatial, high occurrence of multidrug-resistant, biofilm producing and temporal dynamics of T. pyogenes interactions are key factors to better understand how immunity acts on infections with bacteria and how they evade immune surveillance; thus, highlighting the need for prudent use of antimicrobial agents in veterinary medicine (This research was supported by projects No. 2012BAD12B03, No. 313054, No. 20120008110042, No. 2014M561102 and No. GDT2014110043).
BRUCELLOSIS IN GREY SEAL IN BALTIC SEA
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Background

Finnish Food Safety Authority Evira participates in the HELCOM monitoring program to assess the health status of seals in the Baltic Sea area. Organ samples are collected from hunted seals or, to a lesser extent, from seals found dead. In the year 2013 we detected for the first time brucellosis in one of these samples. There are two marine Brucella species, B. pinnipedialis affecting pinnipeds (sea lions, walrus and true seals) and B. ceti affecting cetaceans (dolphins, porpoises and whales). Brucellosis is a zoonosis.

Objectives

The aim of this study was to examine the distribution and possible infection routes of brucellosis in grey seals in the northern Baltic Sea.

Methods

Livers of grey seals (Halichoerus grypus) (n=95) were inspected for parasites and pathological lesions. Samples for bacteriological examination (n=18) were taken from livers with macroscopic inflammatory lesions (n=10) and from normal livers (n=8) for control and cultivated on fastidious anaerobe agar plates. The plates were incubated at 37 °C in microaerophilic atmosphere, and observed for bacterial growth daily for 10 d. The identification was based on Stamp stain, biochemical tests and PCR.

Conclusions

Brucellosis does not seem to be rare in grey seals in the northern Baltic Sea. Of 10 samples with lesions 3 were positive. All normal livers were negative. The bile ducts of positive livers contained abundant numbers of flukes (Pseudamphistomum truncatum), which were positive for Brucella sp. in PCR. Flukes might be one of the possible vectors of brucellosis in marine environments.
Background

Mycobacterium avium subspecies paratuberculosis (MAP) is an obligate, Gram positive, acid-fast bacterium which has adapted to the gastrointestinal tract of ruminants and other animals and can cause fatal inflammatory disorder called Johne's disease. The potency of microorganism to infect human and its close association with Crohn's disease is the main public health issue of the infection, worldwide.

Objectives

The aim of this study was to find out some information about the frequency of MAP in the slaughtered animals and further characterization of its genome, in Southern Iran.

Methods

The study was conducted between October 2013 to August 2014 in which total of 450 specimens from ileocecal lymph node, ileocecal valve and surfaces of 150 slaughtered sheep were collected. The animals were categorized according to the sex and age. IS900 PCR assay was employed to confirm the positive cases.

Conclusions

Out of 450 specimens, 12 were found positive using IS900 PCR assay. All the positive samples were from the intestinal tissues and lymph nodes. Statistical analysis of the results, using Fisher's Exact test, confirmed no significant difference between the frequency of infections in the ram and ewe. Furthermore, infection in the lambs was significantly lower than sheep over 1.5 year age. In general, employing the IS900 PCR assay is recommended to monitor the MAP infected carcasses. The sequence analysis of the positive cases showed a 93% identity when it was compared with gene bank database.
Background
Ear infections are a common problem in dogs. The infections can be very persistent and difficult to treat. The common treatment is with antibiotics and in chronic infections the dogs go through multiple treatments.

Objectives
The aim of this study was to map the most common causes of ear infections in dogs and test their resistance to antibiotics.

Methods
In the period of 2010 to 2013 the Department of Microbiology received 246 samples from dogs for microbial culture. Of these 93 were ear samples and we managed to isolate pathogenic bacteria and/or fungi from 83 of dogs. In the study period the main cause of ear infections in dogs' ears was the bacterium Staphylococcus intermedius. In addition we commonly isolated Proteus mirabilis, Pseudomonas aeruginosa and Candida spp. The antibiotic sensitivity of the isolated pathogens varied considerably and we often saw multiresistant Proteus mirabilis and Candida spp.

Conclusions
Dogs with ear infections are usually treated with antibiotics as soon as the infection is detected. Our study shows that multi-resistant bacteria are common in dogs with recurrent ear infections. It is very important to detect the pathogen and obtain information about antibiotic sensitivity before starting treatment. This will ensure more effective treatment and possible prevent chronic ear infections caused by multiresistant bacteria.
Background
Salmonella enterica serovar Enteritidis is one of the most common serotypes implicated in Salmonella infections in both humans and poultry worldwide. Most Salmonella infections in humans are considered to be associated with foods of animal origins contaminated by Salmonella. Indeed, the most common sources of Salmonella Enteritidis infection are believed to be chickens and chicken products.

Objectives
This study was to estimate the genetic relationship between Salmonella Enteritidis isolates from chickens and humans in South Korea.

Methods
A total of 127 chicken isolates were collected from clinical cases, on-farm feces, and slaughtered chicken meat between 1998 and 2012 and 20 human clinical isolates were obtained from patients who showed clinical signs between 2000 and 2006 in Korea. We compared pulsed-field gel electrophoresis (PFGE) patterns and multilocus variable-number tandem-repeat analysis (MLVA) profiles of the isolates.

Conclusions
PFGE analysis with all the isolates revealed 28 patterns and MLVA identified 16 allelic profiles. PFGE and MLVA showed common types shared by most human and chicken isolates although there was relatively poor congruence between the two analyses. Overall findings demonstrate the transmission of the serovar Enteritidis between humans and chickens.
Canine leishmaniasis is a zoonotic disease by the protozoan parasite *Leishmania* transmitted by the bite of an infected phlebotomine sandfly. *Leishmania infantum* is the most common and important cause of canine leishmaniasis worldwide. Other *Leishmania* spp. reported from dogs include *L. mexicana*, *L. donovani*, and *L. braziliensis*. Leishmaniasis can be categorized by two types of diseases in dogs: a cutaneous reaction and a visceral reaction also known as black fever, the most severe form of *leishmaniasis*.

Infection does not invariably lead to illness. In fact, most infected dogs remain asymptomatic and may never develop clinical manifestations. In endemic regions, the prevalence of disease is often less than 10% and only about 1 in 5 infected dogs are considered likely to develop clinical disease.

Diagnosis of canine leishmaniasis is based on the presence of clinical signs together with positive specific antibody assay.

**Objectives**

The aim of this work was to develop a serological ELISA assay to detect IgG and IgM antibodies against Leishmania in serum or plasma samples derived from all mammals.

**Methods**

Microtiterplates were coated with antigen preparations of *Leishmania infantum*. The presence of antibodies against Leishmania is detected by protein A/G-HRP. A sample collection of about 200 positive samples and 400 negative samples was used for development and evaluation of the assay.
Conclusions

Here we show the performance characteristic of the newly developed assay. Due to the improved antigen design, purification method and test setup a superior assay performance was achieved compared to other test methods.
Background
Bovine mastitis was one of the common and frequent disease in cows, caused huge economic losses to dairy industry.

Objectives
The objective of this study was to investigate the distribution of bovine mastitis pathogens and their antibiotic resistance in China.

Methods
A total of 3941 milk samples from clinical mastitis cows (n=963), subclinical mastitis cows (n=2772) and healthy cows (n=206), which were collected from 40 cities in 150 dairy farms in China, Bacteria were isolated and identified, And disc diffusion test (K-B method) was used to determine the antibiotic resistance of the main pathogens, including Streptococcus agalactiae, streptococcus dysgalactiae, Staphylococcus aureus and Escherichia coli.

Conclusions
The results showed that 24 species of bacteria and fungi include 4337 microorganisms were isolated from 3445 milk samples (87.41%), the other 496 milk samples were detected as negative ones. A total of 2386 bacteria were identified as bovine mastitis related pathogens: Streptococcus agalactiae (35.54%), Streptococcus dysgalactiae (23.55%), Staphylococcus aureus (16.89%), Escherichia coli (12.99%), breast Streptococcus (5.28%), klebsiella (1.63%), Proteus (1.59%), Pseudomonas aeruginosa (1.22%), stellite Nocardia (0.59%), suppurative Corynebacterium (0.38%), Candida albicans (0.17%) and Streptococcus pyogenes (0.17%). the total pathogens detection rate was 62.12%.

Drug susceptibility test showed that the four major pathogens have different resistance to all antibiotics, the resistance rate was 10%~100%. Especially to penicillin G, streptomycin, and cotrimoxazole respectively, the resistance rate was 30%~100%.
O SEROGROUPS OF ENTEROPATHOGENIC (EPEC) AND SHIGATOXIGENIC (STEC) ESCHERICHIA COLI FROM

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Background

*Escherichia coli* producing the attachment-effacement (AE) lesion (EPEC) and/or Shiga toxins (STEC) cause enteritis and (bloody) diarrhoea in young calves and in humans, and are also present in the intestines of healthy cattle. Besides the O157:H7 serotype, EPEC and STEC can belong to more than sixty O serogroups. Of them, 8 have been most frequently identified worldwide: O5, O26, O103, O111, O118, O121, O145 and O165, with some also causing diarrhoea in young calves.

Objectives

This study aimed at identifying the pathotypes and the O serogroups of STEC and EPEC isolated from <1-month-old diarrhoeic calves in Wallonia, Belgium.

Methods

(i) 233 enterohaemolysin-producing *E. coli* were isolated at ARSIA between November 2008 and February 2014 from diarrhoeic calves after growth on EHLY Medium\textsuperscript{®}. They were tested with a triplex PCR targeting the *stx*\textsubscript{1}, *stx*\textsubscript{2} (Shiga toxins) and *eae* (AE lesion) genes.

(ii) triplex PCR-positive *E. coli* were assayed with two pentaplex PCR targeting the specific genes coding for the nine O serogroups listed above and for the O104 serogroup.

Conclusions

(i) 206 isolates tested positive with the triplex PCR. The most frequent pathotypes were *eae*+*stx*\textsubscript{1}+ (102 isolates), *eae*+ (78 isolates) and *eae*+*stx*\textsubscript{1}+*stx*\textsubscript{2}+ (13 isolates).
(ii) the most frequent serogroups of EPEC and STEC were O26 (57 isolates) and O111 (36 isolates). A few additional isolates tested positive for the O103, O5, O145, O121 and O157 serogroups.

(iii) the future is to compare these EPEC and STEC with those isolated from healthy cattle and from humans, to identify host- and age-specific properties.
Background

Mastitis is the inflammation of the mammary gland following infection by most often bacteria. Clinical mastitis is characterized by macroscopic modification of the milk while only high cell counts observed during subclinical mastitis. According to the bacterial species, mastitis can also be subdivided as either contagious (Staphylococcus aureus, Streptococcus agalactiae, Mycoplasma bovis, coagulase-negative staphylococci, Corynebacterium sp., etc) or environmental (Escherichia coli and coliforms, Streptococcus uberis, Streptococcus dysgalactiae, Enterococcus sp., etc).

Objectives

The overall objective of this study is to compare the results of detection of mastitis-associated bacteria by classical bacteriology and by quantitative (q) PCR.

Methods

A total of 120 milk samples were collected from mammary gland quarters with high cell counts (> 300,000 Somatic Cell) between January and March 2014 in seven farms in Wallonia. Samples were inoculated onto Columbia 5% sheep blood, McConkey’s, Chapman’s and modified Edwards’ agar plates. Isolated colonies were picked up and stored at -80°C till further use. After new growth, colonies were Gram stained and identified using appropriate API sets.

Conclusions

A total of 231 isolates grew on Columbia blood agar. Of them, 133 isolates also grew on Chapman’s agar including 63 S. aureus and 63 CNS; 70 isolates on Edwards’ agar including 14 S. agalactiae, 12 S. uberis, 6 S. dysgalactiae and 10 Enterococcus sp.; and 15 isolates on McConkey’s agar including 3 E. coli, 3 Klebsiella sp. and 8 Pantoea sp. These results are being compared with the results of the qPCR detection assay.
Background
Mastitis is one of the most frequent and costly disease in dairy cattle caused by different bacteria.

Objectives
The purpose of this study was to determine and compare the prevalence of different staphylococci, their antimicrobial susceptibility and toxin production, obtained from mammary infected cows in Kosovo.

Methods
Total 152 cow’s samples with clinical mastitis were collected. Cows were of different breeds and different lactation. Eight different commonly used antibiotics were analyzed for antimicrobial activity against staphylococci while SET-RPLA kit for enterotoxin detection.

Conclusions
Our results showed that, staphylococci were present in 89 out of 154 cows from which 58 were coagulase negative and 31 coagulase positive. *S. aureus* was most dominant (27/89) followed by *S. epidermidis* (25/89) and *S. chromogenes* (15/89). Interestingly, staphylococci where found more frequent in some dairy cow breeds than others. Most of the strains (76/89) were resistant to two or more different antibiotics. The highest resistance was observed against penicillin and ampicillin (>65%). While the lowest resistance was against erythromycin (<3%). Regarding the enterotoxin production, 40 out of the 89 strains produced at least one of the toxin types with SEA and SEC being the most common. In Kosovo is very common antibiotic misuse because of lack of local laboratories, hence the study suggests that, the uncontrolled use of antibiotics in treatment of different infective diseases might cause resistance against many antimicrobials. Therefore, attention should be paid on selection of antibiotics; timing of treatment, dosage and the uncontrolled and careless use of antibiotics should be more strictly regulated.
MALDI-TOF AS A TOOL IN IDENTIFICATION OF AVIAN MYCOPLASMA SPECIES
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Background

MALDI-TOF is a key method in identification of microorganisms. Large databases of reference and field strains have been made commercially available. Identifying *Mycoplasma* species has always been a time-consuming process using serological tests and molecular methods which require a large number of primers.

Objectives

Presently, only 3 avian *Mycoplasma* species are available in commercial databases. To evaluate if MALDI-TOF is a useful tool, first a larger database needed to be constructed. Secondly, strains isolated from infected birds were compared and matched against the database.

Methods

Fourteen reference and 8 field strains, obtained from collections held at our facility and from the University of Liverpool were grown in broth. An ethanol/formic acid extraction method was performed on each strain and then spotted on a MALDI target plate. For each strain, minimum 20 mass spectra of high quality were acquired to create the MSP using MALDI Biotyper 3.1 software. Forty-nine clinical isolates from poultry and wild birds were matched. Twenty-seven were correctly identified with a matching score of ≥ 2.0, 6 with a score between ≥ 1.7-1.99 and 16 remained unidentified.

Conclusions

MALDI-TOF is a rapid and useful tool in identifying *Mycoplasma* species. Fourteen isolates did not match probably due to missing strains in the database or possible unknown *Mycoplasma* species. More clinical isolates will be tested in the future and all non-identified isolates will be verified with 16S sequencing.

Acknowledgments

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Background

Bovine mastitis caused by *Prototheca* can assume high significance because of economic losses and the potential risk to public health. Many studies highlighted the high level of antibiotic resistance of *Prototheca*, as well as the unsuccessful therapies.

Objectives

To determine the *in vitro* susceptibility of *Prototheca* isolates to different antibiotics and alternative treatments based on essential oils.

Methods

Sixteen isolates of *Prototheca* were identified by multiplex PCR reaction, typed using RAPD-PCR, and tested by the disk diffusion method for their susceptibility to 28 antibiotics. The efficacy tests to essential oils (thymol, carvacrol and cinnamaldehyde) were performed using emitters releasing compounds in a controlled way in jars, containing the *Prototheca* strains streaked on Sabouraud agar.

Conclusions

All the strains were resistant to Amikacin, Ampicilin, Aztreonam, Cefepime, Ceftazidime, Chloramphenicol, Ciprofloxacin, Erythromycin, Fosfomycin, Imipenem, Levofloxacin, Meropenem, Mupirocin, Nitrofurantoin, Oxacillin, Penicilin G, Piperacillin, Quinupristin/Dalfopristin, Rinfamicin, Streptomycin, Tetracycline, Ticarcillin/clavulanic acid, Tobramycin and Vancomycin. In contrast, Colistin sulphate, Gentamicin, Kanamycin and Netilmicin were effective against all the strains tested.

The growth of *P. zopfii* genotype 1-2, and *P. blaskeae* was completely inhibited by thymol, carvacrol and cinnamaldehyde.

The results of the study revealed 4 out of 28 antibiotics tested efficient against *Prototheca* spp. while all the strains were inhibited by the tested essential oils.
Thus, *in vivo* studies are needed to evaluate if these compounds could rise as alternative treatments for bovine mastitis caused by *Prototheca* spp.
Background

*Vibrio salmonicida* is the cause of cold-water vibriosis in farmed Atlantic salmon. The disease manifests as a hemorrhagic septicemia, with a mortality ranging from 3-90 %. In the 1980s, the disease was a major challenge to the Norwegian aquaculture industry, but it is now being effectively controlled by the use of oil-based vaccines. Nevertheless, in recent years there have been some reports of disease outbreaks. In 2013, 13 Norwegian fish farms were diagnosed with cold-water vibriosis, all localized in the three northernmost counties of Norway.

Objectives

The knowledge on virulence factors of *Vibrio salmonicida* and the subsequent immune response of the fish is limited. As bacteria adapt to their current environment, a bacterium isolated from a diseased fish will not be identical to the same species cultivated *in vitro*. The aim of this study was to explore the phenotype of bacteria present *in vivo*.

Methods

We have grown *Vibrio salmonicida* in semi-permeable implants in live fish and analyzed bacterial protein expression by two-dimensional gel electrophoresis and tandem mass spectrometry. The experiment was approved by the Norwegian Animal Research Authority (approval no. ID6228).

Conclusions

Our findings may contribute to the knowledge on disease progression in cold-water vibriosis, as well as to provide novel targets for more effective vaccines. Also, the
study may provide insights in the course of disease for other bacterial infections in fish.
Background

MALDI-TOF-mass spectrometry (MS) has emerged as a powerful technique for the routine identification of microorganisms in clinical microbiology laboratories allowing an easier and faster diagnosis than conventional phenotypic and molecular methods. We have evaluated the suitability of MALDI-TOF for the rapid identification of *Lactococcus garvieae* the etiological agent of lactococcosis, a septicemic infection affecting different wild and farmed fish species, and recognized also as an opportunistic emerging human pathogen.

Objectives

Assessment of the reliability of MALDI-TOF MS for the identification of *Lactococcus garvieae*

Methods

A total of 35 isolates from different host and environments were obtained from the culture collection of VISAVET were used in the study. The identification results obtained by MALDI TOF were compared with those obtained by biochemical identification (API 32 Strep) and by a species-specific PCR assay (Zlotkin et al. 1998. J Clin Microbiol 36, 983-985). Mass spectra acquisition and analysis was performed on a Bruker UltraFlextrem platform (Bruker Daltonics) using MALDI Biotyper™ 3.0 software in the automatic mode using a matrix of saturated solution of α-HCCA. In addition, a subset of isolates was subjected to PFGE.

Conclusions

The majority of peaks were obtained in the range from m/z 2000 to 10000. The proteomic results matched with those of genotypic approach. Our results also showed some differences in MS spectra in *L. garvieae* isolates recovered from different origins_hosts suggesting the possible identification of molecular biomarkers for *L. garvieae*. Our study demonstrates that proteomics identification using MALDI TOF MS could be a reliable approach for identifying and discriminating this microorganism.
IDENTIFICATION OF ARCANOBACTERIUM PLURANIMALIUM BY MALDI-TOF MS, BY SEQUENCING 16S RDNA AND GENE PLA AND BY DEVELOPMENT OF A LOOP-MEDIATED ISOTHERMAL AMPLIFICATION (LAMP) ASSAY

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Background

Genus Arcanobacterium consists of four species namely A. pluranimalium, A. haemolyticum, A. phocae and A. hippocoleae. More recently, A. canis and A. phocisimile were described as novel species. A. pluranimalium was initially recovered from a harbour porpoise and a fallow deer, from a dog with pyoderma, from abortion material, semen, abscesses, viscera, navel illness and peritonitis of sheep and from milk samples of cows with mastitis. More recently, an A. pluranimalium strain was recovered following necropsy of a juvenile giraffe. However, the clinical importance of A. pluranimalium in animal infection remains unclear.

Objectives

Because of this widespread presence of A. pluranimalium in clinical samples of various animals there is an urgent need of phenotypic and genotypic tests for a rapid and reliable identification of this bacterial species.

Methods

The A. pluranimalium strains investigated in the present study were analysed phenotypically, by MALDI-TOF MS analysis and by 16S rDNA sequencing. In addition, the previously sequenced gene pla encoding pluranimaliumlysin of A. pluranimalium was used for molecular identification of this bacterial species and for the development of oligonucleotide primers for a loop-mediated isothermal amplification (LAMP) assay.

Conclusions

Phenotypic methods, MALDI-TOF MS analysis, sequencing 16S rDNA and target gene pla and the newly designed pla LAMP assay of the present study allowed the identification of all nine investigated A. pluranimalium strains of ovine and bovine
origin and from a juvenile giraffe. This phenotypic and genotypic approach might help to elucidate the role this species plays in animal infections.
Background

Enzootic bovine leucosis was first reported by serological method in Korea as early as in 1980s and recent prevalence in dairy cattle was over 50%. So far there has been no report on the bovine leukemia virus (BLV) genotype circulating in Korea since partial env gene sequence which belonged to genotype 1 was deposited in 2009.

Objectives

This study was performed to identify the genetic diversity of BLV in Korea.

Methods

Total 185 whole blood samples were collected from dairy cattle in two farms in southeast region in Korea. BLV gp51 genes were amplification for diagnosis as described previously by Fechner et al. Gp51 full gene (903bp) for sequencing was amplified. The gp51 PCR products were purified, sequenced and analyzed using DNAstar program and Mega 6 Program.

Conclusions

Total 185 samples were analyzed with PCR, and total 78 samples were positive for BLV env DNA nested PCR (37 samples out of 119 in farm A and 41 out of 66 in farm B). Further PCR reaction to amplify full gp51 gene was performed. 19 out of 37 positive samples from farm A and 31 out of 41 positive samples from farm B were amplified successfully. The genotype of sequences from farm A was divided into two types, genotype 1 and genotype 3. All 31 isolates from farm B were found to belong to genotype 1. This is the first report of BLV genotype 3 circulation in Korea.
Background

The Severe Fever with Thrombocytopenia Syndrome (SFTS) caused by SFTS virus (SFTSV) which belongs to the *phlebovirus* in bunyaviridae family is an emerging tick-borne mediated infectious disease for human, and the fatality rate is 12% in China. SFTSV seems to have wide host range in that SFTSV positive ticks were isolated from farm animals and wild rodents. Therefore, it is important to monitor SFTSV positive animals to prevent circulation or transmission of SFTSV.

Objectives

As there is no gold standard method to detect SFTSV specific antibodies from field animals, we are developing competitive enzyme-linked immunosorbent assay (c-ELISA) technique using nucleocapsid protein (NP) of SFTSV.

Methods

The recombinant NP of SFTSV was obtained by the expression of cloned genes in *E. coli* and the SFTSV was amplified in Vero cells for 7 days. The purified recombinant protein or formalin-inactivated SFTSV was used to immunize laboratory animals. Mono- and polyclonal antibody were tested for their antigen specificity in immunofluorescence assay (IFA) and western blot.

Conclusions

We developed direct c-ELISA using SFTSV specific monoclonal antibody, and generated the SFTSV positive serum from immunized cattle. The c-ELISA result shows 98.08% agreement in IFA result using 418 cattle field serum samples. We also observed the cELISA is applicable to detect SFTSV positive sera from immunized cattle, chicken and goat. This technique could be usefully used as a serological diagnostic method for various species of SFTSV susceptible field animals.
Background

*Mycoplasma bovis* is a cattle pathogen causing broad spectrum of diseases including mastitis, pneumonia and arthritis. The infection is very difficult to control due to long-time persistence of the organism in asymptomatic carriers, lack of knowledge on the specific role of environmental factors in the development of clinical disease, poor response to antibiotic treatment and the lack of effective immunotherapy.

Objectives

Here we report whole-genome sequence analysis of 41 *Mycoplasma bovis* isolates collected during recent outbreak of disease in Danish cattle herds. Our objectives were to determine the extent of genomic diversity in the test population, identify dominant clones, reconstruct their phylogenetic ancestry and infer transmission history.

Methods

Genome sequencing was performed on an Ion Torrent PGM sequencer. Genomic libraries were prepared by using Ion Xpress Plus fragment library kit and amplified using Ion PGM Template 200 OT2 kit according to manufacturer’s instruction. Raw sequence reads were pre-processed using FastQC, mapped to reference genome (PG45) using Burrows-Wheeler aligner and single nucleotide polymorphisms (SNPs) were identified using REALPHY server.

Conclusions

Unique molecular markers were identified for individual strains. Phylogenetic clustering based on SNPs revealed two distinct clades indicating a recent common ancestry. Substantial variations in both the number and sequence identities of individual variable surface proteins were observed with respect to type strain PG45 that may have contributed to increased virulence. The genetic distances inferred from SNPs and temporal distances (sampling dates) showed a strong
negative correlation suggesting that introduction of strains into the herds took place much before sampling time.
Background

Bovine digital dermatitis (BDD) and Contagious ovine digital dermatitis (CIDD), are infectious foot diseases causing severe lameness in cattle and sheep, respectively. The causative agents of digital dermatitis (DD) in dairy cattle are considered to be Treponema bacteria, however little work has been done regarding DD in beef cattle. Similarly, although it is suggested CIDD emerged from BDD little has been done to investigate the aetiology of the disease in sheep or the transmission of DD.

Objectives

1. Isolate and characterise beef cattle and sheep DD treponemes from lesions and compare with dairy cattle DD treponemes.
2. Identify reservoirs of infection of DD.

Methods

Beef cattle and sheep DD lesions were subjected to culture and PCR analysis for the commonly detected DD treponemes from dairy cattle lesions.

The gastrointestinal (GI) tract of beef cows and sheep was investigated as an infection reservoir of DD treponemes by analysing GI tract tissues for the presence of treponemes. Farm equipment which comes into contact with lesions was analysed by PCR and culture methods for the presence of treponemes.

Conclusions

From 16S rRNA gene analysis, treponemes isolated from beef cattle and sheep lesions belonged to the groups of treponemes associated with dairy cattle lesions. The close similarity poses concerns for species-species transmission.

Several GI tract tissues were positive for DD treponemes, providing evidence towards the GI tract as an infection reservoir for DD treponemes. Additionally, farm equipment
was also found to harbour DD treponemes after coming into contact with an infected foot indicating a possible transmission route.
Background

Campylobacter jejuni is a Gram-negative microaerophilic bacterium, and is the major cause of human gastro-enteritis worldwide. It is a zoonotic pathogen and chickens are generally accepted as the most important source of infections. Since the use of antibiotics in animal feeds is no longer an acceptable option and the prevention is limited to hygiene measures, novel control methods are needed.

Objectives

The purpose of this research is to investigate whether colonization of chickens by C. jejuni can be controlled by passive vaccination in a cost effective manner by using nanobodies, the antigen-binding domains of heavy-chain antibodies.

Methods

Anti-Campylobacter nanobodies were identified after immunization of an alpaca with heat-killed C. jejuni cells. The binding of these nanobodies on different C. jejuni strains, isolated from chickens and their environment as well as human clinical isolates, was verified. Nanobodies that are able to bind all of the tested strains were selected for further research. We presumed that these nanobodies recognize conserved epitopes expressed on C. jejuni. The ability of one of these nanobodies to agglutinate Campylobacter cells was tested. This nanobody was multimerised by linking it to magnetic beads. The results confirm that the nanobody recognizes antigens on living C. jejuni cells and that multimers of this nanobody are able to agglutinate the C. jejuni cells.

Conclusions
The nanobodies with a broad *Campylobacter* specificity can be interesting for diagnostic and therapeutic applications, such as for the reduction or inhibition of the colonization of chickens by *C. jejuni*. 
Background

- *Escherichia coli* (*E. coli*) is a bacterium that commonly lives in the intestines of people and animals. There are four major categories of diarrheagenic *E. coli*, namely: enterotoxigenic *E. coli* (*ETEC*), enteroinvasive *E. coli* (*EIEC*), enteropathogenic *E. coli* (*EPEC*) and enterohemorrhagic *E. coli* (*EHEC*). These categories of *E. coli* differ in their epidemiology and pathogenesis and their O: H serotypes and for the presence or absence of several genes responsible for their virulence.

Objectives

- The objective of the study was the analysis of virulent genes and biofilm production in *E.coli* strains isolated from animals with diarrhoeic problems.

Methods

The strains were isolated from stool samples of animals affected by diarrhoea. Genetic analysis were performed by multiplex PCRs. Biofilm assays were performed by safranin method with O.D. readings at 492 nm on 96 wells plate.

Conclusions

The isolates were all coming from symptomatic animals from farms in which repeated episodes of diarrhoea are present. All isolates were negative for the *aggR* gene (Aggregative adherence fimbria), the *pet* gene (Plasmid-encoded toxin), *aap* (dispersin) and other genes. 10 out of 35 were positive for *irp2* (yersinia bactin biosynthesis gene) and 18 out of 35 strains carried the *astA* gene which encodes for EAST1 (Entero Aggregative heat-StableToxin1). This protein is thought to play a role in EAEC pathogenicity even if not all EAEC strains harbour the *astA* gene. The analysis for biofilm formations on the first 22 isolates, showed low capability in biofilm formation. Only 8/22 showed values above 120 O.D., but further analysis will be performed with different stains.
FEMS-1392
Veterinary microbiology

DETERMINATION OF ANTIMICROBIAL RESISTANCE AND VIRULENCE GENES PATTERNS AMONG CAMPYLOBACTER FROM BOILERS IN POLAND

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Background

Campylobacter is the most common zoonotic agent deriving from broiler flocks and broiler carcasses in Poland. The increased resistance of this bacteria to antibiotics is a matter of special concern, representing a significant public health problem. The genetic features responsible for pathogenesis of campylobacteriosis are still under investigation.

Objectives

The aims of the study were to test Campylobacter strains for antimicrobial resistance patterns and to screen for the presence of virulence-associated genes involved in different steps of infection.

Methods

During the project, 29 broiler farms located in 15 voivodeships all over Poland were selected for sampling at the slaughterhouse level. In total, 252 isolates from caeca (150 C. jejuni and 102 C. coli) and 197 strains from the corresponding carcasses (142 C. jejuni and 55 C. coli) were tested. A microbroth dilution method was used to establish the antimicrobial resistance by the minimum inhibitory concentrations (MICs) to 7 antimicrobials. Additionally, the following genes: cadF, flaA, virB11, iam, flhA, cdtA, cdtB, cdtC, ciaB, docA and wlaN were detected by PCR.

Conclusions

The most common resistance profile of C. jejuni was ciprofloxacin-nalidixic acid and tetracyclines. C. coli were mostly resistant to ciprofloxacin-nalidixic acid-tetracycline and streptomycin. The results regarding virulence patterns demonstrated that the majority of C. jejuni strains had virulence and cytotoxin gene pattern: flaA, flhA, cadF, ciaB, cdtA, cdtB, cdtC and docA. Among C. coli, the simultaneous presence of the flaA, flhA, cadF, ciaB, cdtA, cdtB, iam and docA markers was most often found.
Background
Campylobacteriosis is the most common foodborne gastroenteritis in industrialized countries and the number of cases is growing continuously. This bacterial infection is mainly related to the consumption of poultry meat, when it is contaminated by Campylobacter jejuni. Although it is a known fact that C. jejuni has a highly prevalent in broiler flocks, the source of infection in herds remains still unclear. Nowadays, it would be interesting to knowledge the role of the different animals in the transmission of the infection.

Objectives
The objective of this research was to determine the prevalence of bacteria in different types of animal species.

Methods
Both birds and mammals remaining in direct or indirect contact with infected broilers for C. jejuni were analyzed. Individual animals were sampled by rectal swab and all samples were processed similarly, being the positive samples confirmed by PCR. This organism was isolated from all species that had been in direct contact with infected broilers, but it cannot be isolated from the animals not having direct contact with them.

Conclusions
According to the results obtained, it is concluded that C. jejuni can be transmitted between different animal species, acting as vector and/or reservoirs of this organism in broilers.
DETECTION OF HUMAN PAPILLOMAVIRUS DNA AND P53 CODON 72 POLYMORPHISM IN PROSTATE CANCER IN TURKISH POPULATION

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Background: The possible relationship between human papillomavirus infection, tumor suppressor gene p53 and prostate cancer (PCa) has been previously investigated, however, the etiological role of HPV infection and p53 codon 72 polymorphism in the PCa pathogenesis remains highly controversial.

Objectives: The aim of the study was to assess the possible role of human papillomavirus in the development of prostate cancer and the distribution of the p53 codon 72 polymorphism in Turkish population.

Methods: We investigated the presence of HPV in 60 formalin fixed and paraffin embedded prostate cancer tissues, as well as in 36 benign prostatic hyperplasia tissue (BPH) samples by using real-time PCR and pyrosequencing. Furthermore, the same group of patients were also screened for the presence of the Arg72Pro polymorphism of the p53 gene, a p53 polymorphism related to HPV.

Conclusions: No HPV DNA was detected while using real-time PCR in any of the 36 BPH samples. One of the sixty (1.67%) PCa samples was found to be positive for HPV DNA by using a real-time PCR. After using pyrosequencing analysis, the only HPV type detected in the PCa sample was HPV-57. Compared to the control group, Arg /Pro genotype was observed more frequently in prostate cancer patients (p=0.044), and also Pro allele frequency is higher in prostate cancer patients (p=0.021). Our findings do not support the involvement of HPV in the etiology of prostate carcinogenesis, and the proline allele could be a risk factor for the development of prostate cancer in the Turkish population.
Background

Viruses are the numerically dominant life forms on Earth. They represent a huge reservoir of genetic diversity, infecting organisms from small to large. In the oceans, every second $10^{23}$ viral infections take place.

Objectives

To fully comprehend the impact marine viruses can have on their host population dynamics and subsequently biodiversity, ecosystem functioning and global energy & matter fluxes, it is essential to study virus-host interactions under different environmental conditions.

Methods

Here I will present experimental results on physiochemical variables affecting algal virus growth characteristics, as well as field data showing how a changing ocean due to global warming can control the magnitude of viral lysis as compared to the traditional loss factor, grazing.

Conclusions

These examples illustrate the importance of environmental factors underlying the ecological importance of viral infection in marine ecosystems. Further research is warranted to identify more regulatory factors and understand the mechanisms by which they influence virus-host interactions.
Recombinant Lipidated Dengue Envelope Protein Domain III Induces Durabel Neutralizing Antibody Responses and Protective Immunity

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Background

The immunogen and immunopotentiator are two important components of vaccines. Combination of these two components into a single construct will have a great potential to increase the reactivity of immunogen. The lipid moiety of the recombinant lipoprotein provides a danger signal to activate antigen-presenting cells via toll-like receptor 2, which may further enhance immune responses.

Objectives

We selected dengue-4 envelope protein domain III (LD4ED III) as the immunogen and converted this candidate in lipidated form in Escherichia coli-based system. In the present study, the immunogenicity and protective immunity of LD4ED III in mice were evaluated.

Methods

Recombinant proteins were purified by immobilized metal affinity chromatograph columns. Groups of mice were immunized subcutaneously twice with 10 μg of D4ED III or LD4ED III in PBS at a 4-week interval. Mice immunized with PBS alone served as controls. Immune responses in mice were examined. All animal studies were approved and performed in compliance with the guidelines of the Animal Committee of the National Health Research Institutes.

Conclusions

The LD4ED III expressed in E. coli retains the proper conformation, which has the capability to compete with dengue virus for cellular binding sites. In addition, LD4ED III is more immunogenic than D4ED III to induce immune responses. Most importantly, LD4ED III elicits a durable neutralizing antibody response and inhibits viremia in LD4ED III vaccinated mice in the absence of exogenous adjuvant. These results suggest that lipidated dengue envelope protein domain IIIIs are potential dengue vaccine candidates.
Background

Obesity which develops due to multifactorial reasons, was associated recently with human Adenovirus-36 (Ad-36).

Objectives

The aim of this study was to investigate the prevalence of Ad-36 antibodies in obese adults and also investigate the DNA of Ad-36 in their adipose tissue.

Methods

In this cross-sectional and case-control based study, 49 obese adults (BMI ≥ 30 kg/m²) and 49 non-obese adults (BMI ≤ 25 kg/m²) were included in this study as patient and control groups, respectively. Adipose tissue samples obtained by the lipoaspiration method, were studied by PCR methods. Simultaneously, the presence of Ad-36 antibodies and serum leptin and adiponectin levels were assessed by serum neutralization assay (SNA) and ELISA, respectively.
Conclusions

Ad-36 antibody was detected in 6 (12.2%) of 49 patients by SNA and was statistically significant (p<0.05). We couldn’t detect Ad-36 DNA in adipose tissue; however, we detected significantly higher Ad-36 antibody levels in the obese group compared to the non-obese group. Mean BMI and leptin levels were higher in the Ad-36 positive group, while adiponectin levels were found to be lower in the Ad-36 positive group. Although no statistically significant difference was found in cholesterol and triglyceride levels between the two groups (p > 0.05), lower mean serum cholesterol and triglyceride levels were found in the Ad-36 positive patients. There is a need for extended serial, particularly cohort and human-based, studies in order to have a clear understanding of the Ad-36-obesity relationship.
PREVALENCE OF ASTROVIRUS IN FECAL SPECIMENS OF CHILDREN WITH GASTROENTERITIS

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Background
Norovirus genogroup I (NV GI) and NV GII sapovirus (SaV) genogroups I, II, IV, and V, human rotavirus A (HRV), adenovirus serotypes 40 and 41 (AdV), and human astrovirus (AstV) are the most common causes of viral gastroenteritis and result in large outbreaks of viral diarrhea. An estimated 1.8 million children die each year from largely preventable enteric illnesses, with the large majority of these mortalities occurring in developing countries. Gastroenteritis is a major cause of morbidity and mortality in children worldwide.

Objectives
Astroviruses are one of the major viral pathogens responsible for gastroenteritis. In this study performs to determine the prevalence of astrovirus in fecal specimens of children with gastroenteritis.

Methods
In this study 2490 stool samples from children less than five years with gastroenteritis from five cities of Iran were collected. The prevalence of human astroviruses was tested in patients with acute gastroenteritis by using conventional duplex reverse transcription (RT) -PCR and electrophoresis.

Conclusions
Young children were much more likely to be admitted with acute gastroenteritis. In this study astrovirus was detected in 1.6% of samples. There is no relation between age, sex and type of virus. rotavirus was detected in 50% of astrovirus positive samples. rotavirous was detected with ELISA. astroviruses are an important viral agents of gastroenteritis in Iran. Because of co-infection of astrovirus, improved personal hygiene and rotavirus vaccination will reduce the incidence of co-infections.
DEAMINASE ACTIVITY OF APOBEC3 IS REQUIRED FOR EFFICIENT RESTRICTION OF PORCINE ENDOGENOUS RETROVIRUS

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Background

Porcine endogenous retroviruses (PERVs) present a unique concern associated with xenotransplantation because they have been shown to infect certain human cells in vitro. Human APOBEC3G (huA3G) is a single-strand DNA cytosine deaminase, which inactivates the coding capacity of the virus by deamination of cDNA cytosines to uracils. This reaction occurs within (-) DNA strand during reverse transcription, resulting in G-to-A mutation in the (+) strand.

Objectives

A recent report showed that huA3G also could inhibit viral replication by cytidine deaminase-independent mechanisms. Two contrasting results with regard to huA3G-dependent inhibition of PERV transmission have been reported. Some group suggests that the mechanism of inhibition does not require the DNA deaminase activity of huA3G. In contrast, other group report that PERV restriction is attributed to cytidine deamination.

Methods

To determine whether DNA deamination is required for huA3G-dependent inhibition of PERV transmission, we established a new set of 293-PERV-PK-CIRCE (human 293 cells infected with PK15-derived PERVs) clones stably expressing huA3G. In addition to huA3G, we included porcine APOBEC3G(poA3Z2-Z3) and murine APOBEC3(mA3). 293T cells were infected with virus-containing supernatant from 293-PERV-PK-CIRCE clones expressing huA3G, poA3Z2-Z3 and mA3. To search for antiviral mechanism of APOBEC3, genomic DNA from 293T cells was prepared at 10h postinfection and the PERV gag gene from 3D-PCR(differential DNA denaturation PCR) product was sequenced.

Conclusions

huA3G, poA3Z2-Z3 and mA3 induced G-to-A hypermutations in the PERV genome effectively. Based on these results, we conclude that the APOBEC3 restricts PERV replication by deaminase-dependent mechanism.
IS THERE ANY ROLE OF ADENOVIRUS 36 AS A RISK FACTOR IN GYNECOMASTIA? THE FIRST CLINICAL STUDY WORLDWIDE FOR PATIENTS WITH GYNECOMASTIA

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Background

Gynecomastia was indicated as a diseases which develops due to multifactorial causes.

Objectives

Based on adipose tissue affinity of Ad-36, we hypothesized, Ad-36 may also play a role in the development of gynecomastia by possibly accompanying increased regional adiposity.

Methods

In this cross-sectional and case-control based study, 33 adult male (BMI ≤ 25 kg/m²) and 15 adult male (BMI ≤ 25 kg/m²) with anatomic disorders without gynecomastia pathology were included in this study as patient group (PG) and patient control group (PCG), respectively. Breast reduction samples obtained by the lipoaspiration/subcutaneous mastectomy method, were studied by PCR methods.
Simultaneously, the presence of Ad-36 antibodies and serum leptin and adiponectin levels were assessed by serum neutralization assay (SNA) and ELISA, respectively.

**Conclusions**

Ad-36 antibody was detected in 8 (24.2%) out of 33 PG by SNA and was statistically significant (p<0.05). Mean leptin levels were higher in the Ad-36 positive group, while adiponectin levels were found to be lower in the Ad-36 positive group. We couldn't detect Ad-36 DNA in breast reduction samples; however, we detected significantly higher Ad-36 antibody levels in the PG compared to the PCG. Ad-36 antibody positivity stayed in models in multivariate logistic regression analysis (with backward modeling) and has been identified as a risk factor. We report firstly Ad-36 may be a risk factor in gynecomastia. Moreover, with regard to leptin, adiponectin and serum cholesterol levels, our results suggest that there might be a relationship between Ad-36 and gynecomastia.
FEMS-2735
Viral infections and host

THERAPEUTIC POTENTIAL OF A NEW KLEBSIELLA PNEUMONIAE BACTERIOPHAGE: ISOLATION, GENETIC CHARACTERIZATION AND PHAGE DEVELOPMENT IN SLOWLY GROWING BACTERIA
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Background

*Klebsiella pneumoniae* is a facultative anaerobic Gram-negative bacterium associated with pyoderma in animals. *K. pneumoniae* can acquire resistance to carbapenems, thus becoming insensitive to almost all available antimicrobial agents. Phage therapy has the potential to replace antibiotic treatment. Lytic bacteriophages and/or their gene products such as lysins, can be easily used as therapeutic agents against bacteria as they are host specific and generally show no side effects.

Objectives

We focused on isolation and characterization of new bacteriophages against *Klebsiella pneumoniae*, which can be potentially used in phage therapy in animals.

Methods

We collected *K. pneumoniae* swab samples from the infected animals that could not have been treated with a standard antibiotic therapy. Afterwards, new lytic phage PRA33 was isolated from sewage and, with the use of electron microscopy analysis, it was structurally defined as a member of the *Siphoviridae* family. Phage plaque morphology analysis (the plaque clarity and the ability to increase its diameter in time) and determination of phage host range were carried out. Then, analysis of kinetics of adsorption and one-step growth curves were determined in bacteria growing with slow growth rates in a chemostat system. We also performed phage stability tests in various liquid media. Phage genome was sequenced *de novo* and the identification of putative ORFs was undertaken.

Conclusions
Based on the results of our experiments, we propose PRA33 phage to be a promising candidate for application in phage therapy against *K. pneumoniae* causing infections in animals that are otherwise difficult to treat.
Background

Chikungunya (also named breakbone fever) is a highly emerging disease in many tropical settings with great socioeconomic impact. Chikungunya-Viruses are transmitted to humans by bloodsucking mosquitoes (Aedes aegypti, Aedes albopictus). The symptoms of Chikungunya include fever which can reach 39°C (102,2°F) a petechial or maculopapular rash usually involving the limbs and trunk, and arthralgia or arthritis affecting multiple joints which can be debilitating. The fever typically lasts for two days and abruptly comes down. Alphaviruses can be noticed as import or travel associated infection.

Objectives

The aim of this work was to develop an serological assay to detect IgG and IgM antibodies against Chikungunya and to evaluate in endemic outbreak settings.

Methods

An IgG-capture and IgM-capture ELISA was developed. Both take advantage of native antigens produced with a proprietary technique. In house measurements as well as external evaluations in many endemic regions of the world conducted by well know tropical institutes revealed excellent clinical sensitivity and specificity as well as high positive and negative predictive values. Data from the current outbreak in the Caribbean will be discussed.

Conclusions

The newly developed ELISA seems to be a superior tool to diagnose past and acute Chikungunya infection in common and outbreak settings all over the world. It will assist diagnosis of travel returners with unknown fever as well as military in endemic operation area. To further improve Chikungunya diagnostic a Lineblot is currently under development as tool for conformation of ELISA results as well as for small labs with limited lab equipment.
VACCINE-TYPE ATTENUATING MUTATIONS IN HIGHLY PASSAGED STRAIN OF VARICELLA ZOSTER VIRUS

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Background: Varicella-zoster virus (VZV) is causative agent for chickenpox and zoster. Live attenuated vaccines have been developed based on Japanese Oka strain and Korean MAV/06 strain. Comparison of the complete sequences between vaccine and wild-type strains suggested at least 24 mutations important for attenuation in vaccine strains. Certain strains with high in vitro passage history such as the strains Ellen and 32p72 contained some of the vaccine-type mutations.

Objective: In this study, we attempted to understand vaccine-type attenuating mutations in highly passaged VZV strains.

Methods: Genome sequences of clinical strains with different passages were determined by next-generation sequencing and compared with those of other VZV strains.

Conclusion: Vaccine-type mutations were found at position 106262 (T→C) in the Korean clinical strain YC01 passage 32 and at positions 560 (T→C), 105169 (A→G), 106262 (T→C) and 107252 (T→C) in YC01 passage 61. Similar mutations were also found in high-passaged another clinical strain YC02 at positions 560, 106262 and 107252. Same mutations at 106262 and 107252 were found in the strains Ellen and 32p72. Thus, in the course of in vitro passaging of VZV, mutations at 106262 and 107252 seemed to occur first, and followed by mutation at 560. Direct PCR sequencing of these positions in YC01 and YC02 strains at various passages identified the passage numbers when the attenuating mutations occurred. Further studies of in vitro passaging under attenuating conditions such as low temperature or guinea pig cells will help to understand the mechanism of attenuating mutations in VZV.
FEMS-0929
Viral infections and host

LIPIDATED DENGUE-2 ENVELOPE PROTEIN DOMAIN III INDEPENDENTLY STIMULATES LONG-LASTING NEUTRALIZING ANTIBODIES AND REDUCES THE RISK OF ANTIBODY-DEPENDENT ENHANCEMENT
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Background
Dengue virus is a mosquito-transmitted virus that can cause self-limiting dengue fever, severe life-threatening dengue hemorrhagic fever and dengue shock syndrome. The existence of four serotypes of dengue virus has complicated the development of an effective and safe dengue vaccine. Recently, a clinical phase 2b trial of Sanofi Pasteur’s CYD tetravalent dengue vaccine revealed that the vaccine did not confer full protection against dengue-2 virus. New approaches to dengue vaccine development are urgently needed. Our approach represents a promising method of dengue vaccine development and may even complement the deficiencies of the CYD tetravalent dengue vaccine.

Objectives
To develop a vaccine with long-lasting neutralizing antibodies and reduces the risk of antibody-dependent enhancement.

Methods
Two important components of a vaccine, the immunogen and immunopotentiator, were combined into a single construct to generate a new generation of vaccines. We selected dengue-2 envelope protein domain III (D2ED III) as the immunogen and expressed this protein in lipidated form in Escherichia coli, yielding an immunogen with intrinsic immunopotentiating activity.

Conclusions
The formulation containing lipidated D2ED III (LD2ED III) in the absence of exogenous adjuvant elicited higher D2ED III-specific antibody responses than those obtained from its nonlipidated counterpart, D2ED III, and dengue-2 virus. In addition, the avidity and neutralizing capacity of the antibodies induced by LD2ED III were higher than those elicited by D2ED III and dengue-2 virus. Importantly, we showed that after lipidation, the subunit candidate LD2ED III exhibited increased immunogenicity while reducing the potential risk of antibody-dependent enhancement of infection in mice.
Background:

Enterovirus 71 (EV71), a positive-stranded RNA virus, is the major cause of hand, foot and mouth disease (HFMD) with severe neurological symptoms. Seven-day-old toll-like receptor 9 knockout (TLR9KO) mice infected with a non-mouse adapted EV71 strain developed neurological lesion-related symptoms, including hindlimb paralysis, slowness, ataxia and lethargy. Histopathological examination revealed a massive damage in the limb muscles, brain, and spinal cord. The TLR9KO mouse could be useful as EV71 animal model to study anti-EV71 drugs and vaccines.

Objectives:

A novel nanoparticle-based EV71 VP1 peptide vaccine was evaluated in the EV71 mouse model. The clinical symptoms of mice after EV71 infection were monitored.

Methods:

Seven-day-old TLR9KO mice were infected with non-mouse adaptive EV71 and showed neurological diseases. We found that EV71 alone cannot activate toll-like receptor 9 (TLR9) signaling. On the contrast, supernatant from EV71-infected cells could activate TLR9. Furthermore, we identified the EV71-infected cells-derived danger signal activate TLR9 to protect mice from EV71 challenge. To investigate whether TLR9KO mice could be used as an animal model for EV71 vaccine development, EV71-infected TLR9KO mice were treated with sera from vaccinated mice. We designed a VP1 peptide of EV71 that formulated with emulsion type nanoparticles adjuvant and immunization with wild type mice. Sera from immunized mice were administered to EV71-infected TLR9KO mice, and clinical neurological symptoms of mice were observed.

Conclusions:

Sera from EV71 VP1 peptide immunized mice could reduce neurological symptoms of TLR9KO mice from challenged with EV71.
FEMS-1230
Viral infections and host

ALGAL VIRUS PRODUCTION IS NEGATIVELY AFFECTED BY PHOSPHORUS AND NITROGEN LIMITATION, BUT THIS MAY BE COUNTERACTED BY NUTRIENT ASSIMILATION DURING INFECTION

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Background
Viruses are a main cause of phytoplankton mortality, thereby controlling their community composition and driving biogeochemical cycling in the marine environment. Phytoplankton growth and production is often limited by nitrogen (N) and/ or phosphorus (P), which can affect the virus growth characteristics upon infection. The influence of nutrient limitation on phytoplankton – virus interactions are, however, still poorly studied. As climate change will lead to an expansion of stratified and consequently nutrient limited oceanic regions, it is important to understand how phytoplankton mortality rates in the future marine environment will be affected by these changes.

Objectives
Our goal was to study the effects of N- and P-limitation on virus host interaction and the potential relieve of these effects by the uptake of nutrients during viral infection.

Methods
Therefore, cultures of the the phytoplankton species *Micromonas pusilla* and *Phaeocystis globosa* were grown semicontinuously under limitation of either P, N, or NP until the moment of infection. Upon infection the abundances of host cells and viruses were monitored to obtain one-step growth curves.

Conclusions
Nutrient limitation led to elongated viral latent periods (up to 3-fold) and strongly reduced (up to 8-fold) burst sizes, whereby the outcome was dependent on both the species and the limiting nutrient involved. Furthermore, the negative effects of P-limitation on virus production in the picophytoplankter *M. pusilla* could be overcome by spiking the infected and P-limited cultures with small pulses of inorganic and organic P, simulating bacterial remineralization and supply by lysis of neighboring cells, respectively.
SAFFOLD VIRUSES IN PEDIATRIC PATIENTS WITH DIARRHEA IN THAILAND

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Background

In recent years, several new viruses associated with diarrheal diseases in human have been reported. Saffold virus (SAFV) is one of a newly identified human Cardiovirus reported for the first time in 2007.

Objectives

This study investigated the prevalence of SAFV and characterized the genotypes distributed in children with diarrhea in Chiang Mai, Thailand during 2012-2013.

Methods

A total of 608 fecal specimens collected during January 2012 to December 2013 from children with diarrhea in Chiang Mai, Thailand were investigated for SAFV by RT-nested PCR and performed sequence analysis.

Conclusions

Nine out of 608 (1.5%) were positive for 4 genotypes of SAFV, SAFV1, SAFV2, SAFV3, and SAFV4. SAFV mono-infection was found in 5 cases, while co-infection with other viruses that cause diarrhea was observed in 4 cases. This study provides more information about SAFV genotypes circulating in pediatric patients with diarrhea in Thailand.
FEMS-2782
Viral infections and host

THE CYTOKINES EXPRESSION IS MODULATED BY EPIGENETIC MECHANISMS IN RESPONSE TO INFECTION WITH IPNV
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Background

The massive salmon farming associated to aquaculture industry has brought a significant increase in infectious diseases, thereby generating economic losses. The infectious pancreatic necrosis virus (IPNV), agent of high mortalities in fish farmed, has capacity to activate the immune response mediated by interferon in their host; the aim of this study is clarify whether the activation of the antiviral response caused by IPNV is through epigenetic mechanisms.

Objectives

We observed that expression of cytokines, in salmonids cells infected with IPNV was associated to promoter methylation status. Moreover, DNA methyltransferase inhibitors treatments were able to modulate the expression of these cytokines.

Methods

Studies of methylation in CpG islands of the promoters of cytokines in infected salmonids cells with IPNV were conducted. Through bisulfite sequencing, change in methylation of the promoter affecting the expression of interferon was evaluated. Furthermore, treatments with DNA methyltransferase (DNMT) inhibitor 5-azacytidine and 5-Aza-2'-deoxycytidine were performed to evaluate the expression of cytokines.

Conclusions

These results show that cytokines expression is regulated by promoter methylation status which could be correlated with the modulation of DNMT activity when cells are infected with IPNV. Our work suggested that epigenetic mechanisms could participate in the regulation of host genes expression in IPNV infection, and provided a new insight into understanding the mechanisms of viral infection.
FEMS-2475
Viral infections and host

EPIDEMIOLOGICAL, CLINICAL MANIFESTATION AND GENOTYPE CHARACTERIZATION OF HEPATITIS-C VIRUS AMONG THE HIV PATIENTS IN KASHAN
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Background
Due to shared transmission routes, HCV infection is highly prevalent among people infected with human immunodeficiency virus (HIV). Little is known about the characteristics of HCV genotypes in among HIV/HCV coinfected patients in Kashan

Objectives
In order to provide valuable information for HCV management in this particular population, we investigated the HCV genotypes in HIV-infected In Kashan-Iran.

Methods
This study was conducted in 2013 in kashan, Iran. The study population consisted of all HIV infected having records in behavioural concelling center and prison in Kashan. Demographic information and HCV, HIV-related risk behaviours were obtained through an interviewer–assisted questionnaire. After taking consent form, 10 cc blood taken from HIV patients and serum samples were screened for HCV infection using enzyme-linked immunosorbent assay (ELISA), and detection of HCV RNA was performed by PCR amplification. HCV subtypes were determined by direct sequencing of amplicons

Conclusions
54(85%) out of the 63 HIV infected patients, were HCV positive which all of them were male..there was a significant correlation between HCV frequency and occupation (P<0.0001) and level of education (p<0/05). 100% of HIV/HCV coinfected cases had history of illicit drug use,92.6% history of prison and and 40.7% had high risk sexual behavior. There was found genotype 1 in 75.9% and genotype 3 in24.1% of HCV patients .94.4% of HCV patients had subtype a. The mean of AST,ALT,TLC,CD4 in HCV patients were 50.6,58.5,397.2,398.8. There was no clinical symptom of chronic Hepatitis c.
The majority of HIV infected persons in Kashan city are HCV positive and predominant genotype is 1 and predominant subtype is a.
PREVALENCE OF HEPATITIS C IN INJECTING DRUG USERS IN KASHAN -IRAN

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Background
Drug injection is a very important risk factor for viral hepatitis and human immunodeficiency virus (HIV) infection

Objectives
The present study was performed to evaluate the prevalence of hepatitis C among injection drug users (IDUs) and to identify the related risk factors for these infections in this group.

Methods
This descriptive-analytical study was conducted in 2011 in kashan, Iran. The study population consisted of 300 IDUs in MMT, DIC and counselling centers. Demographic information and HCV related risk behaviors were obtained through an interviewer-assisted questionnaire. IDUs serum samples were screened HCV infection using enzyme-linked immunosorbent assay (ELISA). Data analyzed using Spss.

Conclusions
Of the 300 IDUs, 288(96\%) were male. The majority of IDUs 127(42.3\%) were in 30-39 age group with mean age 34.9±9.7. The majority of IDUs 224(74.7\%) had more than 10 years history of addiction. The most common age of onset addiction was 15-20 year 134(44.7\%). The prevalence of HCV was 142(47.3\%). It was found that there was a significant correlation between using shared syringe, age and times of prison and HCV infection.

There was high prevalence of HCV among IDU. High risk behaviors such as tattooing, unsafe sex, needle sharing are common so regular screening of IDU, education of personal Health about using sterile syringe, HBV vaccination and treatment of addiction and HCV infection is recommended.
DETECTION AND PHYLOGENETIC ANALYSIS OF TORQUE TENO VIRUS (TTV) AMONG BLOOD DONORS AND HBC OR HBV-INFECTED PATIENTS IN QATAR

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Background
TTV has been linked to non A-G hepatitis

Objectives
The aim of this project was to investigate the rate of infection and to determine the genotypic features of TTV in Qatar

Methods
A total of 644 blood samples representing different nationalities, non-Qatari (526) and Qatari (118) nationals (mostly from Arab and South East Asia countries) were studied for the presence of TTV DNA by nested PCR. The majority (573) of the blood samples belonged to healthy blood donors, while 53 and 54 of the blood samples belonged to hepatitis C virus (HCV) and hepatitis B virus (HBV)-infected patients, respectively.

Conclusions
Results: we showed that the infection rates of TTV in blood donors, and those HCV or HBV-infected patients were 81.4%, 84.9% and 90.75%, respectively. Significant association found between TTV viremia and nationality, or age was detected. Sequence analysis of PCR fragments amplified from the 5'-untranslated region (5'-UTR) of all (531) TTV positive samples showed that 65.5% (348/531) of the PCR fragment sequences were classified into main genotype 3, followed by genotype 5 (24%), 2 (5.8%) and 1 (4.7%). Interestingly, genotype 4 was not detected among the studied subjects. Phylogenetic and pairwise analyses using sequences from TTV viremic samples also showed an overall close similarity to the main genotype 3. In conclusion, there was no significant difference in the detection rates of TTV among Qatari nationals and non-nationals, and most TTV genotypes, particularly genotype 3, were detected.
Background
Human papillomavirus (HPV) have epithelial and mucosal tropism. HPV type 17 belongs to cluster $b_2$ of Betapillomavirus. Distinct subtypes with dissimilarities of 2 to 10% at the nucleotide level from their reference HPV can be identified. Molecular cloning experiments enabled to recognize two subtypes (17a and 17b) isolated from epidermodysplasia verruciformis (EV).

Objectives
The aim of this study was to investigate a possible novel subtype of Betapapillomavirus HPV type 17.

Methods
The isolate was collected from an oral mucosa of an asymptomatic woman. Partial L1 region was amplified using My09/11 degenerated primers. The complete E6, E7, and LCR genes were amplified with specific primers. The Ethics Committee of the College of Medicine at the University approved the protocols for collection and informed consent.

Conclusions
The viral DNA was sequenced and partially characterized. Within these sets, the DNA sequence was altered at 38 positions (15 in L1, 13 in E6, 8 in E7, and 2 in LCR gene). Partial L1 analysis showed high dissimilarity facing to prototype, reaching 5% of nucleotide substitutions. The E6 oncoprotein presented the highest modification among the sequences studied. The amino acid modified at position 62 (S-T) affected one zinc binding domain (CxxC(C)29 CxxC) of this protein. E2 binding factors, transcription factors, and TATA signals were seen in LCR region. Despite the EV-associated HPV 17 has cutaneous tropism, the oral cavity is an appropriated niche to host HPV which naturally infects other sites. Based on these findings, we believe to have found a novel subtype of this type.
Background
Rotavirus (RV) is a primary cause of acute diarrhea in infants and young animals, and despite attenuated RV vaccines have been able to reduce the global disease burden associated with RV gastroenteritis, this infectious disease is still a problem in developing countries. Several reports indicate that immunobiotics have the ability to reduce severity of RV mediated diarrhea. Previously, we demonstrated that some immunobiotic strains were able to beneficially modulate Toll-like receptor (TLR)-3 mediated immune response in porcine intestinal epithelial (PIE) cells.

Objectives
To evaluate the capacity of immunobiotics to modulate RV-induced antiviral response in PIE cells.

Methods
(i) PIE and MA104 cells were inoculated with several strains of RVs to investigate the susceptibility and the ability to induce the inflammatory cytokines against RV infection, (ii) The capacity of immunobiotics to beneficially modulate RV-induced immune response in PIE cells was investigated, by evaluating inflammatory cytokines, type I interferons, TLR signaling pathways, and TLR negative regulators.

Conclusions
Challenge of PIE cells with RV reduced cell viability and increased the expression of IFN-β, MCP-1, IL-6, IL-8, and TLR3. Immunobiotics were able to improve cell viability, and to reduce RV-induced inflammatory cytokines expression. Those effects were related to the capacity to modulate TLR negative regulators single such as SIGIRR, Tollip, A20, Bcl-3, IRAK-M, and MKP-1. Our results demonstrated that immunobiotics can be used as immunomodulators to alleviate viral induced diarrhea in livestock animals such as the pig.
FEMS-1505
Viral infections and host

NON-VIABLE IMMUNOBIOТИC LACTOBACILLUS RHAMNOSUS CRL1505 IMPROVES RESPIRATORY IMMUNITY AND REDUCES INFLUENZA VIRUS-ASSOCIATED LUNG INJURY
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Background
Symptoms of influenza infection are variable ranging from mild respiratory distress to massive organ failure resulting in death. Seasonal influenza is usually self-limiting but in susceptible patients it may progress to acute lung injury, which is characterized by augmented pulmonary microvascular permeability, hypoxemia and respiratory failure. The ongoing threat of influenza epidemics and pandemics has emphasized the importance of developing novel, safe and effective therapeutic or preventive strategies against infections from divergent influenza viruses.

Objectives
To examine the effect of nasal administration of heat-killed *Lactobacillus rhamnosus* 1505 (Lr1505) on immune response to influenza infection in mice.

Methods
Six-week old BALB/c mice were treated with $10^8$ cells of Lr1505 by the nasal route during two consecutive days. Lr1505-treated and untreated control mice were then nasally challenged with Influenza virus. Lung tissue damage and respiratory and systemic immune responses were studied at several time points after viral challenge. Results demonstrated that non-viable immunobiotic strain protected infected mice by reducing pulmonary injury and lung viral loads through several mechanisms: a) inflammatory cytokines were down-regulated diminishing inflammation ($p<0.01$), b) IFNs with direct antiviral activity were enhanced ($p<0.01$), c) regulatory cytokines IL-10 was up-regulated ($p<0.05$), d) maturation markers and co-stimulatory molecules were up-regulated in lung antigen presenting cells ($p<0.01$), e) IFN-$\gamma$ and IL-10 producing CD4$^+$ lymphocytes were recruited to the lungs.

Conclusions
Our results strongly suggest that administration of Lr1505 may represent an interesting alternative to modulate respiratory immune response and reduce influenza
virus-associated pulmonary damage.
Background. Freshwater cyanobacterium *Microcystis aeruginosa* frequently forms massive natural blooms where the cell densities are comparable to those in laboratory media (10^7 to 10^8 cells/ml). The representative genome of this species contains abundant and diverse potential anti-viral defense genes. Our culture analyses showed distinct CRISPR arrays in this species. This suggested the *Microcystis* cyanophage may have co-evolved with its phage in a frequency-dependent selection.

Objectives. The aim of this study is to understand the co-evolutionary dynamics of the *Microcystis* cyanophage in natural settings.

Methods. The CRISPR diversity of *Microcystis aeruginosa* was examined in a small eutrophic pond using amplicon analysis of the leader-end of CRISPR fragments with a MiSeq mini-gene sequencer.

Conclusions. During the sampling period, a CRISPR-related genotype (CT19) occurred frequently in the *Microcystis* population. A qPCR targeting CT19 showed the copy numbers were from 4.9×10^2 and 3.2×10^5 copies mL^{-1} and accounted for 0.2% to 18% of the *Microcystis* population. We obtained 176,800 leader-end CRISPR fragments using amplicon sequencing. Of these, 116,466 were classified into CT19 consisting of two major variants (28% and 72%, respectively). These major CTs co-existed and their numbers oscillated throughout the sampling periods. A few new variants were found to acquire new spacers or the lack of spacers at the leader-end of the major variants. This indicates *Microcystis* cyanophage co-evolution creates a few new genetic variations and also maintains the genetic diversity in the population.
PRODUCTION IN YEAST OF HUMAN BOCAVIRUS 1-4 VP2 VIRUS-LIKE PARTICLES AND GENERATION OF VP2-SPECIFIC MONOCLONAL ANTIBODIES AS NOVEL TOOLS FOR BOCAVIRUS SEROLOGY

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Background

Human bocavirus 1 (HBoV1), first described in 2005, was considered a causative agent of previously unexplained respiratory tract diseases. Recently, 3 new members of genus Bocavirus, HBoV2-4 were described. HBoV2-4 occurs mainly in the gastrointestinal tract but rarely in the respiratory tract, contrary to HBoV1.

Objectives

Recombinant viral antigens have been proven useful for serologic diagnosis of viral infections. Production of HBoV1-4 antigens in yeast expression system has not yet been reported. In the current study, the capsid proteins VP2 of HBoV1 - 4 were expressed in yeast S. cerevisiae. Electron microscopy demonstrated that all purified recombinant proteins self assembled into virus-like particles (VLPs) exhibiting the typical icosahedral appearance of parvoviruses with a diameter of approximately 20 nm. HBoV1 - 4 VP2 VLPs were stable in yeast and were easily purified by caesium chloride gradient ultracentrifugation. Four monoclonal antibodies (MAbs) of IgG1 subtype were generated by immunization of mouse with recombinant HBoV1 VP2 VLPs. Three of them specifically recognized only HBoV1 VP2 protein; one MAb was cross-reactive with HBoV2 and HBoV4 VP2 proteins. Recombinant HBoV1-4 VP2 VLPs and VP2-specific MAbs were employed to develop serological assays to detect virus-specific IgG antibodies in human serum specimens.

Methods


Conclusions
Yeast expression system proved to be simple, efficient and cost-effective, suitable for high-level production of HBoV1-4 VP2 as VLPs, that resemble native virus in regards of morphology and antigenicity.
REMARKABLE DIVERSITY AMONG NOVEL DENSOVIRUSES FROM CRICKETS

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Background
Densoviruses are members of the Paroviridae family infecting invertebrates. Since 2009, a cricket densovirus, AdDV, caused a severe epidemic in the $600-million cricket industry in North-America.

Objectives
During the last 18 months, we also received samples of diseased crickets from North America, Europe and Japan that were negative for AdDV. Electron microscopy demonstrated that these samples contained densovirus-like particles. Objectives of this study included characterization of these virus(es), cloning, sequencing, and X-ray crystallography.

Methods
A SISPA method was used to detect the pathogens (J Virol 88:12152). Sequencing of the amplicons identified those with identities to known viruses allowing by primer extension to obtain the complete genomes and cloning in suitable vectors to obtain infectious clones. Sequencing revealed that some were novel circoviruses and a novel ambisense densovirus (Genome Announc. 1(2):e00079-13, 1(3):e00328 and 131(6):e00914-13). However, the greatest surprise was a densovirus with a segmented genome (AdSDV). NS- and VP- coding sequences are in separate segments of about 3.3 kb. Both NS- and VP- segments are flanked by identical hairpin terminal sequences but lack ITRs. The two ORFs for VP arose through recombination between a mosquito brevidensovirus and AdDV. The downstream ORF with AdDV-VP1up-like phospholipase A2 could be spliced to the C-terminus of the major capsid protein. Currently, the capsid structure (X-ray crystallography) and transcription strategy of AdSDNV.

Conclusions
A brevidensovirus that normally infects only mosquitoes has adapted itself through recombination with AdDV to crickets. These findings necessitate a revision of the definition and taxonomy of paroviruses.
TIME SINCE ONSET OF DISEASE AND INDIVIDUAL CLINICAL MARKERS ASSOCIATE WITH TRANSCRIPTIONAL CHANGES IN UNCOMPLICATED DENGUE

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Background
Dengue virus (DENV) infection causes viral haemorrhagic fever that is characterized by extensive activation of the immune system.

Objectives
The aim of this study is to investigate the kinetics of the transcriptome signature changes during the course of disease and the association of genes in these signatures with clinical parameters.

Methods
Sequential whole blood samples from DENV infected patients in Jakarta were profiled using affymetrix microarrays, which were analysed using principal component analysis, limma, gene set analysis, and weighted gene co-expression network analysis.

Conclusions
Time since onset of disease associates with the shift in transcriptome signatures from immunity and inflammation to cell cycle and repair mechanisms in patients with non-severe dengue. The strong association of time with blood transcriptome changes hampers both the discovery as well as the potential application of biomarkers in dengue. Clinical diagnosis (according to the WHO classification) does not associate with differential gene expression. However, network analysis did show that that key clinical markers, including platelet count, fibrinogen, albumin, IV fluid distributed per day and liver enzymes SGOT and SGPT, strongly correlate with gene modules that are enriched for genes involved in the immune response. The expression level of these gene modules may support earlier detection of disease progression as well as clinical management of dengue.

Background
Phomopsis longicolla (teleomorph = Diaporthe) is an ascomycete phytopathogenic fungus. Mycoviruses were reported to debilitate their hosts [1]. The KY isolate and derived subcultures of P. longicolla exhibited abnormal growth and reduced virulence. Initial examination revealed that the isolate harbors Phomopsis longicolla hypovirus 1 (PlHV1) [2]. This ssRNA virus belongs to a group of betahypoviruses that, unlike alphahypoviruses, were not reported to reduce virulence of their hosts.

Objectives
The objective of the study was to investigate potential viral (or other) determinants of hypovirulence and characterise structure and diversity of PlHV1 population in the KY isolate of P. longicolla.

Methods
Illumina-based DNA and RNA deep sequencing was performed of a number of the KY subcultures and the type strain of Phomopsis longicolla Hobbs, anamorph (ATCC® 60325™).

Conclusions
The analyses of the NGS data revealed that the virome of the KY isolate consists of PlHV1 recombinant with a previously undescribed hypovirus, population of deletion mutants of PlHV1, and a novel RNA virus related to members of Narnaviridae family.
CHARACTERIZING THE VIROME OF THE ENTOMOPATHOGENIC FUNGUS BEAUVERIA BASSIANA

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Background

Entomopathogenic fungi are of scientific interest because they enable analysis of virus-host interactions and can be used as biocontrol agents of insect pests. The deuteromycetous fungus Beauveria bassiana has a widespread geographical distribution and a wide host range. Mycoviruses have been described mostly in phytopathogenic fungi and are occasionally associated with hypovirulence, while in entomopathogenic fungi presence of mycoviruses has been reported only rarely.

Objectives

The aim of the present study is to detect and analyse the viruses present in a collection of B. bassiana isolates sourced from worldwide locations.

Methods

Population studies, RT-PCR, cloning and sequencing, hybridization experiments, time-course studies and transmission electron microscopy (TEM) were used to characterize the virome of B. bassiana.

Conclusions

A population study revealed that 17/75 (22.7%) B. bassiana isolates harbor dsRNA elements. Two members of the Partitiviridae family, B. bassiana partitivirus-1 and -2 (BbPV-1 and BbPV-2), have been sequenced, while TEM revealed the presence of virus-like particles. Hybridization experiments revealed that BbPV-1 and BbPV-2 are present in seven B. bassiana isolates derived from different hosts and geographical origins. Additionally, four members of the Totiviridae family, originating from the Iberian Peninsula and the Canary Islands, have been partially sequenced. Furthermore, two previously uncharacterized viruses, B. bassiana polymycovirus-1 (BbPmV-1) and B. bassiana non-segmented virus-1 (BbNV-1), have been detected and fully sequenced and the prevalence of further PmV-like viruses in B. bassiana is currently under investigation. Finally, a time-course study revealed a strong negative
correlation between the copy numbers of viral dsRNA and the developmental stages of the fungus.
The new mesophilic filamentous anoxygenic phototrophic bacterium from sulfide hot springs.

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Background

Filamentous anoxygenic phototrophic bacteria (FAPB) belong to the deep-branching phylum Chloroflexi, and are considered to be the most ancient lineage of phototrophs. The unambiguous reconstruction of the evolution of FAPB is complicated by poor representativeness of mesophilic FAPB.

Objectives

The above mentioned pointed our efforts to the search and study of new mesophilic FAPB in mesothermal cyanobacterial mats from hot springs in Barguzin valley (Buryat Republic, Russia).

Methods

Mat samples were collected from mesothermal zones of Umkhey (36 – 40°C) and Kuchiger (35 - 39°C) sulfide hot springs. The enrichment culture containing mesophilic green filamentous anaerobic bacterium Um-2 was obtained from Umkhey mat sample at the Pfenning medium (pH 7.0) at 28°C under illumination. The Um-2 cells (diameter about 0.8 µm) were combined to trichome. The main cellular pigments was bacteriochlorophyll c (maximum absorption at 740 nm). Gas vacuoles were not found. According to the phylogenetic test results (by 16S), Um-2 formed the branch belonging to mesophilic FAPB cluster. The nearest neighbor of Um-2 was “Candidatus Chloroploca asiatica” Um-3 (KJ605349) (91% of similarity). The Um-2 phylotype representatives was detected in Umkhey and Kuchiger mat samples using PCR test with FAPB-specific pufLM primers. The sequences of rRNA (16S rRNA coding) and pufLM genes were deposited in GenBank under accessions KP341999 and KP342000 respectively.

Conclusions

Thus the enrichment culture of new mesophilic FAPB belonging to the new genus and species of the Chloroflexales order was obtained. It was shown that the new bacterium is presented in the microbial community of sulfide springs of Barguzin valley.
Background
Despite of many studies on antibacterial activity of titanium dioxide (TiO$_2$), its antifungal properties are not yet well evaluated.

Objectives
The aim of our study is to exam the activity of nanosized TiO$_2$ against yeast cells.

Methods

TiO$_2$ used was synthesized by sol-gel method and characterized as anatase with particles size about 20 nm. Its antifungal activity was examined against *C.albicans*, *C.tropicalis*, *C.lusitaniae*, *C.glabrata* and *C.krusei*, using the following experimental setup: concentration of TiO$_2$ powder – 1 mg/ml, 100 ml fungal suspension with cell density about $10^4$ CFU/ml and BLB lamp (50 Hz 8W T5 with emission 365 nm) situated at distance 10 cm. At regular intervals of time, samples were taken and serial dilutions were prepared. The number of viable cells was detected by spread plate method. For determination of the post-irradiation effect, the treated suspensions were tested after 48 h dark period.

Conclusions
The data obtained about antifungal activity of TiO$_2$ are in agreement with photocatalytic properties of metal oxide nanoparticles After 120 min UV-A photocatalytic treatment with 1 mg/ml TiO$_2$, the number of colonies of *C.albicans* and *C.tropicalis* decrease from about $10^4$ CFU/ml to less than 5 CFU/ml with 100% reduction index. Final reduction index in *C.lusitaniae* and *C.glabrata* is 99.643% and 99.898%, respectively. These data indicate strong photocatalytic fungicidal effect of TiO$_2$ on the tested fungi. At the same conditions, the colonies of *C.krusei* are reduced with 98%. Our data show that the nonhydrolytic synthesized nanosized TiO$_2$ possesses antifungal activity against isolates of the genus Candida with some differences in the speed of photocatalytic process.
Background
Ciprofloxacin ranks among the primary drugs used as treatment in urinary system infections in adults.
It is known that Oxalobacter formigenes colonization in intestines is disrupted by antibiotic intake.
Due to the Oxalobacter formigenes colonization deficiency, the fact that high level serum oxalic acid results in formation of renal calcium oxalate stones has been determined.
Additionally, serum oxalic acid level increase is thought to have negative effect on urinary and cardiovascular system.

Objectives
The purpose of our study is to search whether there is an increase in serum oxalic acid level because of intestinal flora disorder in the patients with urinary system infection and the individuals given ciprofloxacin as treatment.

Methods
In our study, 40 people who have urinary system infection and take ciprofloxacin treatment have been included. Patients serum oxalic acid levels were evaluated both in pre-treatment and post-treatment evaluation which was a month later. Serum oxalic acid was measured by a commercially available kit (SunRed Biological Technology, Serial number: 201-12-2153), using Human Oxalic Acid ELISA method.

Conclusions
In conclusion, when pre-treatment and post-treatment serum oxalic acid levels were compared, no statistical difference was found (p=0.47). It was found that ciprofloxacin treatment didn’t affect serum oxalic acid levels of the patients with urinary system infection. In the light
of this data, it is concluded that these patients don’t need to be followed up for any possible complications because of high level serum oxalic acid.
POTENTIAL SOURCES OF COMMUNITY CLOSTRIDIUM DIFFICILE INFECTIONS: OVERLAP OF PCR-RIBOTYPES ISOLATED FROM ANIMALS AND THE ENVIRONMENT
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Background
Anaerobic bacterium Clostridium difficile has been traditionally considered as a human nosocomial enteropathogen. Although its natural habitat is human and animal intestine, the ability to form dormant endospores enables C. difficile to survive also in aerobic environment. Recent emergence of community acquired C. difficile infections and infections in animals implies that environment might be a reservoir of C. difficile and a source of infection.

Objectives
Our aim was to investigate diversity and overlap of C. difficile PCR-ribotypes isolated from animals and different non-hospital environments in Slovenia.

Methods
Strains included in the comparison originated from domestic animals (calves, piglets, horses, poultry and birds, goats, sheep, dogs and cats) and different environments (puddles and soil from rural and urban areas, compost and water from rivers and ponds) in Slovenia. Isolates were characterized by PCR-ribotyping and toxinotyping.

Conclusions
This work is unique in presenting a large number of C. difficile strains from several rural and urban environments and compare them to animal and human isolates. Altogether 80 PCR-ribotypes (47 in animals and 58 in the environment) were identified and 25 were shared between animals and the environment. Forty-six of
these PCR-ribotypes were found also in humans in Slovenia. Twenty-seven PCR-ribotypes were identified as non-toxigenic with more than half found only in the environment, mainly puddles and soil, which seem to represent an independent environment. Our results show that *C. difficile* genotypes that are commonly isolated from animals can also be isolated from different environments, indicating that non-hospital environment could represent an important reservoir.
Background

When a high-voltage direct-current is applied to two beakers filled with water, a horizontal electrohydrodynamic (EHD) bridge forms between the two beakers. In this work we studied the transport and behavior of yeast cells and ThP1 human-monocytic-cell-line in this special environment.

Objectives

The behavior of *Escherichia coli* in an EHD-bridge set-up was recently investigated: *E.coli* cells survive the procedure; the main direction of cell transport is from the cathode to the anode beaker. The goal of this work was to test in how far these results are specific for *E.coli*, thus studies with yeast cells and ThP1 cells were conducted.

Methods

Cells were added to one or to both beakers, and the transport of the cells through the bridge was investigated using optical and microbiological techniques. One experimental series consisted of three different configurations: carrier solution in both beakers, only in the anode or only in the cathode beaker. As carrier solutions for yeast 5% glycerol and ThP1 cells 4.5% glucose were used.

Conclusions

The behavior of cells in an EHD-bridge depends largely on their size and their surface charge. The heavier the organism, the slower the transport; the higher the surface charge, the more pronounced the preferred flow direction.

*S.cerevisiae*: transport was observed in both directions. The absence of a preferred flow direction means that electric forces play a smaller role which is corroborated by the zeta potential.
ThP 1 cells survive the transport and showed similar behavior as the *E.coli* cells: pronounced transport towards the anode.
POST-ANTIBIOTIC AND POST-ANTIBIOTIC SUB-MINIMAL INHIBITORY CONCENTRATION EFFECTS OF CHLORHEXIDINE AGAINST ORAL BACTERIA

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Background
The post-antibiotic effect (PAE) is defined as the suppression of bacterial growth for a particular duration after a brief exposure to an antimicrobial agent. Chlorhexidine is a widely used biocide found in antiseptic products; however, its PAE remains unclear.

Objectives
In this study, PAE, post-antibiotic sub-minimum inhibitory concentration (MIC) effects (PA-SME), and sub-MIC effects (SME) of chlorhexidine on oral bacteria were investigated.

Methods
For PAE measurement, bacteria were exposed to 10x MIC chlorhexidine for 1 min, which was then eliminated by washing. For determining PA SME, bacteria were exposed to 0.1, 0.2, and 0.3x MIC chlorhexidine during the postantibiotic phase and to sub MIC chlorhexidine for measurement of SME. PAE, PA SME, and SME of chlorhexidine were observed.

Conclusions
The PAE lasted for 0.9 h for Streptococcus mutans, 0.1 h for Streptococcus gordonii, and 0.35 h for Lactobacillus acidophilus. The PA SME against oral bacteria lasted for longer duration as the chlorhexidine concentrations increased. The PA SME against oral bacteria lasted for substantially longer than SME did. The present study illustrates the existence of chlorhexidine-induced PAE, PA SME, and SME against oral bacteria, thereby extending the pharmacodynamic advantages of chlorhexidine.
PINWORM INFECTION AMONG FOREIGN LABORERS IN TAIWAN

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Background

According to the demographic ageing of labor force participation in Taiwan, we imported massive foreign laborers to Taiwan for nursing or other labor force work since October, 1989. The purposes of this study was to evaluate the effectiveness of single examination to diagnose Enterobiasis by scotch tape perianal swab technique in non-constant time.

Objectives

The survey covered southeast asian laborers from four countries including philippines, thailand, Indonesia and vietnam. The data were collected from 391 valid questionnaires, 391 valid stool samples and 391 valid scotch tape perianal swab samples.

Methods

Single scotch tape perianal swab technique was performed by foreign laborers in non-constant time. Stool samples were also obtained after the scotch tape perianal swab. This was a cross-sectional study. Descriptive statistics were applied to the data.

Conclusions

Scotch tape perianal swab technique in non-constant time could increase the detection rate of Enterobius vermicularis from 0% to 2.0%(8/391). Occupational health personnel should educate the foreign laborers to get into the habit of washing hands before a meal in their daily practice. The employers should arrange the examination of scotch-tape anal swab technique for caretakers periodically.
SPECIFIC KILLING OF BACTERIA USING A TOXIN-INTEIN BASED AND-LOGIC GATE

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Background

Synthetic biology aims reprogram bacteria to design and construct sophisticated new biological systems by gene regulatory networks. However gene regulatory networks (gene expression, protein or molecule concentration) are continuous, thus fitting them into a deterministic model like Boolean approximation (0/1) is noisy.

Objectives

We would like to implement a model aiming at killing specific bacteria in mixed populations. We will design an AND-logic gate to solve Boolean satisfiability (SAT) problem. Our model is based on conjugation where plasmids will act as wires (plasmid inside cell is 1; no plasmid is 0).

Methods

To avoid the noise associated with transcriptional regulators, our system is based on half toxins (thus non-functional) coupled with split intein technology. We are designing two types of plasmids: input plasmids (A and B) and clause plasmid (CP). Only CP is conjugative. A population of bacteria could have already implemented the input plasmids in different combinations: A and B; only A; only B or no one. The output in our system is cell death. The CP will infect these bacteria by conjugation and it will evaluate the inputs and will specifically kill bacteria harboring both plasmids (A and B). When both plasmids are together in the same cell, the expression of the toxin-intein fusion triggers the reaction between two inteins domains, allowing the reconstitution of the toxin and killing the cell.

Conclusions

We have successfully completed the evaluation of Boolean-SAT problem by the construction of an AND-logic gate and using the inputs plasmids A and B, and the CP.
OUTBREAK OF BOTULISM IN MEMBERS OF A FAMILY FOLLOWING THE CONSUMPTION OF KUSHK

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Background
Botulism is a serious neuroparalitic disease caused by the Clostridium botulinum

Objectives
In this article, we report a outbreak of the foodborne botulism due to consumption of kushk in 5 members of a family

Methods

case presentation

A 41 and 46 year old women presented with dyopia and blured vision from 4 days ago. She had progressive symptoms including dry mouth, dysphagia and swallowing disorder, constipation, dyspnea, general weakness of neck muscle and lower and upper limbs. Pupils were dilated and no reactive to light. There was no gag reflex. Deep tendon reflexes were normal and the muscle force of upper limb was 4/5.

The third case was a 13 year old boy presented some days after two above mentioned cases. His complains were petosis, dyopia, dry mouth, vertigo and weakness of lower and upper limbs but had no constipation, dysphagia and swallowing disorder and dyspnea. The forth case was a 12 year old boy with history of diabet and similar symptoms and sign and the fifth case was a 8 year old girl with neck rigidity and petosis.

They were members of a family and had history consumption of kushk. Interval between involvement of children was longer than adult and incubation piod. Disease was more severe in adult than children. EMG was done in one of the woman and was suggestive of botulism. After clinical diagnosis, serum of patients was obtained and the patients received antitoxin. Their symptoms resolved gradually.

Conclusions

The diagnosis of botulism should be made based on the patient's initial sign and symptoms and history of consumption of suspected food
FEMS-2657
Free subjects

BLOOD BORN DISEASES IN INTRAVENOUS DRUG ABUSERS
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Background
Drug injection is responsible for numerous minor to life-threatening and fatal complications such as the transmission of human immunodeficiency virus (HIV), sexually transmitted diseases, and viral hepatitis

Objectives
this paper has tried to offer a brief look at the epidemiology of drug abuse, while focusing on blood born infections seen in IDUs.

Methods
The study was carried out using a cross sectional design. A questionnaire was distributed among 135 IDUs. serological tests (ELISA Anti HCV, Anti HIV, HBs Ag) were taken to confirm the existence of blood borne diseases

Conclusions
A total of 135 cases between the ages of 15-57 were studied. opium was most frequently first drug used (58.5%), followed by cannabis (20.7%) and heroin (11.1%).

nearly 68.9% of the population began abusing drugs before the age of 20. 61.4% of the cases had a history of sharing needles Other results showed that 51.5% had an accompanying blood borne disease. Further analysis indicated a meaningful relationship (P< 0.001) between needle sharing and accompanying blood borne diseases. Of the 51.5% who had an accompanying blood borne disease, 17.1% were found to have either reactive ELISA of AntiHIV alone, or in combination with Hepatitis. In other words, it can be concluded that 8.1% of the total population had reactive ELISA of AntiHIV. Results also showed that 11.1% of the total population had HBs Ag positive while 47.4% had Positive ELISA Anti HCV
FEMS-2662
Free subjects

MICROBIAL CAUSES OF WOUNDS AND SKIN INFECTIONS IN INJECTING DRUG USERS
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Background
60–80\% of hospital admissions in IV drug abusers are due to infectious complications and skin and soft-tissue infections seem to be some of the most common infections among this group.

Objectives
The goal of this study was to identify the characteristics of wounds and skin infections of this group to hopefully have early recognition and as a result better prevention and management strategies.

Methods
This descriptive cross sectional study was carried out on 135 IDUs... all current injection drug users who had at least one wound as a result of injecting drugs were enrolled in this study. The wounds were physically examined and smear samples were also cultivated on Blood Agar and EMB Agar environments for microbiological assessments.

Conclusions
A total of 135 cases between the ages of 15-57 were studied. T. Most wounds (45.2\%) were located in the lower limbs such as the foot. The majority (44\%) of wounds were as old as six months up to one year. Each individual had at least 4 wounds with an average size of 1.8 cm\(^2\). 22.2\% of the population had some form of infection such as abscess or cellulitis. Thrombophelebitis was seen in 37\% and. Only 10.4\% showed necrotizing ulcers.

Microbiological assessments found that the majority of wounds (54.1\%), had gram positive cocci. This included staphylococcus (36.3\%), streptococcus (17.8\%). 7.4\% of the cases showed gram negative species. 30.4\% of the bacterial cultures showed negative or nontypable results, since the culture environments used were very routine and non-specific. Fungi were seen in 8.1\% of the cases.
MICROBIAL CAUSES OF WOUNDS AND SKIN INFECTIONS IN INJECTING DRUG USERS

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Background

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Conclusions

A total of 135 cases between the ages of 15-57 were studied. The majority (44\%) of wounds were as old as six months up to one year. Each individual had at least 4 wounds with an average size of 1.8 cm\textsuperscript{2}. 22.2\% of the population had some form of infection such as abscess or cellulitis. Thrombophlebitis was seen in 37\% and Only 10.4\% showed necrotizing ulcers.

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OUTCOME OF HIV/AIDS PATIENTS WITH PNEUMONIA
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Background
Pneumonia is also a major cause of prolonged hospitalization, mortality and morbidity among HIV infected people that costs millions each year

Objectives
The goal of this study was determine of outcome and prognostic factors of pneumonia in HIV positive individuals.

Methods
In a descriptive method, 80 HIV positive patients that hospitalized in Loghman-hakim hospital between 2005 to 2011 with pneumonia have been studied and their demographic findings include age, sex, marriage, job, social history such as smoking, I v drug using and imprisonment, medical history of co infections such as pulmonary TB, HCV and HBV, and also clinical features include their initial signs and symptoms, lab data and imaging, and at last their outcome gathered and record in questionnaire. Then gathered data enter SPSS#13 software, analyzed by descriptive tests

Conclusions
80 HIV positive patients hospitalized with pneumonia in loghman-hakim hospital since 2005 to 2011 entered the study. Mean age was 37.4(±8.2) years. Death was the first with 43.8%, then recovery with 30% and pulmonary complications with 16.3% were the most common outcomes. Mortality was more common in the elderly. Most of the patients had history of smoking, injection drug use and imprisonment history and mortality rate in these patients were more common. The mortality rate was also more common in patients with anemia, high ESR levels, CRP + , high urea and high creatinine levels and low CD₄ counts and pleural effusion.
Background

Iron is an essential trace element required for growth of all microorganisms. However, iron in environment is mostly insoluble form and cannot be readily utilized by microorganisms. In iron starvation condition, actinomycetes have developed specific mechanisms involving in the production of small organic metal chelator called siderophore.

Objectives

Actinomycetes were isolated from coastal and mangrove sediments in Thailand. Genomic DNA were extracted by phenol-chloroform method. Hydroxamates and catecholate siderophore biosynthetic genes were detected by PCR using desD and entF specific primers, respectively. Siderophore production was determined by CAS assay. Siderophore producing actinomycetes were identified by 16S rRNA gene sequence analysis.

Methods

A 1.2 kb fragment of desD gene that encodes for desferrioxamine, a hydroxamate siderophore was detected in 7 isolates. A 203 bp amplified fragment of entF gene encoding enterobactin, a catecholate siderophore was detected in 3 isolates. All isolates produced siderophore on Chrome Azurol S agar. High siderophore producing Streptomyces parvulus S2-SC3 was selected for sequence analysis of desD gene. A 1.2 kb amplified PCR fragment showed 90% sequence similarity with desferrioxamine synthetase from Streptomyces lividans strain 1326. Phylogenetic analysis revealed that these siderophore producing actinomycetes were members of the genus Micromonospora, Streptomyces and Verrucosispora.

Conclusions

PCR offers a fast way to screen for siderophore producing actinomycetes. However, some producing strains were failed to detect by our PCR screening, several actinomycetes strains may carry diverse siderophore biosynthesis gene which could not be detected by currently available primers. Our results suggested that novel siderophore may exist in these actinomycetes.
TAXONOMIC DIVERSITY OF ACTINOMYCETES FROM THAI MANGROVE SEDIMENTS

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Background

Actinomycetes are large group of high GC Gram positive bacteria that widely distributed in nature. They have proved to be an important source for industrially useful bioactive metabolites, notably antibiotics. In Thailand, there are very few studies on microbial diversity in mangrove forests. In this study, we report on the diversity of cultivable actinomycetes in mangrove sediments and provide taxonomic evidence of their novelty taxa.

Objectives

Actinomycetes were isolated from mangrove sediments and taxonomically characterized. Representative isolates were selected and identified based on 16S rRNA gene sequence analysis. Putative novel taxa were subjected to polyphasic taxonomic characterization using standard methods.

Methods

Over 100 strains of actinomycetes were isolated from mangrove sediments using media selective for actinomycetes. Phylogenetic analyses based on a 16S rRNA gene sequences showed that the majority of isolates were dominated by genus Streptomyces. In addition, members of the genera Amycolatopsis, Jiangella, Micromonospora, Nocardia and Verrucosispora were also found. Some isolates may represent a new species. For example, Jiangella strain 3SM4–07ᵀ was most closely related to Jiangella alkaliphila JCM 15620ᵀ (99.0%), However, they shared mean DNA–DNA hybridization values of only 31.5±2.6%.

Conclusions

Our results provide further evidence that mangrove sediments harbor taxonomically diverse actinomycetes. The diversity of these organisms and their novelty support the view that this under studied habitat is a rich source of novel actinomycetes which can be further exploited for biotechnological purposes.
Background

Eucalyptus are the most important raw material supply for pulp industries in Thailand. Leaf and shoot blight caused by Cryptosporiopsis eucalypti, Cylindrocladium sp. and Teratosphaeria destructans is a serious disease in eucalypt plantations. The search of actinomycetes for biological control agents is one of the promising approaches.

Objectives

In this study, we isolated actinomycetes capable of inhibiting the growth of fungal pathogens causing leaf and shoot blight on eucalyptus. The potential strain to use as biocontrol agent was selected for further study.

Methods

Actinomycetes were isolated from roots and rhizospheric soil of eucalyptus using starch casein agar and humic acid-vitamin agar supplemented with antimicrobial antibiotics. The isolates were screened for their ability to inhibit eucalyptus fungal pathogens using dual culture technique.

Conclusions

A total of 478 actinomycete strains were successfully isolated from roots (95 isolates) and rhizosphere soil (383 isolates) of eucalyptus. Among these, 439 isolates (91.8%) were streptomycetes and 39 isolates (8.16%) were non-streptomycetes. All isolates were evaluated for their in vitro antagonistic effect on plant pathogenic fungi. The result showed 273, 120 and 241 isolates were antagonistic to Cryptosporiopsis eucalypti, Cylindrocladium sp. and Teratosphaeria destructans, respectively. Among these, 48 isolates (10%) displayed activity against all three fungal pathogens. The isolate EUSKR2S82 showed the strongest inhibitory effects against all tested fungi.
The identification of this strain using 16S rRNA genes sequence revealed that isolate EUSKR2S82 shared 99.4% similarity with *Streptomyces ramulosus* NRRL B 27-14\textsuperscript{1}. 
FEMS-2276
Free subjects

CULTIVABLE ACTINOMYCETES ASSOCIATED WITH GLOMUS MOSSEAE SPORES AND THEIR PLANT GROWTH PROMOTING ACTIVITY
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Background

Actinomycetes are prolific producers of bioactive compounds. Much of our research effort has been directed at assessing the diversity of actinomycetes in various environments. Currently, we are interested in mycorrhizal helper bacteria which may be a good source for novel taxa for bioprospecting.

Objectives

In this study, we report on the isolation of actinomycetes from spores of \textit{Glomus mosseae} and provide preliminary evidence of their potential in agriculture as plant growth promoter.

Methods

Actinomycetes were isolated from spores of \textit{Glomus mosseae} using the dilution plate technique and media designed for the selective isolation of members of specific actinomycete taxa. All isolates were identified by 16S rRNA gene analyses. The isolates were examined for their ability to produce indole-3-acetic acid, siderophores and solubilize phosphate \textit{in vitro}.

Conclusions

Six strains were isolated from spores of \textit{Glomus mosseae} using media selective for actinomycetes. Phylogenetic analyses based on a 16S rRNA gene sequences showed that the isolates belonged to the genera \textit{Lysibacter} and \textit{Streptomyces}. All isolate produced siderophore, four isolates produced IAA and two isolates solubilized phosphate at varying level. \textit{S. thermocarboxydus} S3 produced 11.23±0.02 mg/ml IAA and high activity of phosphate solubilization and siderophore production. The results provide evidence that actinomycetes were associated with arbuscular mycorrhizal spores of \textit{Glomus mosseae}. The ability of these organisms to produce plant growth promoting agents support the possibility of using these actinomycetes for agricultural purposes.
PROFERROROSAMINE A SECRETION BY ERWINIA RHAPONTICI P45 INHIBITS GROWTH OF THE FIRE BLIGHT PATHOGEN ERWINIA AMYLOVORA

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Background
The classic diagnosis of the fire blight pathogen E. amylovora (EA) takes the cell morphology on King’s B agar into account. Sometimes, an unusual pink coloration of EA colonies can be observed on this agar. This coloration is due to the co-cultivation of E. rhapontici or E. persicina and their production of the iron (Fe²⁺) chelator proferrorosamine A (pFRA), that accumulates in E. amylovora. Dependent on the proximity of a pFRA synthesizer, EA colonies can also exhibit an attenuated growth.

Objectives
To get a deeper insight into the mechanism of the Fe²⁺ chelator, we aimed to identify the genes involved in proferrorosamine A synthesis.

Methods
pFRA negative mutants of E. rhapontici P45 were isolated in a phenotypic transposon mutagenesis screen.

Conclusions
We identified a 9.3 kb cluster of seven genes that encode proteins involved in pFRA synthesis. Wild type E. rhapontici P45, but not pFRA negative mutants, showed a growth inhibition effect on EA in vitro and detached flower assay.
COMBINED EFFECT OF OSMOTIC AND UV-B IRRADIATION ON A N2 FIXING CYANOBACTERIUM FROM A RICE PADDY

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Background
Cyanobacteria play a crucial role in the productivity of terrestrial and aquatic systems and have developed a series of defense mechanisms against abiotic factors as UV-B light and osmotic stress.

Objectives
In order to study the protective strategies of Calothrix BI22 spp., this cyanobacterial isolate from a subtropical rice field was exposed to low doses of UV-B alone or in combination with osmotic stress.

Methods
After the stress exposure, oxidative damage was determined by TBARS and proline accumulation quantified. The activity of enzymes (superoxide dismutase and catalase) involved in the decrease of reactive oxygen species (ROS), O₂⁻ or H₂O₂, were determined and the in vivo production of ROS was followed by confocal microscopy. Photosystem II yield and O₂ photoevolution were also studied as proxies of the effect of the stress on the cyanobacterium physiology.

Conclusions
Osmotic stress, in general, caused a more pronounced oxidative damage but in combination with UV-B the damage drastically increased. Among the enzymes involved in the antioxidant defense, CAT had a higher activity under osmotic stress but in the presence of both types of stresses its activity doubled. SOD, on the other hand, had higher rates only under UV-B light. The accumulation of ROS followed a similar pattern as the antioxidant enzymes studied. Photosynthesis was affected in all of the situations studied. This study showed that this cyanobacterium had a differential response when exposed alone to each type of stress than in combination.
Background
Agarose gel electrophoresis has been widely used as the standard method to separate, identify, and purify DNA/RNA fragments in the life sciences research, because it’s easy to perform, relatively inexpensive, and excellent in analytical performance characteristics. However, technologies to separate for short DNA fragments in high resolution are still lacking.

Objectives
In response to the above challenge, we hypothesized that the tailoring agarose molecules with various pendant groups would provide the rapid analysis of an array of small to large DNA fragments in the gel electrophoresis.

Methods
We examined this hypothesis by tailoring the agarose molecules with various pendant groups such as octadecyl chains, and aromatic groups, and then directly fabricate the three dimensional agarose gel for electrophoresis. This concept of the hydrophobically-modified 3D microporous gel can be regarded also as a novel microfluidic-based mold for being demonstrated and also used in the bio-microelectromechanical system (Bio-MEMS). The molecular structure (degree of substitution and molecular weight) of the modified agarose was confirmed by 1H-NMR, and MALDI-TOF (Matrix: CHCA) analysis. Interestingly, the tailoring agarose gel provided the distinctive separation of small DNA fragments efficiently and also enhanced the discriminatory power in the gel electrophoresis based on the pendant groups’ characteristics.

Conclusions
Overall, the results from this study may be useful in understanding the role of the hydrophobic/hydrophilic balance in the separation of DNA fragments and further in the extracellular microenvironments in tuning a variety of cellular activities.
TEMPERATURE- AND NITROGEN SOURCE-DEPENDENT GROWTH OF LISTERIA MONOCYTOGENES IMPLICATES AN IMPORTANT ROLE OF 2-OXOGLUTARATE AS INTERNAL SIGNAL DURING ADAPTATION TO CHANGING ENVIRONMENTS

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Background
The pathogen Listeria monocytogenes lives either saprophytically in the environment or within cells in the vertebrate host. Thus it has to adapt its lifestyle to its ecological niche and the availability of nutrients.

Objectives
The aim of this study was to investigate the impact of different nitrogen sources and temperatures on growth of L. monocytogenes.

Methods
Nitrogen source dependent growth analysis was conducted in defined minimal medium at 24°C or 37°C with either glutamine or ammonium as nitrogen source. Nitrogen source- and temperature-dependent gene transcription was validated by qPCR.

Conclusions
A preference for ammonium over glutamine was observed in growth experiments. This might be ascribed to the interrupted TCA cycle in L. monocytogenes, causing accumulation of the intermediate 2-oxoglutarate. 2-oxoglutarate has to be removed to allow a permanent glucose metabolism by continuous channeling of pyruvate in the TCA cycle. During growth on ammonium twice the amount of 2-oxoglutarate is removed compared to growth on glutamine.

Reduced growth on glutamine was more obvious at 24 °C than at 37 °C. When grown on the same nitrogen source, but at different temperatures, the removal of 2-oxoglutarate is mainly determined by the amount of glutamate used for the de novo biosynthesis of amino acids which is expected to be elevated at the optimal growth temperature of 37 °C.

It is assumed that in L. monocytogenes the intracellular 2-oxoglutarate concentration serves as internal signal that allows the adaptation of L. monocytogenes to two environmental parameters, temperature and nitrogen source, at the same time.
INVESTIGATION OF AUTOANTIBODIES IN CANCER PATIENTS

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Background

Malignant diseases are often related with the induction of autoimmunity, characterized by the generation of autoantibodies against a wide range of autoantigens. Serum autoantibodies have been identified in patients with solid tumors and in patients with hematological malignancies.

Objectives

The purpose of the study was to investigate the prevalence of antinuclear antibodies (ANA) in sera of the patients with various cancer types.

Methods

Serum samples were obtained from 54 patients with cancer (37 breast, 1 myxoid liposarcoma, 1 brain, 2 lung, 1 colon, 3 gastric, 1 pancreas, 2 lymphoma, 1 multiple myeloma, 1 nasopharynx, 1 renal, 1 prostate, 1 vulva, 1 endometrial) and with rheumatic compliant. A total of 100 healthy blood donors served as the study control group. ANA patterns were searched by using the HEp-2010/Liver (Monkey) indirect immunofluorescence assay (IFA) kit (Euroimmun AG, Germany). In addition these samples were further processed by line immunoassay (LIA), (Euroimmun AG, Germany).

Conclusions

Of 54 patients, 18 were ANA-positive by IFA and LIA. Homogen (8), granular/speckled (2), nucleolar (2), anticentromere (2) and mixed (4) patterns were detected by IFA. SS-A/Ro (8), SS-B/La (6), antidsDNA (2), antiScl (2) and CENP-B (2) positivity were observed by LIA. In healthy control group, ANA was not detected. There was statistically significant difference between the results of the patients and of the control group. Of 54 patients, 29 were diagnosed with cancer while 20 were diagnosed with rheumatic disease firstly; and 5 were diagnosed with both simultaneously. In
conclusion, the association between rheumatic diseases and malignant diseases need further investigation.
ANTI-DFS70 ANTIBODIES IN 500 HEALTHY BLOOD DONORS
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Background
Autoantibodies against DFS70 (dense fine speckled 70) antigen have been recently identified among the antinuclear antibodies (ANAs) in patients with systemic autoimmune rheumatic diseases.

Objectives
The typical DFS has been described as indirect immunofluorescence (IIF) staining pattern which is uniformly distributed throughout the interphase nucleus and, most notably, are localized on metaphase chromosomes. We undertook this study to examine the frequency of anti-DFS70 antibodies in a large number of healthy blood donors and to evaluate the significance of these antibodies, accordingly.

Methods
Sera of 500 healthy blood donors (459 men, 41 women) were analyzed for ANAs and anti-DFS70 antibodies by IIF with HEp-2 cells as a substrate.

Conclusions
ANAs were determined in 0.8% of all blood donors by IIF. Positive results were detected in different serum dilutions: Three subjects (0.6%) 1:320 nucleolar pattern, and 1 subject (0.2%) 1:1000 homogeneous/nucleolar pattern. There were 3 anti-DFS70 antibody positive blood donors. In this study, anti-DFS70 autoantibody was detected 0.6% in healthy blood donors. Among anti-DFS70 antibody-positive subjects the percentage of male was 0.65% (3 of 459 subjects). The prevalence of anti-DFS70 antibody positivity decreased with increasing age. According to our results, anti-DFS70 autoantibody is detected more commonly in male than female healthy blood donors. More comprehensive studies are needed to be done to investigate the anti-DFS70 autoantibody specificity for healthy individuals.
ANTI-DFS70 ANTIBODIES IN SYSTEMIC AUTOIMMUNE RHEUMATIC DISEASES

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Background
Systemic autoimmune rheumatic diseases are major cause of morbidity and mortality. The presence of various autoantibodies, such as antinuclear antibodies produced against intracellular antigens, are characteristic of systemic autoimmune rheumatic disease (SARD).

Objectives
In this study, we aimed to investigate the prevalence of anti- DFS70 antibodies in SARD and to evaluate the significance of these antibodies, accordingly.

Methods
In our study we created test groups as; 107 healthy blood donors, 418 Rheumatoid arthritis (RA), 101 Systemic Lupus Erythamatosus (SLE) patients, 36 Systemic sclerosis patients, 71 Sjögren syndrome patients, 43 Ankylosing spondylitis and 2555 Connective tissue disease (CTD) pre-diagnosed patients. Serum samples were evaluated by indirect immunofluorescence assay (IFA). Anti-DFS70 positive samples were also tested by ELISA test.

Conclusions
Anti DFS70 antibody was detected 1.4% in RA, 2.97% in SLE, 1.4% in Sjögren syndrome and 1.05% in disease group with pre-diagnosed CTD. It was not detected in AS and SSc patient groups. Totally 39 out of 47 samples were also found to be anti DFS70-positive by ELISA method. According to our results, anti-DFS70 autoantibody is detected more commonly in certain disease sub-groups of SARD than healthy individuals.
NONTOXIGENIC CLOSTRIDIUM DIFFICILE MEMBRANE FRACTION AS A VACCINE CANDIDATE

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Background
Clostridium difficile is a leading cause of infectious diarrhea and pseudomembranous colitis. Standard treatment for C. difficile infections (CDI) includes the administration of antibiotics. However, the rate of CDI recurrence after initial therapy is approximately 20% and even increases when antibiotic therapy is repeated.

Objectives
Therefore, alternatives or additional measures to current antibiotic therapy are required to achieve lower rates of CDI recurrence. Vaccination is one of prevention measures by which low recurrence rate is expected. In this study, we demonstrate the potential of nontoxigenic C. difficile membrane fraction (ntCDMF) as a vaccine candidate.

Methods
Mice immunized with ntCDMF showed serum IgG and intestinal IgA titers of 4,660 and 156, respectively, as compared to 30 and 13, respectively, for non-immunized controls. In Caco-2 cell adhesion assay for 9 different C. difficile strains, the number of adherent bacterial cells pretreated with the immune serum was 5.8–9.3 CFU per 100 Caco-2 cells, which was significantly lower than in the case treated with non-immunized serum (21.1 to 36.6). Similar findings were observed for intestinal fluid, where the number of adherent bacterial cells ranged from 19.6 - 29.1 and 25.9 - 42.0 CFU per 100 Caco-2 cells for immunized and control mice, respectively.

Conclusions
These results support the idea of ntCDMF-derived C. difficile vaccine capable of preventing the adhesion for C. difficile to the intestinal cells, as well as emphasize its potential as a preventive measure against CDI.
Background
This paper reviews the toxicity and antibacterial activity of ten cyanobacterial strains isolated from fresh waters in Serbia.

Objectives
All investigated cyanobacterial strains showed toxic effect to *A. salina*. The highest toxicity was observed in the case of strains belonging to *Microcystis* genera (*Microcystis* L1 and P1) with detected larval mortality of 98% and 96%, respectively. The observed LD50 values were from 0.24 mg ml\(^{-1}\) (*Anabaena* P1) to 3.37 mg ml\(^{-1}\) (*Oscillatoria* K3). The intracellular microcystin concentrations, calculated as microcystin-LR equivalents, were in the range of 0.1–3.96 µg mg\(^{-1}\) dry weight. All cyanobacterial strains revealed antibacterial activity and inhibited the growth of at least one bacterial species. The intracellular extract of *Aphanizomenon* K2 strain showed the most evident inhibitory effects to 6 bacteria. The MIC values of the tested strains were in the range of 0.00014 gL\(^{-1}\) - 0.5805 gL\(^{-1}\).

Methods
The toxicity of intracellular extracts of cyanobacterial strains was tested by *Artemia salina* bioassay and expressed as the percentage of larval mortality. The intracellular hepatotoxin (microcystin) content of cyanobacterial strains was determined by colorimetric protein phosphatase 1 inhibition assay (PP1). Methanolic intracellular extracts of cyanobacterial strains were tested for antibacterial activity using agar diffusion method and broth microdilution assay.

Conclusions
The results suggest that toxic effect on larvae *A. salina* found in the most tested strains is likely to be a consequence of their microcystin production. The obtained results indicate that toxins are probably not responsible for the expressed antibacterial activity of the tested strains.
LABELLING OF EICOSAPENTAENOIC ACID WITH STABLE ISOTOPE (13C) IN BACTERIA

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Background

Eicosapentaenoic acid (EPA) is an essential omega-3 polyunsaturated fatty acid (PUFA) well known for its beneficial effects on human and animal health.

EPA is present at relatively high levels in a number of marine animals with its primary dietary source being microalgae and some bacteria. This essential bioactive plays a critical role in marine life; however its transfer between different trophic levels is yet to be fully understood.

Objectives

A simple and affordable method to study and trace the fate of EPA through the trophic food chain was developed.

Methods

*Shewanella* sp. is a marine bacterium, known to produce EPA [1, 2]. In this work, we describe a simple method to label the EPA present in the bacterial strain IRL 567 (*Shewanella* sp.) with the stable isotope $^{13}$C. Labelling assays were performed at small scale (100 ml shake flask) and bench scale (1lt stirred tank bioreactor). By incubating the bacteria with $^{13}$C-acetate in culture medium, we demonstrated that EPA is *de-novo* synthetized utilizing the simplest carbon source -acetate- as precursor. $^{13}$C incorporation into the EPA molecule was determined by mass spectrometry (ESI TOF MS), finding that 95.5% of synthetized EPA being labelled in the shake flask and 88.6% in the bioreactor, yielding a concentration of labelled EPA of 5.36 mg/l and 2.90 mg/l, respectively, with between 2 to 8 $^{13}$C atoms contained in EPA structure.

Conclusions

Labelled EPA could be a valuable research tool to understand the assimilation and metabolic routes of fatty acids, essential components in human and animal diets.
Background
The occurrence of candidemia is on a rise worldwide. Non-albicans Candida species have emerged as major causes of candidemia in many countries. Added to it is the problem of antifungal resistance in Candida isolates.

Objectives
The aim of our study was to investigate the isolation of Candida spp in blood cultures and to evaluate their antifungal susceptibility during a 8-year period (2007-2014) in a tertiary care hospital.

Methods
The blood cultures were incubated in the automated blood culture system BACTEC9240 (Becton Dickinson). Positive blood cultures were examined microscopically directly for yeast or pseudohyphae and subcultured on Sabouraud dextrose agar (Liofilchem Italy). Candida isolates were identified using automated VITEK 2 system (bioMerieux) or Api 20CAUX (bioMerieux). Antifungal susceptibility was carried out by automated VITEK 2 system (bioMerieux) using AST Y01 or AST Y07 test card.

Results: During the study period there were 67 candidemia cases. The 51.35% of candidemias occurred in the ICUs, the 40.54% in the medical wards and the rest of 8.11% in surgical wards. C. parapsilosis was the predominant species (35.15%), followed by C. albicans (32.43%), C. glabrata (10.81%), C. tropicalis (8.12%) and C. norvegensis, C. guilliermondii, C. kefyr, C. famata, Cryptococcus neoformans with (2.70%). All tested isolates were susceptible to amphotericin B and voriconasole. Among non-albicans strains increasing resistance to fluconasole was found: C.parapsilosis (8.33%), and C. tropicalis (8.33%).

Conclusions
Candidemia was more frequent in ICUs followed by medical and surgical wards. C. parapsilosis was the predominant cause of candidemias in our Hospital. Amphotericin B and voriconasole were active against all tested isolates.
ASSESSMENT OF IMMUNE PROTECTIVE CAPACITY OF THE RECOMBINANT IRON SUPEROXIDE DISMUTASE (FESOD) FROM BORDETELLA PERTUSSIS

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Background
Whooping cough (pertussis) is a highly contagious respiratory infection caused by Bordetella pertussis. Although availability of effective pertussis vaccines seems to decrease the incidence of the disease, B. pertussis circulation in population has not been eliminated. Thus, finding new protein candidates with high immune protective capacities is necessary to enhance the efficacy of current acellular pertussis (Pa) vaccines (1).

Objectives
The aim is to evaluate immune protective capacity of iron superoxide dismutase (FeSOD) protein from B. pertussis with the aim of developing new generation pertussis vaccines.

Methods
FeSOD gene was cloned, expressed in Escherichia coli and the recombinant protein was purified. FeSOD was formulated with Aluminum hydroxide (Alum) or Monophosphoryl Lipid A (MPLA) and mice were immunized intraperitoneally. IgG1, IgG2a and IFN-γ levels were determined and bacterial colonization on mice lungs were evaluated.

Conclusions
IgG1 and IgG2a responses were significantly increased in both mice groups immunized with FeSOD-Alum and FeSOD-MPLA, the level of IgG2a was relatively higher in mice vaccinated with FeSOD-MPLA. Immunization with FeSOD-MPLA formulation provided a significant decrease in bacterial count in mice. Moreover, antigen specific-IFN-γ response was significantly increased in the group vaccinated with FeSOD-MPLA. These findings, altogether, suggested that the recombinant FeSOD protein formulated with MPLA can be a possible acellular pertussis vaccine candidate.

References:
Background

MIRRI (Microbial Resources Research Infrastructure, www.mirri.org) is an initiative within the European Strategy Forum on Research Infrastructure (ESFRI) that includes 16 partners (14 public microbial Culture Collections / mBRCs), supported by 21 collaborators; the preparatory phase is currently funded by the European Commission.

MIRRI aims to construct a pan-European distributed infrastructure that boosts research and development in the field of biotechnology by improving access to the microbial resources present in European public collections, their associated data and expertise.

Objectives

One of the MIRRI objectives is to create clusters bringing together the expertise available at different partner and non-partner institutions to respond to concrete demands of the MIRRI stakeholders. These expert clusters will provide solutions in aspects such as regulatory framework (e.g. biosecurity, ABS, IPR), managerial best practices, taxonomy or microbial applications, among others.

Methods

To design the content, composition and rules of operation of the clusters, the relevant stakeholders have been consulted (face to face meetings, workshops and surveys). An analysis of the comparable RIs of the ESFRI 'Food and Health' group was performed to bridge the gap between information sharing and facilitating a collaboration landscape.

Conclusions

Here, we present the concept of the MIRRI Clusters of expertise as a tool to share and generate knowledge within the MIRRI stakeholder community (culture
collections, policy makers, scientists, bioindustry, etc.) to foster innovation within science, research and development. The authors gratefully acknowledge the contribution of the MIRRI consortium and the consulted persons to this work.

This project has received funding from EU FP7 (grant agreement 312251).
Background
The bacterial flagellum is a complex organelle requiring the coupling of gene expression to the assembly pathway. In *Escherichia coli* and *Salmonella enterica* serovar Typhimurium the *flhDC* operon encodes the essential FlhD$_4$C$_2$ flagellar-specific master transcriptional regulator. The activity of FlhD$_4$C$_2$ is influenced by many flagellar-specific and global regulatory stimuli. FlhD$_4$C$_2$ activity responds to cellular and environmental factors through regulators including OmpR, RcsB and CRP. Flagellar-specific signals known to influence FlhD$_4$C$_2$ activity are controlled by the action of FliT and FliZ. *flhDC* expression is also sensitive to cell growth.

Objectives
Our objective in this study was to investigate the impact of growth rate on flagellar formation in *E. coli* and *S. Typhimurium*

Methods
Our methods to study our objective included the use of steady-state chemostat cultures. Our measure was flagellar abundance using a functional FliM-GFP fusion protein. We will show that flagellar abundance correlates with growth rate, where faster growing cells produce more flagella in both species. We will present data showing that the impact of transcriptional, post-transcriptional and flagellar-specific regulation of *flhDC* with respect to the growth rate response of the flagellar system leads to a marked difference in flagellar abundance when the species are compared.

Conclusions
Our data suggests that even though both flagellar systems have a high degree of genetic similarity the way *E. coli* and *S. Typhimurium* assimilate signals during flagellar regulation may reflect the lifestyle of these two bacterial species.
Bacterial type VI secretion systems (T6SSs) are recently discovered nanomachines used to inject effectors into prokaryotic or eukaryotic cells. T6SSs are therefore involved in both inter-bacterial competition and bacterial pathogenesis.

Objectives
The aim of this work is the analysis of T6SS in the soil bacterium Pseudomonas putida. P. putida is known for its capacity to colonise the root of crop plants providing growth advantages to the plant and, importantly, protection against plant pathogens. Since T6SS is mainly used by environmental bacteria for interbacterial competition, we analysed whether this secretion system of P. putida might be involved in such protection.

Methods
- In silico analysis of P. putida KT2440 genome to identify potential T6SSs
- Competition assays to determine P. putida T6SS activity and bacterial targets.
- qRT-PCR and transcriptional fusions to study P. putida T6SS expression and regulation

Conclusions
P. putida contains three putative T6SSs named as H1, H2, and H3. These clusters contain the genes encoding the T6SS conserved core components and some accessory proteins, including regulatory proteins and toxins-immunity pairs. Additional T6SS-related genes are found scattered on the chromosome. At least the H1-T6SS is active and used by P. putida to kill other bacteria, including the phytopathogen Pseudomonas syringae. Expression of this system is maximal in stationary phase and it is controlled by the global regulators RetS and GacS-GacA, and by two alternative sigma factors, RpoS and RpoN.
Bacterial nanomachines

A PSEUDOMONAS FLUORESCENS TYPE VI SECRETION SYSTEM IS INVOLVED IN ANTIBACTERIAL ACTIVITY

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Background

To persist in an ecological niche, bacteria have developed several strategies to enable them to resist in the environment. One of these mechanisms able to struggle with other bacterial species is Type VI Secretion System (T6SS). This allows the bacterium to inject toxins directly into prey’s cytoplasm and could cause death. A mucoid environmental strain of Pseudomonas fluorescens, MFE01, has antibacterial activity carried by T6SS. MFE01 is not virulent against eukaryotic cell models (amoebas, plant or animal cell models). Currently, genomic analysis reveals only one T6SS core component cluster and three hcp genes were found (named hcp1, hcp2 and hcp3).

Objectives

Aims of this study are to assign a role to each Hcp protein and to test the potential application of this secretion system to prevent infection by nosocomial strain (like Pseudomonas aeruginosa) in burn wound.

Methods

Mutations of hcp1, hcp2 and hcp3 were performed. Furthermore, T6SS apparatus was also inactivated by tssE gene mutagenesis. Competitor activity were established in different conditions: immobilized experiment, swarming or swimming conditions. Hereafter, we will use Hacat keratinocyte model to study the ability of MFE01 to protect against infection by etiologic agents.

Conclusions

MFE01 T6SS plays a crucial role in bacterial competition in ecological niche. Hcp2 was involved in antibacterial activity whereas Hcp1 immobilized prey cell. Hcp1 and Hcp2 proteins had a synergic effect which Hcp1 inhibits prey cell mobility and then Hcp2 can killed it. Presently, no role is attributed to Hcp3.
Bacterial nanomachines

MICRO- AND MACRO-SCALE INSIGHTS ON THE TRANSFORMATION OF SELENITE TO SELENIUM NANOPARTICLES BY MIXED MICROBIAL AGGREGATES

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Background
Selenium oxyanions can be reduced by diverse microorganisms resulting in the formation of selenium nanoparticles (SeNP). This process has been mostly investigated using pure bacterial cultures. However, using pure cultures for treatment of wastewater containing selenium oxyanions is not recommended. Anaerobic granular sludge, which consists of aggregates of mixed microbial communities, may also reduce selenium oxyanions and would thus be appropriate for developing treatment systems.

Objectives
The aims were: (i) to investigate which microorganisms prevailed in a granular sludge that was exposed to selenite and reduced this oxyanion to SeNP, (ii) to investigate at the macro- and micro-scale the location of the produced SeNP, and (iii) to identify proteins associated to the SeNP.

Methods
High-throughput pyrosequencing, electron microscopy and proteomic approaches were employed.

Conclusions
A large change in the microbial community occurred when the inoculum granular sludge was exposed to and reduced selenite during a 20 day period. Most abundant microorganisms in the selenite reducing granular sludge were affiliated to Veillonellaceae and Pseudomonadaceae families. Most of selenium bioreduction occurred in the outer layer of the granular sludge, where most biomass contents are localized. At the micro-scale, electron tomography showed that SeNP were produced inside the cells. A proteomic analysis of extracted SeNP revealed that most of their associated proteins were proteins found in the outer and inner membranes. The used granular sludge readily reduced selenite and the combined results allowed proposing mechanisms for the bacterial synthesis of SeNP in these mixed microbial aggregates.
YERSINIA PSEUDOTUBERCULOSIS TYPE III SECRETION IS RELIANT UPON AN AUTHENTIC DUAL FUNCTIONAL N-TERMINAL YSCX SECRETOR DOMAIN.

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Background
Various Gram negative bacteria employ a type III secretion system (T3SS) to deliver effector proteins into eukaryotic cells to form mutualistic or pathogenic interactions with their host. A T3SS is comprised of about 25 different proteins many of whose functions still remain obscure. In *Yersinia* Ysc-Yop T3SS, YscX and YscY are two proteins which have not been well characterised. However, a Ysc-Yop T3SS is non-functional in the absence of either *yscX* or *yscY*, suggesting that both YscX and YscY are crucial Ysc-Yop T3SS constituents.

Objectives
An attempt to understand the role of YscX and YscY was initially investigated by studying the functional interchangeability between genetically conserved members of the YscX-YscY protein families. It suggested that YscX might be functionally unique to *Yersinia* despite reciprocal binding with YscY family members. Intrigued by the specificity of YscX function, we scrutinized the role of YscX N-terminus in secretion of itself and other T3S proteins.

Methods
Site directed mutagenesis and defined domain swapping revealed YscX N-terminus to be a critical aspect of *Yersinia* T3S as it prevented the polymerisation of surface localized YscF needle. This was neither due to a defect in YscX secretion potential nor any observable defect in forming a bipartite interaction with YscY or a tripartite interaction with YscV as measured by standard protein interaction assays.

Conclusions
Therefore, the YscX N-terminus must perform dual functions; on the one hand it is a secretion recognition motif and on the other, a non-redundant recognition signal important for the correct assembly of Ysc-Yop T3SS.
NEW INSIGHTS ON THE ROLE OF SPAS AUTOCLEAVAGE IN SALMONELLA TYPE III SECRETION SYSTEM

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Background

The type III secretion system (T3SS also known as ‘injectisome’) is organized in three main structures: a multi-ring basal body that houses the export apparatus, a needle, and a translocon in the host cell membrane. To assemble a functional T3SS, specific substrates must be targeted to the apparatus in the correct order. The substrate specificity switch from early to intermediate substrates occurs once the needle has reached its full length.

The Salmonella T3SS export apparatus protein SpaS, is a multifunctional component involved in the switch. It consists of a transmembrane region and a C-terminal cytoplasmic domain that undergoes autocleavage at a conserved NPTH motif.

Objectives

Elucidating the timing of autocleavage and why it is critical for SpaS function.

Methods

In this study we test expression and secretion levels of T3 proteins by immunoblotting and blue-native gel electrophoresis to study the composition of the assembled T3SS. Our data are complemented by injectisome purification and its visualization by electron microscopy.

Conclusions

Studying autocleavage in its physiological context we could demonstrate that the kinetics of SpaS autocleavage are not regulated by injectisome state of assembly or secretion and that the autocleavage mechanism is independent from needle length control. Rather, autocleavage is a fast process occurring before SpaS incorporation into the injectisome base, which is demonstrated by the fact that a functional injectisomes can assemble on pre-expressed fully cleaved SpaS. We also found that autocleavage at the NPTH motif is not a prerequisite for switching since also SpaS cleavage by an endogenous site-specific protease leads to functional switching.
Background

Magnetotactic bacteria are motile prokaryotes with the ability to swim along the Earth’s geomagnetic field lines due to the presence of intracellular structures called magnetosomes. Magnetosomes are composed of a mineral core of magnetite or greigite surrounded by a 2-4 nm thick lipid bilayer membrane (1). In recent years, the magnetosomes have attracted great attention because of their potential usefulness in biotechnological and biomedical applications (2).

Objectives

In this work, we explore different methods to isolate magnetosomes from Magnetospirillum gryphiswaldense and check their biocompatibility on eukaryotic cells.

Methods

Two mechanic cell lysis procedures are compared based on the amount and quality of the magnetosomes obtained. The isolated magnetosomes were structurally characterized by TEM as cuboctahedral magnetite particles with an average diameter of 45(±3) nm (3). The aggregation behaviour of magnetosomes in solution is followed by Dynamic Light Scattering technique. The Zeta potential was always lower than -30mV indicating moderately stability. Infrared spectroscopy (FT-IR) revealed the presence of poly-β-hydroxybutyrate under some extraction conditions.

The biocompatibility is evaluated in vitro in cytotoxicity assays on murine macrophage ANA-1 cell line. Cells are incubated with 30 pg of magnetite per cell and viability is measured using flow cytometer by annexin-propidium staining. Magnetosomes labeled with fluorescein isothiocyanate are used to track the location of the internalized particles in the cells.

Conclusions
No evident signs of cytotoxicity were found after 48 hour of incubation.


Background

Salmonellosis caused by *S. enterica* is an important food-borne disease worldwide and pathogenicity and antibiotic resistance of these bacteria is an important health concern. *C. elegans* is a current nematode model to test pathogenicity of *Salmonella*.

Objectives

We determined pathogenicity, antibiotic resistance, serotypes and plasmid profiles of 32 non-typhoidal food-borne *Salmonella* isolates and searched for a correlation between these characteristics among the isolates.

Methods

Pathogenicity of isolates was tested in *C. elegans* model. Nematodes (20/group) were fed with *Salmonella* isolates grown on NGM agar (0.6 at OD600). The time required for 50% of nematodes in each group to die (TD50) was calculated. *S. Typhimurium ATCC14028* and *E. coli OP50* were used as positive and negative controls, respectively. Plasmids were isolated by Kado-Liu method and examined by agarose gel electrophoresis (0.7%). Serotyping of isolates was done by slide agglutination test and their antibiotic resistance was investigated by disc diffusion method.

Conclusions

26 of *Salmonella* isolates belonged to Infantis, 4 to Enteritidis, 1 to Kentucky and 1 to Telaviv serotypes. 6 isolates harboured 1-3 plasmids ranging from 1.2 to 35.8 kb. TD50 values were 4.2±0.5 days for *S. Typhimurium ATCC14028* fed, 8.0±0.02 days for *E. coli OP50* fed, and between 3.5-7.2 days for food-borne *Salmonella* fed nematodes. The significance of differences in TD50 values were tested by t test (p<0.05). 22 of isolates were pathogenic in *C. elegans*; 20 of these isolates were multi-drug resistant, 2 were ciprofloxacin resistant and 21 were nalidixic acid resistant. Multi-drug resistant isolates identified as pathogens in nematodes may pose serious health risk for consumers.
Background

*Acinetobacter baumannii* is an emerging opportunistic pathogen which cause severe infections in immunocompromised patients and exhibit resistance to many conventional antibiotics. Factors responsible for *A. baumannii* pathogenesis are numerous; some of them are biofilm formation and hemagglutination, which are dependent on lectin production.

Objectives

The aims were to determine the lectin production ability of the *A. baumannii*, to determine the lectin specificity to different blood types, and to semi-quantify lectin produced by *A. baumannii*.

Methods

The study was carried out with 3 reference strains (ATCC BAA-747, ATCC 19606, NCTC 13423), 8 environmental and 20 MDR *A. baumannii* wound isolates. Lectin production screening was preformed using the hemagglutination assay. The supernatant of overnight cultures were examined using 3 % erythrocyte suspension of different blood types (O Rh+, A Rh+ i B Rh+) and results were recorded after 30 min. The semiquantitative determination of lectin production was examined by *ex vivo* modified hemagglutination assay with erythrocyte of A blood type in microplates using two-fold culture supernatant dilutions (from 1/2 to 1/2048).

Conclusions

All examined isolates were able to produce lectins *in vitro* and expressed hemagglutination activity to all examined blood types. The semiquantitative metod showed that titer is in a range from 1/4 to 1/64. The clinical strains produced lectines in sligtly higher amount comparig to the environmental isolates, but without significant statistical difference (P>0.05). The results indicate that lectine production can be a new target for development of novel anti-*Acinetobacter* agents.
Background

The main complication of colitis would be seen after antibiotic treatment by β lactams, quinolone and aminoglycosides antibiotics. Recently, Klebsiella oxytoca has been known to cause this type of diarrhea.

Objectives

Investigating the prevalence and characterizations of K. oxytoca isolated from patients with antibiotic associated diarrhea. K. oxytoca isolates were also tested for cytotoxin production.

Methods

This study was carried out from May 2011 to Dec 2013. Fecal samples were collected from hospitalized patients who received antibiotics. Initial cultivation was performed on specific media. Clinical isolates were confirmed by PCR using the specific K. oxytoca polygalacturonase (pehX) gene. The double disk diffusion test was used to detect ESBLs producing strains. Tracking ESBLs encoding genes were performed by PCR. The bacteria’s cytotoxin production was conducted using cell culture on Hep2 cell lines.

Conclusions

Five (12%) isolates had cytotoxin activity less than 30%, 12 (30%) strains had moderate cytotoxin activity between 30 and 60% and 23 (58%) strain had cytotoxin activity equal to/or greater than 60%. The cytotoxin producing K. oxytoca can be described as one of the causes of antibiotic induced colitis. Drug discontinuation and creating opportunities for the establishment of intestinal normal flora or appropriate medication after antibiogram would perform better chance earlier in patients with hemorrhagic colitis caused by antibiotics.
Background

*Haemophilus parasuis* is the etiological agent of porcine Glässer's disease.

Objectives

Based on previous studies, three recombinant outer membrane proteins of *H. parasuis* serovar 5 (P2, P5 and D15) were designed and produced for studying their immunogenicity and potential use as vaccine antigens.

Methods

Each gene was amplified and cloned into a pBAD/TOPO vector, this plasmid being enable to express each protein with a tag of histidines for facilitating the subsequent purification. *Escherichia coli* was transformed with the vector and then, it was cultured with arabinose to induce protein expression. As the three proteins were expressed at insoluble fraction, it was necessary to develop a denaturation process using urea, in order to solubilize and refold them. Refolding was performed at the same time as purification by means of immobilized nickel-histidine affinity chromatography. Each of these proteins was retained in the stationary phase and washed with buffers containing decreasing urea concentrations until removing totally urea; the proteins were then eluted with imidazole. Rabbits were used to evaluate the capacity of these proteins to induce a humoral response before testing them in Glässer's disease natural host. Groups of animals were immunized thrice with each of these proteins, and blood was collected after each inoculation. Sera were used to perform indirect ELISAs, in which purified proteins were used as antigen for coating plates.

Conclusions

An increasing in antibody titres with seroconversion can be found after immunizations for recombinant P2, P5 and D15 proteins.
Background

Crohn's disease (CD) is a chronic gastrointestinal inflammatory disease. Adherent invasive Escherichia coli (AIEC) strains, which have the capacity to attach and invade intestinal epithelial cells, and also survive within macrophages; have been associated with CD.

Objectives

To identify immunogenic proteins in the outer membrane of AIEC isolates obtained from Chilean patients with CD, and determine their role in pathogenicity.

Methods

AIECs were isolated from biopsies of Chilean patients with CD. AIEC outer membrane proteins (OMPs) were obtained from cultures grown at 20 °C and 37 °C, and separated by SDS-PAGE. Immunogenic proteins were identified by immunoblot using sera from patients with CD and MALDI-TOF/TOF. Genes coding identified proteins were knocked out and mutant strains were characterized according to: 1) Capacity to adhere and invade intestinal cells, and to survive within macrophages. 2) Differential expression of outer membrane proteins by (2D SDS-PAGE and MALDI-TOF/TOF. 3) Capacity to stimulate production of inflammatory cytokines.

Conclusions

Siderophore receptors IutA and ChuA were identified as immunogenic OMPs. No effect in adherence and invasion capacity was observed after knocking out their genes, however, mutant strains did not survive within macrophages. Additionally, mutation altered the outer membrane profile by apparently affecting expression of virulence-associated proteins. A decrease in IL-1, IL-2, IL-12, IL-4 and IFN-gamma levels was detected in supernatants of macrophages infected with the mutant, while TNF- alpha level did not change. Therefore, iutA and chuA would be required for AIEC intracellular survival in the host, in order to capture iron and express virulence factors. Finally mutating these receptors the strain becomes unstable and avirulent bacteria, which can not survive oxidative stress because other virulence genes are repressed by iron deficiency.
Background
Klebsiella pneumoniae (K. pneumoniae) is one of the main pathogens in nosocomial infections. Type 3 pilus (T3P) is a virulence factor required for biofilm formation and adherence to epithelial cells. Three promoters control the expression of T3P: mrkA (codes for the pilin) mrkHI (code for two activator proteins) and mrkJ (a repressor protein). H-NS nucleoid protein structures the bacterial genome, mainly Gamma-proteobacteria. Hypermucoviscosity that is attributed to the capsule formation, is a hallmark of pathogenic strains. No reports have been described for the role of H-NS protein in regulating T3P and capsule in K. pneumoniae.

Objectives
1. Determine the role of H-NS protein in transcription of T3P.
2. Analyze both the biofilm and capsule formation in the absence of H-NS.

Methods
Transcriptional expression of mrkA, mrkH, mrkJ and mrkJ genes was carried out by qRT-PCR. Using EMSA, promoter regions (PCR) of mrkA, mrkJ and mrkHI were incubated with purified H-NS protein and DNA-proteins complexes were observed. Biofilm formation was quantified by the method of 96-wells polystyrene microplates. The abundance of capsule was determined by measuring spectrophotometrically the supernatants of bacterial cultures at exponential phase.

Conclusions
K. pneumoniae hns mutant affected the growth at exponential phase and resulted in a hypermucoviscosity phenotype. The absence of H-NS affected the biofilm formation and differentially regulated the mrk promoters: derepressed and repressed to mrkHI- mrkJ and mrkA, respectively. H-NS directly bound to the three promoters of T3P. The H-NS protein is a crucial regulator of the main virulence determinants of K. pneumoniae.
EXOGENOUS SUPPLEMENTATION WITH BRANCHED-CHAIN AMINO ACID PROMOTES AEROBIC GROWTH OF SALMONELLA TYPHIMURIUM UNDER NITROSATIVE STRESS CONDITIONS
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Background
An intracellular pathogen *Salmonella enterica* serovar Typhimurium can detoxify cytotoxic nitric oxide (NO) produced in phagocytes of host animals by their metabolism depending largely on the flavohemoglobin Hmp. In hmp mutant *S. Typhimurium*, NO causes deficiency in amino acids including branched-chain amino acids (BCAA). In the BCAA biosynthetic pathway, iron-sulfur cluster-containing enzymes dihydroxyacid dehydratase (IlvD) and isopropylmalate isomerase (LeuCD) have been implicated to be targets of NO, suggesting the importance of BCAA metabolism in the NO-induced amino acid auxotrophy.

Objectives
This study aimed to determine the effect of BCAA supplementation on *Salmonella* resistance to NO under different oxygen conditions, and to test physiological roles of IlvD and LeuCD in the intramacrophage survival of *Salmonella* in the presence or absence of BCAA supplementation.

Methods
Gene mutations deficient in *ilvD* and *leuCD* were constructed in *S. Typhimurium*. With mutant *S. Typhimurium* having combinations of these gene mutations and *hmp* mutation, we measured the effect of BCAA on the susceptibility to nitrosative stress, the NO consumption rate, and the intramacrophage growth.

Conclusions
Under nitrosative stress conditions, BCAA supplementation restored the growth of *hmp* mutant and mutants further lacking IlvD and LeuCD in aerobic and semiaerobic cultures, but not in anaerobic cultures. Intracellular survival of *ilvD* and *leuCD* mutants in macrophages was also promoted by BCAA supplementation. Results suggest that the NO-induced BCAA auxotrophy of pathogenic bacteria due to inactivation of iron-sulfur enzymes in the BCAA biosynthetic pathway could be rescued by bacterial taking up exogenous BCAA available in oxic environments of host animals.
PASTEURELLA MULTOCIDA - A NOVEL PARADIGM FOR BACTERIAL INVOLVEMENT IN CANCER
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Background
Pasteurella multocida toxin (PMT) is mitogenic for many cell lines. It acts via modification of three of the four families of heterotrimeric G-proteins which are- Gq/11, G12/13 and Gi. PMT has been shown to induce anchorage independent cell growth, enhance anti-apoptotic proteins, increase β-catenin signalling in a differentiating adipocyte system, and up-regulate JAK/STAT and MAPK signalling pathway, all of which are implicated in various cancers. These different effects of PMT suggest it may have a carcinogenic potential.

Objectives
Here we are studying PMT as a potential carcinogen by chronically treating cells in vitro with PMT to analyse its involvement in cell transformation.

Methods
The following end points are being monitored to assess the carcinogenic potential of PMT: cell proliferation, stress fibre formation, cellular morphology, levels of normal G-proteins, levels of PMT-modified G-proteins, and anchorage independent growth. Results achieved so far show that PMT-treated cells have an increased cell number, show morphological changes, and differences in protein levels compared to the untreated cells.

Conclusions
Together the data suggests that prolonged exposure to G-proteins modified by chronic treatment with PMT can set cells along a transformation pathway.
SINC, A TYPE III SECRETED EFFECTOR OF CHLAMYDIA PSITTACI, TARGETS EMERIN AND THE INNER NUCLEAR MEMBRANE OF INFECTED CELLS AND UNINFECTED NEIGHBORS

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Background

Chlamydia species encode a type III secretion (T3S) system that enables the translocation of effector proteins into the host cell cytosol, where they target host pathways to benefit the pathogen. We have identified SINC, a novel T3S effector of Chlamydia psittaci, a highly infectious avian pathogen, and medically significant zoonotic pathogen of humans.

Objectives

To identify and characterize the eukaryotic target(s) of SINC.

Methods

Immunofluorescence, immuno-electron microscopy, differential digitonin permeabilization, inhibitors of nuclear transport, BioID (in situ biotinylation analysis of effector-BirA-proximal eukaryotic targets, streptavidin affinity purification, mass spectrometry).

Conclusions

SINC uniquely targets the nuclear envelope (NE) of C. psittaci-infected cells and uninfected neighboring cells. Digitonin-permeabilization studies of infected or SINC-GFP-transfected HeLa cells indicate SINC targets the inner nuclear membrane (INM). SINC localization at the NE was blocked by importazole, confirming SINC import into the nucleus. Candidate partners were identified by proximity to biotin ligase-fused SINC in HeLa cells and mass spectrometry. Several candidates were identified with high confidence including the nucleoporin ELYS, lamin B1 and four INM proteins (emerin, MAN1, LAP1 and LBR), suggesting SINC interacts with host proteins that control nuclear structure, signaling, chromatin organization and gene silencing. GFP-
SINC association with the native LEM-domain protein emerin, a conserved component of nuclear ‘lamina’ structure, was confirmed by GFP pull-down. We conclude that SINC is a novel bacterial effector with the capability of globally altering nuclear envelope functions in the infected host cell and neighboring uninfected cells. These properties may contribute to the aggressive virulence of *C. psittaci*. 


THE ROLE OF BACTERIA AND PUTATIVE TUMOURIGENIC GENES IN THE PATHOGENESIS OF COLORECTAL CANCER

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Background

Colorectal cancer (CRC) remains one of the most prevalent cancers in the Western world. However, the factors leading to the development of colon cancer have not been determined, with genetics, diet and other environmental factors all likely to play a role. Mechanisms by which Bacteroides fragilis and Fusobacterium nucleatum may drive colonic tumour development have been identified. Some B. fragilis isolates contain a metalloprotease toxin known as BFT or fragilysin. BFT has been demonstrated to promote colonic hyperplasia in animal models. F. nucleatum is of relevance due to the consistent finding across several studies that it is enriched on CRC tumours. Its FadA adhesin can mediate invasion into the cell with a resultant increase in expression of host oncogenes.

Objectives

Advances in next generation sequencing technologies have led to a high resolution of the CRC microbial community. The aim of this study is to use a hypothesis led approach to focus on potential key isolates and genes in the development of colonic tumours.

Methods

We are compiling a comprehensive collection of human colonic tissue from patient cohorts representing different stages of disease. These samples are being analysed using a PCR-based assay to determine the prevalence of selected bacterial taxa and genes. Results show primer specificity, PCR optimization and sensitivity of detection that have been experimentally determined.

Conclusions
Overall this work describes the design and validation of a panel of primers with the intention of analysing a fresh collection of human tissue. Preliminary findings using a collection of pilot samples will be presented.
FEMS-3089
Bacterial pathogenicity

• GENOMIC CHARACTERIZATION OF AVIAN PATHOGENIC AND COMMENSAL CHICKEN E. COLI ISOLATES


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Background

Extraintestinal pathogenic E. coli (ExPEC) are a major source of urinary tract infection, newborn meningitis and sepsis in humans. Avian pathogenic E. coli (APEC) cause colibacillosis, a complex systemic infection in poultry. Certain human ExPEC and APEC cannot be clearly distinguished based on molecular epidemiology and their virulence-associated genome content. APEC are considered as a reservoir of virulence- and resistance genes for human ExPEC and a zoonotic risk cannot be excluded.

Objectives

To characterize potential differences between APEC and chicken commensal E. coli, we compared the virulence- and antibiotic resistance-associated genome content of commensal E. coli isolates from healthy chicken with APEC strains.

Methods

E. coli isolates from tracheal and fecal samples of healthy chicken were collected and subjected to virulence gene and antibiotic resistance profiling. The genomes of 127 commensal and APEC isolates were sequenced.

Conclusions

The virulence gene content of commensal chicken and APEC isolates was similar. Comprehensive genome comparison of APEC and chicken commensals will extend our knowledge of bacterial traits that could contribute to pathogenesis and antibiotic resistance. Interestingly, animal husbandry has a great impact on the spread of antibiotic resistance determinants among chicken E. coli isolates. As APEC have a zoonotic potential and serve as potential gene pool for human ExPEC, the increased presence of antibiotic resistance determinants in E. coli from conventionally raised
chicken can facilitate the spread of multiresistant human ExPEC.
PROPERTIES OF THE DSBA PROTEIN (CLA0559) FROM CAMPYLOBACTER LARI RM2100.

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Background

Campylobacter spp. are known to be one of a major cause of gastrointestinal illness throughout the world. Most prevalent species responsible for human infections are C. jejuni and C. coli. Human infections by Campylobacter lari are also associated with gastrointestinal diseases but mostly in developing countries. So far, little is known about C. lari molecular attributes. CLA0559 protein shows high homology to members of DsbA family. As Dsb enzymes control the formation and rearrangement of disulfide bonds during the folding of extracytoplasmic proteins they play an important role in pathogenesis. In silico analysis indicate that C. lari Dsb system might be a novel, different from the ones described for those operating in E. coli or C. jejuni cells.

Objectives

C. lari CLA0559 mutant remains motile but becomes sensitive to DTT. E. coli complementation tests (cadmium resistance, motility test and alkaline phosphatase assay) clearly showed that CLA0559 substitutes for EcDsbA activity and acts in cooperation with EcDsbB. CLA0559 functions in vitro as an oxidase. It what was proven by in vitro RNase activity assay. CLA0559 doesn’t cooperate with ClDsbI1/DsbI2, homologs of DsbB).

Methods

All genetic manipulations were performed using standard molecular biology procedures. For complementation purposes in E. coli cells ClDsbA was expressed from low copy vector. In vitro tests were performed with ClDsbA purified from E. coli using affinity and size exclusion chromatography.

Conclusions

Our results showed that cla0559 gene encodes for ClDsbA protein functioning in an oxidizing pathway in C. lari cells. Its redox partner still remains unknown.
IN VITRO PROPERTIES OF THE TRUNCATED FORM OF HELICOBACTER PYLORI HP0231 PROTEIN.
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Background
Dsb proteins control the formation and rearrangement of disulfide bonds during the folding of membrane and exported proteins. The mechanism of disulfide bond formation in microorganisms is extremely diverse. The H. pylori Dsb system seems to be novel and different from the Dsb system in E. coli. Dsb system plays an important role in H. pylori pathogenesis as H. pylori mutant impaired in disulfide bond formation revealed greatly reduced ability to colonize mice gastric mucosa.

Objectives
Our recent work led to the characterization of the HP0231 functioning mainly in an oxidizing pathway in H. pylori cells. It is intriguing that HP0231 acts as periplasmic oxidase, as EcDsbA, despite its structural resemblance to EcDsbG. Additionally HP0231 acts as chaperone and is involved in the cytochrome c biogenesis. To assess relations between HP0231 structure and its oxidizing activity we tested biochemical activities of the truncated form of HP0231 (HP0231m) containing only catalytic domain.

Methods
All genetic manipulations was performed using standard molecular biology procedures. Correctness of the obtained constructs were verified by sequencing. HP0231m was overexpressed in E. coli Rosetta strain and purified using NGC™ Medium-Pressure Chromatography (Bio-Rad). Purified protein was used to perform insulin reduction assay, chaperone activity assay and izomerization/oxidation RNase activity test.

Conclusions
Our results show that HP0231m mutated protein possess chaperone activity slightly higher than native HP0231 form and at the same time it lost ability to reduce insulin. It displays the same level of oxidation activity as HP0231 and similarly to native HP0231 does not have izomerization activity.
Background
Genital CT infections cause tubal factor infertility (TFI) in some women. Bacterial motif recognition by intracellular pattern-recognition receptors NOD1 and NOD2 can trigger immune response. Whether their functional polymorphisms impact CT infection has not been researched.

Objectives
Our objective was to test the effects NOD1+32656 T>GG and NOD2 1007fs on susceptibility and severity of CT infection in Dutch Caucasian women.

Methods

Susceptibility cohort: We selected 737 women visiting the Amsterdam STD outpatient clinic. Questionnaires were collected in regards to urogenital complaints.

Severity cohort: 490 Dutch Caucasian female patients visiting the UMC Groningen Fertility clinic were selected. Laparoscopy was used to grade the tubal pathology status as TFI grade 0-4. Controls were TFI grade 0 as assessed by either laparoscopy or hysterosalpingography (HSG).

Conclusions
The NOD1 +32656 GG insertion appears protective against CT infection, but predisposes to TFI and a higher occurrence of symptoms in women with a past CT infection. The GG variant might be enhancing successful clearing while acting deleterious in the upper genital tract, causing tubal pathology.
GENOTYPIC AND PHENOTYPIC CHARACTERIZATION OF ATYPICAL ENTEROPATHOGENIC ESCHERICHIA COLI (AEPEC) AND ENTEROHEMORRHAGIC E. COLI (EHEC) ISOLATED FROM OVINE IN BRAZIL

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Background
Atypical Enteropathogenic Escherichia coli (aEPEC) and Enterohemorrhagic E. coli (EHEC) are human pathogens that causes attaching and effacing lesions (A/E) in the intestinal mucosa. EHEC differs from aEPEC because it carries stx genes.

Objectives
Characterizing genotypic and phenotypic samples of aEPEC and EHEC.

Methods
E. coli was isolated from 130 healthy sheep and characterized by PCR technique as aEPEC (eae+/bfpA/stx-) and EHEC (eae+/stx+). 25 samples of aEPEC and 14 samples of EHEC were analyzed by PCR for presence of ehx, tirY-P (Tir phosphorylated), tirS (no phosphorylated), tccp e tccp2. PCR-triplex was used for phylogenetic classification with chuA, vjaA genes and fragments of TspE4.C2. Samples were also tested for sorbitol fermentation (SOR), enzymatic activity of β-D-glucuronidase (GUD), hemolysins production and HEp-2 cell adhesion.

Conclusions
The stx gene subtypes analysis by PCR and cytotoxic effect in Vero cells were realized only in EHEC. The ehx, tirY-P, tirS e tccp2 genes frequency was 40, 60, 16 and 16% in aEPEC and 78, 64, 7 and 28% in EHEC. The tccp gene was present only in aEPEC and only two aEPEC samples were SOR-/GUD+, being the another samples characterized as SOR+/GUD+. Enterohemolysin expression was analyzed in 32% of aEPEC and 66% of EHEC. A diversity patterns adhesion were observed. For the phylogeny, both patotypes were classified as belonging to B1 group. A 100% of EHEC samples presented cytotoxic effect in Vero cells, eight (57%) presented stx1c and seven (60%) stx2d. We concluded that aEPEC and EHEC, isolated from ovine, are two heterogenic groups of bacteria.
Background

*Staphylococcus aureus* is associated with various diseases both in hospitals and in the community. The MRSA strains had manifested primarily in hospitals, but in recent years have appeared in community outbreaks in healthy people, so is called MRSA strains community-acquired (CA-MRSA), and now is important its detection.

Objectives

Identify MRSA strains in vulnerable adults admitted to a Welfare Center

Methods

Pharyngeal, nose and hands swabs were taken, of 100 volunteers in two samples of 50 people each; one in November 2012 and another in May 2014. The presence of *S. aureus* was determined by microbiological methods and biochemical tests. MIC for oxacillin was performed. Detection of genes for resistance to methicillin, *meca* and Panton-Valentine Leukocidin, *lukS-PV / lukF-PV*, was made by PCR. The strains were typed by *spa*-typing and the clonal determination was performed by PFGE.

Conclusions

44 strains of *S. aureus* were isolated: 15 in throat, 13 in nose, and in 16 hands. Only two strains were resistant to methicillin. The dendrogram obtained from PFGE banding pattern shows that almost all strains are identical, which was corroborated by the *spa*-type where 83% of the strains tested showed the same *spa*-type t003. One dominant genotype of *S. aureus* in the population studied was found: *spa*-type t003. The MRSA clone of this type is dominant in Europe, but was not reported in Mexico in addition the type found here is methicillin-susceptible (MSSA). It could be a health problem in this community center.
DETECTION OF COMMUNITY-ACQUIRED METHICILLIN-RESISTANT STAPHYLOCOCCUS SPP IN ICU AND IN AREA HOSPITAL.

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Background
Nosocomial infections are an important cause of morbidity and mortality worldwide. At present, Staphylococcus aureus and Staphylococcus epidermidis are important etiologic agents of hospital infections. S. aureus is common in surgical areas of adult intensive care (ICU), sepsis being the most common process involved. Currently strains of community-acquired MRSA (CA-MRSA) have entered in the hospitals.

Objectives
The aim of this study was to detect the strains of community-acquired methicillin-resistant Staphylococcus in a General Hospital

Methods
Swabs of oral and nasal cavity of 52 patients, both male and female with different pathologies were taken, aged between 28 and 72 years, 14 patients were in the ICU and 38 the hospitalization area. The presence of S. epidermidis or S. aureus was determined by microbiological methods. MIC for oxacillin was measured. The presence of the mecA gene, the Panton-Valentine leukocidin gene, and the arginine catabolic mobile (ACME) gene, were detected by PCR. The strains were typed by spa-type.

Conclusions
We found that 100% of the samples analyzed in the area of hospitalization were strains of S. aureus, of which 37% were resistant to methicillin (MRSA). While 93% of the strains tested in ICU were S. epidermidis, and 31% of these were methicillin resistant (MRSE). All methicillin-resistant strains showed the presence of the mecA gene. One CA-MRSA strain and two CA-MRSE strains were found.

We detected the presence of community-acquired methicillin-resistant Staphylococcus in the hospital analyzed, so it is important to conduct periodic sampling to prevent outbreaks caused by this pathogen.
DETECTION OF COMMUNITY-ACQUIRED METHICILLIN-RESISTANT STAPHYLOCOCCUS AUREUS IN A NURSERY

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Background

*Staphylococcus aureus* is a microorganism of great medical importance. For many years it has been recognized as one of the major pathogens for humans. Infections with methicillin-resistant *S. aureus* (MRSA), are usually acquired in hospitals (strains HA-MRSA). However, in the late 90’s, MRSA strains emerged in healthy adults and children in communities. These strains cause infections in the community. The prevalence of these infections has increased significantly in recent years. Strains of *S. aureus* that cause these infections are called strains MRSA community-acquired (CA-MRSA).

Objectives

The aim of this work was to make the identification and molecular characterization of strains of *Staphylococcus aureus* isolated in a daycare of Mexico City.

Methods

Throat swabs samples from 87 children, between 2 and 6 years old were taken. *S. aureus* was identified by microbiological methods. The *mecA* and Panton-Valentine leukocidin (PVL) genes were detected by PCR. The SCCmec type and spa-type were determined.

Conclusions

25% of the population had *S. aureus* in the throat. From the strains isolated, we found that 22% (five strains) were MRSA, of which only one present the *mecA* gene, the gene of PVL and SCCmec type IV, therefore we found a CA-MRSA strain. The results show there may be strains CA-MRSA in healthy carriers in Mexico City, so strains CA-MRSA are present circulating in the community.
Background
Staphylococcus aureus is a pathogenic bacterium that is associated with various diseases both in hospitals and in the community. Between 20% and 35% of the adult population are carriers of this microorganism in the nasal vestibule, but can also be found in the pharynx of people. The MRSA strains had manifested primarily in hospitals, but in recent years have appeared in community outbreaks in healthy children and adults, with no history of hospitalization. These strains were called community-acquired methicillin-resistant Staphylococcus aureus (CA-MRSA).

Objectives
The objective of this work was to detect the presence of strains CA-MRSA in an orphanage in Mexico City.

Methods
Pharyngeal and nasal swabs of 80 people between 3 and 19 years old of both genders were taken. We identified S. aureus by microbiological methods. The mecA and Panton-Valentine leukocidin (PVL) genes were detected by PCR. The SCCmec type and spa-type were determined.

Conclusions
56% of the population had S. aureus; 12.5% only in the pharynx, 17.5% only in the nose and 26% at both sites. We detected only four MRSA strains (5%), one in the pharynx and three in the nose, which presented the mecA, the PVL genes, and have the SCCmec type IV, so they are CA-MRSA strains. The results show that there are CA-MRSA strains in healthy children and adolescents in an orphanage in Mexico City.
IDENTIFICATION AND REGULATION OF A NOVEL CITROBACTER RODENTIUM GUT COLONIZATION FIMBRIAE (GCF)

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Background

Citrobacter rodentium is a natural mouse bacterial pathogen that has been extensively used as a surrogate model for studying the human pathogens enteropathogenic and enterohemorrhagic Escherichia coli. During infection these pathogens employ surface structures called fimbria to adhere and colonize the host intestinal epithelium; however, for C. rodentium only a small number of its fimbrial operons have been studied.

Objectives

To analyze the regulation and role in colonization of the C. rodentium fimbrial operon gcfFGABCDE.

Methods

The role in colonization of the Gcf fimbriae was evaluated in mouse colonization assays, while the regulation of the gcf operon was assessed using transcriptional fusions and western blot. The promoter of gcf was identified by in silico analysis, primer extension assays and site-directed mutagenesis. Mutant strains and electrophoretic mobility shift assays were used to determine the role of H-NS as a repressor of gcf expression.

Conclusions

Here we report the characterization of Gcf (Gut colonization fimbriae) as an important C. rodentium colonization determinant of the mouse gastrointestinal tract. We demonstrated that the promoter of the gcf fimbrial operon is highly repressed under several in vitro growth conditions by H-NS1, one of the five H-NS paralogs encoded in the C. rodentium genome. H-NS binds to the regulatory region of gcf, further supporting its direct role as a repressor. The gcf operon possesses novel and interesting features that open future opportunities to expand our knowledge of these
essential bacterial structures during infection. Work supported by grants IN209713 (DGAPA) and 154287 (CONACyT).
FEMS-2245
Bacterial pathogenicity

SALMONELLA TYPHIMURIUM STRAINS WITH DISTINCT CLINICAL PHENOTYPES CAN BE DIFFERENTIATED AT THE TRANSCRIPTOMIC LEVEL
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Background
Salmonella Typhimurium infects a wide range of animal hosts, and generally causes a self-limiting gastroenteritis in humans. However, some variants of this serovar, sequence-type ST313, have been associated with an emergent invasive Salmonella disease in sub-Saharan Africa, which usually affects susceptible HIV+ or malarial individuals. A genomic comparison between an ST313 isolate, D23580, and the well-characterized 4/74 strain (sequence-type ST19) shows that both strains share 96% coding genes. Genetic differences include 1000 SNPs, D23580-specific prophages and the presence of pseudogenes.

Objectives
We tested the hypothesis that the two strains had different pathogenic mechanisms that are reflected by altered gene expression patterns and gene fitness in environmental conditions that reflect the infectious process.

Methods
RNA-seq-based transcriptomic data were obtained for strains 4/74 [1] and D23580 grown under seventeen infection-relevant in vitro conditions. Transcriptomic data were validated with a proteomic approach. In addition, a transposon library was generated in the D23580 strain and sequenced after passaging in different media.

Conclusions
Comparative transcriptomics between the two strains revealed that the proportion of differentially-expressed genes varied between 1% to 9% of all genes in various stress conditions. Proteomics for the early stationary phase condition confirmed 60% and 54% of the transcriptomic data in 4/74 and D23580, respectively. We are currently analysing the D23580 transposon library data. The differences observed in the expression and fitness of virulence-associated genes under specific environmental conditions may reflect altered regulatory mechanisms of these Salmonella strains.

Background
Atypical Enteropathogenic *E. coli* (aEPEC) is a human pathotype implicated in endemic infantile diarrhea. aEPEC with an identical virulence gene profile has been isolated from several animals including dogs, suggesting a zoonotic behavior. Adherence to host enterocytes is a key step of aEPEC virulence, being mediated through the expression of LEE locus genes, leading to formation of attaching-effacing (A/E) lesions. However, similar to LEE-positive Shiga Toxin-producing *E. coli* (STEC), aEPEC may contain distinct non LEE-associated effectors molecules and adhesins.

Objectives
Investigate the adhesion profile of aEPEC strains through search of non LEE-associated virulence markers and interaction assays with epithelial cells.

Methods
We analyzed by PCR the distribution of several virulence genes, including 16 *nle* genes, among aEPEC strains isolated from children (n=5) and dogs (n=5). Adherence phenotypes were determined through *in vitro* assays with HEP-2, Caco-2 and T84 cells.

Conclusions
The occurrence of virulence genes was: *ecpA* (100%), *fimH* (80%), *lpfA* (60%), *hcpA* (50%), *toxB* (30%), *iha* (10%). Fifteen *nle* genes were detected in all strains with different combinations. aEPEC of O51:H40 and O4:H16 serotypes carried the *nleB* and *nleE* genes, both located in PAI-122, an important pathogenicity island of STEC O157:H7. All strains showed a localized-like adherence phenotype (LLA) in HEP-2, Caco-2 and T84 cells. Although aEPEC strains are a very heterogeneous group, these findings shows a similar virulence profile between human and canine isolates belonging to the same serotype, suggesting an important role for dogs in the maintenance of the virulence gene pool of aEPEC.
Background
L-asparaginase II (AnsB) enzyme of *E. coli*, known for their use in treatment of children with acute lymphoblastic leukemia (ALL), and has been described as a bacterial periplasmic protein. However, we detected its presence in outer membrane proteins (OMP) extracts of different STEC serotypes, by Western blot using sera from STEC infected-patients and diagnosed with HUS. Recent reports using a mouse T cells co-cultured with wild type *S. Typhimurium* strain, showed that AnsB is sufficient to suppress T lymphocyte blastogenesis.

Objectives
Determine the AsnB presence in outer membrane protein extract of different STEC serotypes, its secretion and effect on T lymphocyte proliferation

Methods
The OMP and soluble proteins fractions were separated by SDS- PAGE 12% and western blot directed to AnsB detection was performed with commercial specific antibody anti-AnsB. A electron microscopy using a secondary antibody labeled with gold particles for visualization by immunogold was done. Peripheral blood mononuclear cells, from healthy individuals were incubated in the absence or presence of STEC O157: H7, O157:H7ΔansB, O157:H7ΔansB/pVB1_ansB, STEC O113:H21, commensal *E. coli* HS strain and *S. Typhimurium*, as a negative and positive control for AsnB effect, respectively. The suppression of proliferation was measured by flow cytometry. Study protocols for peripheral-blood mononuclear cells were approved by the Institutional Review Board of the Faculty of Medicine, University of Chile.

Conclusions
We observed by immunogold that the AsnB enzyme is secreted and some molecules remain associated with the outer membrane in STEC. AnsB expressed in O157: H7 contribute to its pathogenicity by inhibiting T lymphocyte proliferation.
Background

YadA is a trimeric autotransporter adhesin present in *Yersinia enterocolitica* and *Yersinia pseudotuberculosis*. It has a transmembrane domain which forms a highly stable β-barrel anchoring it to the outer membrane and an N-terminal passenger domain which translocates through the β-barrel forming a coiled-coil stalk followed by a sticky head which mediates attachment of bacteria to host cell ECM components (1). The mechanism by which the N-terminal domain is translocated is still under debate. A relatively flexible region has been found near the C-terminus of the passenger domain known as the ASSA region which has been proposed to act as a hairpin during translocation (2).

Objectives

The idea is to mutate residues in the ‘ASSA’ region to obtain stalled translocation intermediates. The intermediates will be analyzed with various methods, followed by structural analysis using solid state NMR experiments.

Methods

Heat stability assays of the mutants indicated a different tertiary structure as well as less stability compared to wildtype. Infrared Spectroscopy indicated loss of α-helical content of mutant YadA by 33%. Electron Microscopy images showed that mutant YadA does not form fibre-like projections on cell surface and cells do not autoaggregate which was proved in Autoaggregation assays.

Conclusions

Preliminary results indicate a translocation intermediate which needs to be further analysed by ssNMR.

References

FUNCTIONAL GENETIC ANALYSIS OF THE ENVZ-OMPR TWO-COMPONENT SYSTEM IN ENTEROHEMORRHAGIC E. COLI PATHOGENICITY

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Background
Enterohemorrhagic Escherichia coli O157:H7 (EHEC) causes severe diarrhea, hemorrhagic colitis, and can also lead to life-threatening diseases like hemolytic uremic syndrome (HUS). Several EHEC virulence factors have been reported and encoded in the virulence plasmid pO157 and the O157 pathogenicity islands (O-Islands), including the locus of enterocyte effacement (LEE) island (148 O-Island encodes the type three secretion system and effector proteins) and the 93 and 45 O-Islands (encode the two Shiga-like toxins).

Objectives
In our previous studies, we utilized the model animal C. elegans for studying EHEC infection in vivo. Using this EHEC-C. elegans model to perform an EHEC transposon library screen, we found that mutations in the ompR gene confer the virulence-attenuated phenotype of EHEC against C. elegans animals. Here, we aimed to test whether the EnvZ-OmpR two-component system can regulate these reported EHEC virulence genes to infect C. elegans.

Methods
We identified several potential OmpR binding sites in the promoter regions of these EHEC virulence genes through in silico analysis. Furthermore, the mRNA transcript levels of these EHEC virulence genes were significantly down regulated in the ompR deletion mutant.

Conclusions
Taken all together, our current data suggested that OmpR is one of the EHEC virulence master regulators to manipulate the expression of virulence factors during infection in vivo.
Background

*Pseudomonas taiwanensis* is a board host range Gram-negative bacterium. Recently, we found that *P. taiwanensis* displayed strong antagonistic activity against rice pathogen *Xanthomonas oryzae* pv. *oryzae* (*Xoo*). Rice bacterial blight caused by *Xoo* is one of the most destructive diseases of rice worldwide.

Objectives

Understanding which genes are required by *P. taiwanensis* to resist the leaf blight of rice pathogen *Xanthomonas oryzae* pv. *oryzae* (*Xoo*).

Methods

Here we combined whole genome sequencing and Tn5-transposon mutagenesis to identify anti-*Xoo* toxin factors and related regulatory pathway. A high quality complete sequencing was accomplished by a combination of Roche 454, Illumina Solexa, Sanger sequencing and Optical mapping.

Conclusions

The complete sequence of the 5.08-Mb genome sequence and 4666 CDS were determined. We used transposon random insertion to identify genes involved in the production and regulation of anti-*Xoo* activity based on analyses of 6000 individual insertion-strains. Our results show that the siderophore pyoverdine biosynthetic gene (*pvd*), Type VI secretion system (T6SS), and EnvZ/OmpR two-component system have important roles in antagonistic activity against *Xoo*. On the other hand, the toxicity of *P. taiwanensis* was negatively regulated by the RpoS sigma factor. We further used MALDI-imaging mass spectrometry (MALDI-IMS) to track pyoverdine in *P. taiwanensis* and mutants. The results showed that pyoverdine was positively regulated by EnvZ/OmpR two-component system and secreted by T6SS. In contrast, pyoverdine was negatively regulated by RpoS. To the best of our knowledge, this is the first report that Pyoverdine has toxicity toward *Xoo* and T6SS can secrete small compounds.
Background

IL-6 is a key proinflammatory cytokine which has been considered to be important in the pathogenesis of periodontal disease. Host modulatory agents directed at inhibiting IL-6, therefore, appear to be beneficial in terms of attenuating periodontal disease progression and potentially improving disease susceptibility.

Objectives

In the current study, we investigated the effect of flavonoid isorhamnetin on the production of IL-6 in murine macrophages stimulated with LPS from P. intermedia, a pathogen implicated in inflammatory periodontal disease, and its mechanisms of action.

Methods

LPS from P. intermedia ATCC 25611 was isolated by using the standard hot phenol-water method. Culture supernatants were collected and assayed for IL-6. We used real-time PCR to quantify IL-6 and HO-1 mRNA expression. HO-1 protein expression and levels of signaling proteins were monitored by immunoblot analysis. DNA-binding activity of NF-κB was analysed by using the ELISA-based assay kits.

Conclusions

Isorhamnetin significantly down-regulated P. intermedia LPS-induced production of IL-6 as well as its mRNA expression in RAW264.7 cells. Isorhamnetin upregulated HO-1 expression at both gene transcription and translation levels in P. intermedia LPS-activated cells. In addition, inhibition of HO-1 activity by SnPP blocked the inhibitory effect of isorhamnetin on IL-6 production. Isorhamnetin failed to prevent LPS from activating either JNK or p38 pathways. Although further research is required to clarify the detailed mechanism of action, we propose that isorhamnetin may contribute to blockade of the host-destructive
processes mediated by IL-6, and could be a highly efficient modulator of host response in the treatment of inflammatory periodontal disease.
Background
*K. pneumoniae* is an opportunistic pathogen that affects immunocompromised patients and commonly associated with nosocomial infections. In the past two decades, the capsular serotype K1 has emerged as the predominant pathogen in causing liver abscess, affecting primarily Asians. While many studies have been reported for K1 and K2 which has comparable virulence to K1, very few of the 82 capsular serotypes have been studied.

Objectives
To determine the association between *K. pneumoniae* serotypes K1, K11, K19 and K31, and liver abscess and complications in kidney, spleen and lungs

Methods
Bacterial suspensions of the four serotypes of *K. pneumoniae* were inoculated intraperitoneally into healthy mice models. After 72 hours, the organs were aseptically removed, and histopathological examination and bacterial enumeration were carried out. The presence of liver abscess and complications in the other organs were characterized by the observation of necrosis and severity of inflammation, respectively.

Conclusions
From the histopathology examination, serotypes K1, K19 and K31 resulted in liver abscess, unlike K11. The spleen was severely inflamed with K1 and mildly inflamed with the other three serotypes. Moderate inflammation was observed in the lungs infected with K1 while the other serotypes caused mild inflammation. The immune system of the mice was able to clear the bacteria in all the organs within 72 hours when infected with all four serotypes. Our preliminary data supports a statistical association between non-K1 *K. pneumoniae* serotypes and liver abscess and organ complications in healthy mice models.
THE EFFECTOR PROTEIN SDHA FROM PISCIRICKETTSIA SALMONIS IS OVEREXRESSED DURING AN INFECTIOUS PROCESS IN CELL LINE SHK-1.

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Background
Piscirickettsia salmonis is the etiological agent of Piscirickettsiosis disease in Chilean Salmon farming and cause significant economic losses in this industry. For similar pathogens like Legionella pneumophila and Coxiella burnetii some effector proteins were described that modify cellular processes of the host to establish intracellular infection. Nowadays there is no evidence for similar effector proteins of P. salmonis. Only one report described a type 4b secretion system (SST4b) in this pathogen.

Objectives
Evaluate gene expression levels of dotH, dotG and sdhA genes during an infectious process induced by Piscirickettsia salmonis on SHK-1 cell line.

Methods
RNA extraction was performed combining Trizol with commercial kit for total RNA. RT reaction was performed with M-MLV and Real Time PCR was performed by commercial SYBR Green master mix kit.

Conclusions
The expression levels of dotG, dotH and sdhA genes are increased in a correlated manner between them during an infectious process induced by Piscirickettsia salmonis. At early times such 2 hours after infection the expression levels are increased and then decrease a few fold-change to increase again at the final days of infection but not as much as in the beginning of the experiment. From this pattern of gene expression we propose that the SST4b is involved in traslocating the effector protein SdhA to the cytoplasm of the infected cell.

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LIPL21 LEPTOSPIRAL PROTEIN BINDS EXTRACELLULAR MATRIX(ECM) COMPONENTS B2 INTEGRINS AND C3/C4 COMPLEMENT FACTORS

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Background
Leptospirosis is a zoonosis with global distribution recognized as a re-emergent disease caused by pathogenic bacteria of the genus *Leptospira*. LipL21 is major leptospiral surface protein identified by proteomics.

Objectives
The goal of this work is to obtain the recombinant protein LipL21 expressed in *E. coli* and to evaluate its binding activity with extracellular matrix components (ECM), integrins cell receptors (αLβ2, αMβ2) and C3b and C4b complement factors.

Methods
The gene LIC10011 encoding for LipL21 was cloned and expressed in *Escherichia coli*. Protein attachment to individual ECM components, αLβ2, αMβ2 integrins, and C3b and C4b was screened by ELISA, and the binding was evaluated by probing the reaction with anti-LipL21 serum. Binding of LipL21 recombinant protein to ECM and integrins was compared with their binding to gelatin, BSA and fetuin negative controls, by using Student’s two-tailed t-test.

Conclusions
LipL21 was expressed in *E. coli* with a 6X HIS sequence tag at N-terminal. The protein was present in its soluble form, and was successfully purified in Ni$^{2+}$-charged resin, as assessed by SDS-PAGE. Binding of LipL21 was statistically significant with cellular fibronectin, collagen IV, laminin and αLβ2, αMβ2 integrins, which may be justified by the occurrence of DGEA integrin binding domain in LipL21 protein. The binding of LipL21 with C3b and C4b was significantly enhanced when the protein was associated with αMβ2 integrin, probably due the presence of binding sites for these components in the ligand. These results suggest that this protein has the potential to cooperate in the bacterial immune evasion and dissemination in the hosts.
CHARACTERIZATION OF VIRULENCE FACTORS IN ESCHERICHIA COLI ISOLATES FROM HAEMOCULTURES

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Background

Extraintestinal pathogenic Escherichia coli (ExPEC) is one of the main etiological agents of Gram-negative bloodstream infections. Capacity of ExPEC to cause systemic infections is due to genes encoding virulence factors, which include adhesins, toxins, polysaccharide coatings, siderophores or iron acquisition systems.

Objectives

The aim of our study was to analyse the presence of virulence genes fimA, bfpA, pap, sfa, eae, afa, cnf1, α-hly, aer, iai, lt, st, ipaH, iucC, cdhI-V and to determine the phylogenetic origin of E. coli strains isolated from septicemic patients.

Methods

This study included 133 E. coli strains isolated by haemoculture from adult patients with bacteriemia. Automated blood culture system (Bactec 9050) was used in haemocultivation. E. coli strains of positive haemoculture were identified on a Microflex MALDI Biotyper (Bruker Daltonik) and confirmed by standard biochemical methods ENTEROtest 16, ENTERO-Rapid 24 (Lachema, Czech Republic). Polymerase chain reaction was used to detect virulence genes and to determine ECOR phylogenetic classification of E. coli strains.

Conclusions

We found that fimA (96%), aer (75%) and iucC (68%) represent most common genes encoding virulence properties. Toxin studies revealed a relatively high incidence of cnf1 (20%) and α-hly (19%). Phylogenetic classification showed that 80% of E. coli strains tested fall into phylogenetic groups B2 and D, representing main groups of virulent E. coli.
Background

Enterotoxigenic *Escherichia coli* is a leading cause of diarrhea, mainly in children and travelers to endemic regions. Currently, there is no effective vaccine to prevent ETEC-caused diarrhea. ETEC colonizes the small intestine by using a diverse set of adhesins including about twenty different pili. However, between 15-50% of the isolates obtained from diarrhea cases worldwide are negative in detection of the known adhesins, suggesting presence of additional unidentified adherence determinants and, at the same time, presenting an obstacle in the development of adhesin-based vaccine candidates.

Objectives

To identify novel fimbrial loci in ETEC strains negative for detection of the known adhesins

Methods

Genomes of 35 ETEC strains isolated from diarrhea cases in Chile (14 isolates), Kenya (5), Mozambique (2), The Gambia (1), Mali (3), Bangladesh (3), India (2) and Pakistan (5), which were negative in detection of 23 adhesin genes, were partially sequenced by a paired-end protocol (Illumina, HiSeq 2000). Genomic sequences were screened for 161 fimbrial usher genes belonging to nine families of fimbriae (alpha, beta, gamma-1, gamma-2, gamma-3, gamma-4, kappa, sigma and pi), in order to locate homologous genes and find putative novel loci encoding usher/chaperone assembled adhesins. Genomic sequences of non-pathogenic *E.*
coli strains (13) and other ETEC strains (H10407, E24377A and B7A) were included as controls.

**Conclusions**
Loci encoding putative novel gamma-2 fimbriae were found in 23 strains, being frequent among ETEC strains negative for detection of known adhesins.

FEMS-1018
Bacterial pathogenicity

COORDINATED REGULATION OF THE MAJOR VIRULENCE GENE PELD OF THE PHYTOPATHOGEN BACTERIUM Dickeya dadantii BY FIS, CRP AND H-NS

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Background
Pathogenic bacteria tightly regulate their virulence genes in response to the variations of their environment. Many transcription factors have been identified for such genes, but their interactions remain poorly understood. The pel genes of the phytopathogen bacterium D. dadantii are a good example of this situation, with a dozen identified regulators, yet an integrative view of their action was lacking.

Objectives
As a first step towards an integrative model, this work aims at taking into account the interactions between the major global regulators of virulence (Fis, CRP and H-NS) in the regulation of the pelD virulence gene. In particular, Fis has 2 sites overlapping the promoter (-34 and -10 from the transcription start site) and an upstream site (-126).

The relationships between those sites are investigated here as well.

Methods
Binding sites were inactivated individually by site-directed mutagenesis and the impact of the modifications was evaluated both in vivo by gfp gene fusion measurements, and in vitro by quantitative DNase I, potassium permanganate footprinting and in vitro transcription.

Conclusions
D. dadantii has established 3 redundant mechanisms to ensure a full repression of pelD in exponential phase: 2 of the Fis binding sites (-34 and -10) directly compete with RNA polymerase, 1 Fis site (-34) prevents the activator CRP from binding and a Fis-regulated (involving the -126 Fis site) reverse promoter represses the pelD promoter. On top of this, H-NS modulates the action of Fis. These results show that the importance of interactions in transcriptional regulation should not be underestimated.
Background

The phenotype of *Salmonella* yqiC gene and its impacts on host responses are little known.

Objectives

To investigate whether yqiC is responsible for *Salmonella* colonization/invasion, bacterial motility/adherence, and interleukin (IL)-8/human β-defensin-3 (hBD3) production in human intestinal epithelium.
Methods
HeLa, Caco-2, LS174T, and THP-1 cells were infected with *Salmonella* Typhimurium.
wild-type SL1344, yqiC-depleted mutant ΔyqiC, its complemented strain ΔyqiC, and filC-deleted mutant ΔfilC (MOI=5) for 2 hours, and treated with plain medium (output pool A: colonizing bacteria) or gentamicin (output pool B: invading bacteria) for 1 hour. At these times cell-associated bacterial numbers were calculated. Next, motility and adherence of these strains were examined by soft agar motility assays and yeast agglutination tests. Last, LS174T cells were treated with these strains, flagellin, and IL-1β for 2 hours, incubated in gentamicin for 1 hour, and the infections were continued for 15 hours. At the end-point, the supernatants were collected to measure IL-8 and hBD3 using ELISA.

Conclusions

Compared with SL1344, ΔyqiC was attenuated in bacterial colonization and invasion in 4 cells (Fig.1), lost swimming ability (Fig.2A), and constitutively expressed type 1 fimbriae (Fig.2B). IL-8 production in ΔyqiC-infected cells was lower than that in SL1344-infected cells, with lower levels than those in ΔfilC-infected cells (Fig.3A). Furthermore, ΔyqiC and ΔfilC induced lower hBD3 secretion than SL1344 did in LS174T cells (Fig.3B).

In conclusion, yqiC is required for Salmonella Typhimurium colonization, and host IL-8 and hBD3 production. This is mediated by its modulation in Salmonella motility and flagellation via downregulation of type 1 fimbriae.
FEMS-1024
Bacterial pathogenicity

ROLE OF FIBRONECTIN IN THE ADHERENCE OF DIARRHEAGENIC E. COLI TO INTESTINAL CELLS AND THE INDUCTION OF SECRETION OF PRO-INFLAMMATORY CYTOKINES
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Background
Fibronectin has been shown to be important for the adherence of diarrheagenic E. coli (DEC), but the biological relevance of the DEC-fibronectin-mediated interaction to intestinal cells remains unclear

Objectives
We evaluated the impact of the DEC-fibronectin-mediated interaction on the induction of secretion of pro-inflammatory cytokines

Methods
We assessed the ability of EAEC, ETEC and EHEC to bind to HEp-2 cells in the presence or absence of purified fibronectin protein and we quantified the amount of IL-8 secreted by infected cells. Additionally, we quantified the expression of FOS, NFKB, IL8, CCL20, IL1A and TNFalpha genes. Alternatively, we quantified the IL-8 secretion of HEp-2 cells transfected with small hairpin RNA (shRNA) for fibronectin or scrambled shRNA infected with EAEC strain.

Conclusions
We found a significant increase in the adherence to HEp-2 cells pre-incubated with fibronectin compared with cells not incubated with this protein for all DEC assayed. However, the IL-8 secretion was significantly reduced in the presence of fibronectin. Real-time PCR assay indicated that of all genes assayed only IL8 gene expression was reduced in HEp-2 cells pre-incubated with fibronectin. Experiments using fibronectin shRNA revealed a decrease in the EAEC adherence to cells, but no changes in the IL-8 secretion.

Overall, all data suggested that fibronectin participates exclusively in the adherence of DEC to epithelial cells, but not in the inflammation originated as a consequence of the infection.
Background
Attenuated *Salmonella* employed as live vaccine carrier could provide protection against many infectious diseases. To guarantee successful vaccination, balance between attenuation and immunestimulation is required. Since aromatic amino acids are not freely available in the host, ΔaroA is commonly used as an attenuating factor. However, we observed that an aroA deficiency also dramatically modified the phenotype of *Salmonella* by increasing bacterial virulence.

Objectives
In the present work the molecular basis for the phenotypic changes in the strains should be determined by transcriptional profiling and gene deletion of apparently relevant genes.

Methods
We were able to demonstrate via transcriptome profiling that *Salmonella* is consuming excessive pyruvate by up-regulating sugar pathways like manXYZ or glpABCQT resulting most likely in an osmotic imbalance. Furthermore, aroA deficient mutants are more prone to express FljB flagella. These metabolic dysregulations could be a possible explanation for the observed phenotype of increased virulence. In accordance after intravenous application of ΔaroA *Salmonella* to mice (86/609/EEC), significantly increased induction of pro-inflammatory cytokines like TNF-α or IFN-β was observed emphasizing the importance of this deletion for *Salmonella*’s phenotype. We also employed such bacteria in bacteria-mediated tumor therapy. We could show that the ΔaroA strains display an increased tumor colonization and an improved anti-tumor response.

Conclusions
Disturbing the bacterial metabolism by mutating aroA in *Salmonella* leads to a modified phenotype that results on one site in attenuation but also improves *Salmonella*’s ability to induce an immune response. Therefore this mutation might be a preferable modification for vaccine and cancer research.
THE CAPSULE OF CAMPYLOBACTER JEJUNI PREVENTS BINDING TO SIGLEC-1 AND SIGLEC-7
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Background
Campylobacter jejuni (C. jejuni) is the leading cause of bacterial gastroenteritis worldwide. Besides diarrhoea, an infection with C. jejuni can precede the acute and severe polyneuropathy Guillain-Barré syndrome (GBS). Lipooligosaccharides (LOS), present in the outer membrane of C. jejuni, cause activation of the innate immune system and sialylation of LOS is critical for the induction of GBS. Sialylated LOS can be recognised by two host immune-receptors, Siglec-1 and Siglec-7. Recent research indicates that C. jejuni LOS are not always exposed to the external environment. We hypothesize that C. jejuni exploits the polysaccharide capsule as an evasion strategy to prevent immune recognition mediated by Siglec-1 and Siglec-7.

Objectives
To determine whether the polysaccharide capsule of C. jejuni prevents the specific binding of bacterial LOS to Siglec-1 and Siglec-7.

Methods
Capsule and sialic acid transferase knock-out mutants were generated. Bacteria were FITC-labelled, incubated with Siglec-1 or Siglec-7 transfected cells and binding of live or heat-inactivated bacteria was measured using flow cytometry.

Conclusions
Absence of the capsule of C. jejuni enhanced binding of C. jejuni sialylated LOS to Siglec-1 and Siglec-7. For Siglec-1, this was observed for both live and heat-inactivated bacteria. In contrast, for Siglec-7 the difference in binding between wild-type and non-capsulated C. jejuni was only observed for live bacteria. Sialic acid mutants showed lower binding to Siglec-1 and Siglec-7. Our results give insight in how the immune system recognizes C. jejuni and define the capsule as a bacterial factor that may influence the onset of GBS.
CTRA-DEPENDENT BRUCELLA ABORTUS CELL CYCLE REGULATION IN CULTURE AND DURING INFECTION

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Background

Brucella abortus is a facultative intracellular pathogen for mammals. Many aspects of its intracellular trafficking have been investigated in HeLa cells and RAW264.7 macrophages. However very little is known about its cell cycle regulation, except that bacteria are blocked in G1 phase during early hours of infection.

Objectives

The main goal of this project is to investigate the regulon of CtrA, a central transcription factor. We also decided to investigate a two-component system (TCS) located upstream of a signalling pathway regulating CtrA phosphorylation status. This TCS involves a histidine kinase, PdhS, and a single-domain response regulator, DivK (Hallez).

Methods

We used RSA-Tools (Van Helden) to look for the consensus sequence bound by CtrA in B. abortus genome. This in silico approach allowed the prediction of a long list of putative CtrA targets. A ChIP-seq analysis confirmed part of these targets. The activity of some of these promoters was monitored by fusing them to a gene coding for an unstable GFP. We performed in vitro kinase assays to test PdhS autophosphorylation and its ability to transfer the phosphate group to DivK.

Conclusions

Promoters bound by CtrA are predicted to control genes involved in cell cycle regulation, such as division, chromosome replication and segregation. The reporter system showed that the activity of some promoters varies in bacteria grown in rich culture medium according to their cell size, as well as during B. abortus intracellular trafficking in HeLa cells. In vitro kinase assays show that PdhS can autophosphorylate and quickly transfer its phosphate group to DivK.
THE DUAL BEHAVIOUR OF CARBON STARVATION GENES PROVIDES A LINK BETWEEN METABOLISM AND PATHOGENESIS OF SALMONELLA

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Background

The ability of the enteric pathogen Salmonella to utilize a diverse set of nutrients, allows it to survive in various nutrient limiting conditions, rendering it one of the most successful pathogens. Salmonella employs sophisticated machinery to either acquire a specific nutrient from the surrounding environment or modify the host’s ecosystem for its own metabolic benefit. Carbon starvation (cst) genes represent one such class that are proposed to be expressed during carbon starvation, the most common stress encountered by Salmonella. Two cst genes, cstA and yjiY, predicted to mediate peptide utilization, were previously reported to be regulated by the global regulator CsrA and YehU/YehT two-component system in E. coli.

Objectives

In Salmonella, cstA was shown to be required for virulence in C. elegans, however, the underlying mechanism remains unknown. The objectives of this study are to establish the role of cst genes in metabolism and to understand how they affect Salmonella pathogenesis.

Methods

We generated knockout for the genes cstA and yjiY in Salmonella Typhimurium. Phenotype microarray on various nutrient sources along with transcriptome analysis was carried out for wild-type and knockout strains. Virulence was assessed using cell culture and animal models.

Conclusions

Phenotype microarray confirmed the importance of cst genes in metabolism of Salmonella. Despite the high similarity in their sequences, cstA and yjiY displayed different effects on motility, adhesion, biofilm forming ability and virulence in the animal model of infection. This study brings forward pleiotropic role of cst genes and highlights the crucial cross-talk between metabolism and pathogenesis of Salmonella.
FEMS-1397
Bacterial pathogenicity

TRANSCRIPTOMIC ANALYSIS IN SALMONELLA ENTERICA REVEALS PRODUCTS OF THE STD FIMBRIAL OPERON AS GLOBAL REGULATORS OF GENE EXPRESSION
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Background
Fimbriae are virulence factors in Salmonella enterica, playing diverse roles in bacterial adhesion (Humphries et al. FEMS microbiology letters, 201:121-125, 2001). Synthesis of Std fimbriae, that are not expressed under laboratory conditions, has been shown to occur in the animal intestine, but the molecular mechanisms involved remain unknown (Weening et al. Infection and immunity, 73:3358-3366, 2005). It was previously described that derepression of the std operon occurs in DNA adenine methylase (dam) mutants and that HdfR, a poorly known LysR-like factor, is necessary for std transcription. However, HdfR-mediated activation of std transcription occurs only in a fraction of the bacterial population, suggesting the occurrence of either bistable expression or phase variation (Jakomin et al. Journal of bacteriology, 190:7406-7413, 2008).

Objectives
This work aims to further understand the molecular mechanisms that might allow expression of the std operon inside the animal and to analyze the effect of its expression in S.enterica.

Methods
A genetic screen for additional std regulators revealed that the std operon itself is a positive regulator, and the downstream genes of the operon, stdE and stdF, are crucial for this control. Surprisingly, transcriptomic analysis revealed that StdE and StdF control a plethora of S. enterica loci including genes involved in motility, chemotaxis, biofilm formation, conjugation, and virulence.

Conclusions
StdEF-mediated control may play a role in the crosstalk between motility, adhesion and invasion, considered essential for optimal Salmonella infection. If StdEF expression is subjected to phase variation during infection, subpopulation formation can be expected to occur in the animal intestine.
Background

Despite continuous efforts to reduce its incidence, mastitis remains a major disease in dairy cows. Main mastitis pathogens include Streptococcus uberis, Staphylococcus uberis and Escherichia coli. Induction of the innate immune response is a key mechanism in the initiation of the host response during infections of the mammary gland in cows by Escherichia coli.

E. coli P4 is a prototypical mastitis strain isolated from a case of clinical mastitis. Previous studied showed that E. coli P4 was virulent in a mouse model of mastitis. Yet, we have found that this strain induced a reduced pro-inflammatory reponse in mammary epithelial cells (MEC) compared to other E. coli isolates.

Objectives

Our objective was to identify genes responsible for the low pro-inflammatory response induced in MEC by E. coli P4 and determine if this reduced pro-inflammatory response contributed to the virulence of E. coli P4 in a murine mastitis model.

Methods

By screening a library of 2000 mutants for increased pro-inflammatory response on MEC, we identified a series of mutations that increase the response of MEC to strain P4. The impact of these mutations on the phenotype of E. coli P4 will be presented in details and discussed.

Conclusions

Altogether, results presented will allow a better understanding of the initial steps of the interaction between mammary epithelial cells and mastitis pathogens. This work will help understand if the low pro-inflammatory response triggered by E. coli P4 contributes to its virulence.
GARDNERELLA VAGINALIS AS A TRIGGER OF RECURRENT E. COLI UTI

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Background

Recurrent urinary tract infection (rUTI), most often caused by uropathogenic Escherichia coli (UPEC), is a life altering, potentially life threatening problem for many women. Asymptomatic carriage of UPEC in bladder reservoirs has long been believed to be a source for rUTI. However, the natural triggers of UPEC emergence from these reservoirs have remained mysterious. Women with bacterial vaginosis (BV), an imbalance of the vaginal microbiota, are at increased risk for UTI.

Objectives

We sought to address the long-standing idea that UPEC bladder reservoirs seed rUTI and to understand why women with BV are more prone to UTI. Translocation of BV bacteria to the urinary tract is likely to occur during sexual activity, which is often a prelude to rUTI. We tested the hypothesis that bladder exposure to BV-associated bacteria may initiate UPEC emergence from intracellular reservoirs.

Methods

Mice containing UPEC bladder reservoirs were challenged by transurethral inoculation with either PBS (control) or the BV-associated bacterium Gardnerella vaginalis. We monitored the consequences of bladder exposure to G. vaginalis by 1) enumerating urinary tract bacterial titers, 2) assessing epithelial exfoliation by immunofluorescence and scanning-electron microscopy and 3) RNAseq analysis.

Conclusions

Here we show that G. vaginalis triggers bladder epithelial exfoliation and emergence of E. coli from intracellular reservoirs. We observed phenotypes consistent with rUTI, including high UPEC titers and neutrophils in urine.. These data strongly suggest that G. vaginalis presence within the urogenital microbiota may be a trigger for rUTI and likely contributes to the substantially increased risk of UTI in women with BV.
THE EFFECTS OF INSULIN AND GLUCOSE ON EXPRESSION LEVELS OF USP, SFA/FOC, CNF 1 GENES IN AN UPEC STRAIN.

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Background: The gene expression of microorganisms and environmental factors are closely related.

Objectives: To investigate possible changes in the gene expression levels of virulence genes [sfa/foc (S and F1C fimbriae), cnf 1 (cytotoxic necrotizing factor), and usp (uropathogenic-specific protein)] in a uropathogenic E. coli standard strain (UPEC C7) in the presence of insulin and glucose.

Methods: The UPEC strain was cultured in broth media (tryptic soya broth-TSB) under 5 different conditions: 0.1% glucose, 20µU insulin, 200µU insulin, 0.1 % glucose+200µU insulin, and control TSB. After incubation for 24 hours at 37°C, total RNA isolations were prepared from all five bacteria cultures. Gene expression levels were determined by quantitative PCR. Changes in gene expression were evaluated using the expression levels of 16sRNA as a “housekeeping gene,” according to the Pfaffl equation.

Conclusions: The medium containing 0.1 % glucose+200µU insulin had increased expression levels of all three genes in the E. coli C7 strain. In contrast, the other media (0.1% glucose, 20µU insulin, 200µU insulin) had a decrease in the expression levels of virulence genes, all compared to control medium.

This investigation shows that some environmental factors, such as glucose and insulin, could determine the pathogenicity of E. coli strains.
Background

*Enterococcus faecium* is a commensal of the mammalian gastrointestinal tract. Recently, it has become an important nosocomial pathogen, causing infections that are difficult to treat. However, little is known about the mechanisms that *E. faecium* employs to colonize and infect mammals.

Objectives

We aimed to clarify the interactions of *E. faecium* with the host and we hypothesized that genes important for colonization and infection, could exhibit temperature-regulated expression control.

Methods

We performed a transcriptome analysis of *E. faecium* E1162, during mid-exponential growth at 25°C and 37°C. Furthermore, we functionally characterized a surface protein of *E. faecium*, that was found to be produced at higher levels during growth at 37°C than at 25°C.

Conclusions

We found thirty-three genes expressed significantly higher at 37°C compared to 25°C. One of the most highly upregulated genes (4.4-fold), is predicted to encode a peptidoglycan-anchored surface protein. The N-terminal domain of this protein is unique to *E. faecium* and closely related enterococci. The C-terminal domain contains...
three proline-rich repeats, leading us to name the protein PrpA for proline-rich protein A. PrpA is a surface-exposed protein, that was maximally produced in exponentially growing cells at 37°C. PrpA is immunogenic as specific antibodies were observed in patients after they suffered from an *E. faecium* bacteremia. Heterologously expressed and purified PrpA was able to bind to fibrinogen, fibronectin and to platelets. Furthermore, a *prpA* deletion mutant was defective in the early stages of biofilm formation. Our data indicate that PrpA may contribute to the pathogenesis of *E. faecium* infections in hospitalized patients.
Background
The intestinal epithelium provides a physical barrier to luminal bacteria. This barrier serves as the first line of defense against bacterial adhesion and invasion. *Campylobacter jejuni* is a Gram-negative commensal bacterium in domestic animal and cause gastrointestinal foodborne disease in human. *C. jejuni* is able to adhere and invade gut epithelium, which lead cause of acute gastroenteritis in human. It has believed that the function of tight junctions (TJs) in human intestinal epithelium is closely related with invasion of *C. jejuni*.

Objectives
The aim of this study was to investigate interaction between *C. jejuni* and host intestinal barrier functions. In this study, we used cultured islands (unpolarized) and sheets (polarized) form Caco-2 cell.

Methods
Invasion of *C. jejuni* was measured by gentamycin protection assay in Caco-2 cells. Confocal microscopy was used to examine the localization of internalized bacteria. And the most of bacteria were observed on cellular lateral part in islands of cell. Interestingly, *C. jejuni* efficiently invaded from basolateral surface compared with apical surface and intracellular *C. jejuni* was significantly increased in disruption of TJs by the treatment with EGTA. Furthermore, *C. jejuni* infection induced the distribution of the TJs component.

Conclusions
These data indicated that the TJs formed by each of neighboring cells might act as physical barrier for prevention of *C. jejuni* invasion, and infection of *C. jejuni* might effect on formation of TJs component. These results suggested that the *C. jejuni* infection inducible TJs disruption might enhance *C. jejuni* invasion into host cell.
CAPNOCYTOPHAGA CANIMORSUS IS ADHERING TO HOST MEMBRANE GLYCOPROTEINS

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Background

*Capnocytophaga canimorsus* (Cc), member of the *Bacteroidetes* phylum, is an oral commensal from dogs which causes rare but severe infections in humans. Cc feeds on sugar moieties of glycoproteins expressed on host cell membranes. Removal of N-linked glycans is achieved by the glycoprotein deglycosylation (Gpd) complex encoded by a polysaccharide utilization locus, a hallmark of *Bacteroidetes*.

Objectives

The aim of this study is to determine whether Cc is adhering to the host cells it is feeding on and, if so, which mechanisms are implicated.

Methods

Cell lines were infected with strain Cc5. Mutant bacteria were constructed by site-directed gene replacements. Adhesion was monitored by microscopy and flow cytometry.

Conclusions

Cc5 adheres to epithelial, endothelial and immune cells. In *Flavobacterium johnsoniae*, another member of the *Bacteroidetes* phylum, gliding motility and adhesion are associated. In Cc5 adhesion was not abrogated in a mutant unable to glide. Adhesion of Cc5 was mainly mediated by the Gpd complex. Cc5 adhered to host cells via the membrane glycoproteins as inhibition of protein glycosylation by tunicamycin led to a decreased adhesion. Removal of serum from the assay, leaving cell glycoproteins as the only feeding source, increased adhesion. If fetuin, a glycoprotein deglycosylated by Cc5, was added to the serum-depleted medium, adhesion decreased. We conclude that adhesion of Cc5 to cells is linked to feeding through the Gpd complex. Further work will be conducted to find out whether adhesion could be important during infection.
Background

Members of the *Mycobacterium tuberculosis* complex are the causative agents of tuberculosis, a major global health threat. The majority of individuals are thought to be infected latently whereby the bacteria are in a non-replicating dormant state, and the individual is asymptomatic. Many of the proteins secreted by this organism have been shown to be essential for the virulence, survival and dormancy, and function in a variety of different roles. The resuscitation promoting factors (Rpfs) are one such family of proteins whose peptidoglycan hydrolyzing activities have been correlated with the resuscitation of dormant bacteria; however, the precise molecular mechanisms underlying this process remain poorly understood.

Objectives

The aim of this work is to gain insight into the expression and localisation patterns of Rpfs during bacterial growth and macrophage infection.

Methods

We have generated a number of Rpf deletion mutants and Rpf specific polyclonal antibodies to monitor Rpf expression in vitro using flow cytometry, and during *Mycobacterium marinum* infection of murine macrophages by immunofluorescence and confocal microscopy.

Conclusions

Having observed Rpfs at the level of individual bacteria, we conclude that a subset of
the mycobacterial population express Rpf at a detectable level during \textit{in vitro} growth, and the infection process.
DISTRIBUTION OF GENES RESPONSIBLE FOR VIRULENCE IN CRONOBACTER STRAINS

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Background
Cronobacter spp. was proposed as a new genus in 2008. All the members of Cronobacter are considered to be potentially pathogenic; however recent epidemiological studies indicated differences in virulence. The most often isolated species from clinical samples were C. sakazakii and C. malonaticus. Some reports revealed the connection between sequence type and virulence of particular strain. It has been shown that sequence type 4 (ST4 – C. sakazakii) is prevalent for meningitides, ST7 (C. malonaticus) is often associated with adult infections. These sequence types are considered as the evidence of virulence.

Objectives
The objectives of this work were to implement PCR specific for virulent proteins and to reveal connection between sequence type (MLST) and virulence genes.

Methods
Several factors responsible for virulence were targeted: the system for iron acquisition, outer membrane protein A, haemolysin and metalloprotease placed on chromosome and type six secretion system, secretion system FHA and outer membrane protease placed on plasmids.

PCR protocols for the detection of corresponding genes were applied to all Cronobacter species and compared with determined sequence type.

Conclusions
The predominance of some virulence genes was observed within particular species, in some cases even with sequence type; for example, haemolysin was detected in all tested C. sakazakii strains and in C. malonaticus presenting sequence type 7.

This work was supported by the Czech Grant Agency (13-23509S).
Background
Haemophilus influenzae (Hinf) is a Gram-negative pathogen colonizing the upper respiratory tract mucosa. Hinf belongs to a group of human-restricted bacteria, which bind to carcinoembryonic antigen related cell adhesion molecules (CEACAMs) on epithelial cells. Adhesion to CEACAMs is thought to be mediated by the Hinf outer membrane protein (OMP) P5 promoting establishment of the pathogens in the human nasopharynx.

Objectives
Aiming at preventing Hinf colonization, we sought to identify the molecular requirements for Hinf binding to CEACAMs.

Methods
Binding assays with soluble receptor ectodomains, followed by flow cytometric analysis or Western Blotting, were used to characterize CEACAM-binding profiles of wildtype and mutant Hinf. OMP P1, and not OMP P5, was identified as the CEACAM-binding adhesin. Multiple amino acid sequence alignment of P1 combined to heterologous expression of wild-type, chimeric, or mutated P1 in E. coli depicted the molecular details of the P1-CEACAM interaction.

Conclusions
Surprisingly, Hinf P5 mutants still avidly bind CEACAMs and Hinf P5 expressed in E. coli fails to mediate CEACAM targeting. Instead, a genetic screen identifies Hinf P1 as the CEACAM-binding adhesin. Deletion of P1 in Hinf and heterologous expression in E. coli demonstrate that P1 is necessary and sufficient to bind several human CEACAMs. Concordantly, when expressed on the surface of E. coli, P1 promotes adhesion to and invasion into epithelial cells. Structure-activity relationship investigations with P1 mutants demonstrate that several flexible extracellular loops allow P1 to engage human CEACAMs. These results provide the first evidence for the involvement of the major outer membrane protein P1 of Hinf in pathogenesis.
FEMS-2695
Bacterial pathogenicity

LOCAL AND SYSTEMIC EFFECTS OF EXPERIMENTAL MURINE HELICOBACTER PYLORI INFECTION.
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Background

Despite its late-in-life pathogenicity (gastric cancer and peptic ulcer disease), due to its potential early-life benefits (protection against childhood-onset asthma, diarrheal disease, and reflux esophagitis), Helicobacter pylori (Hp) might be considered a commensal. The ongoing disappearance of Hp from the human microbiome is concerning as little is known about the influence of Hp on the host’s microbiome and immunity.

Objectives

In this study, we aimed to investigate the interactions of Hp with its host in a mouse model.

Methods

We infected C57/Bl6 mice with cagA+ Hp PMSS1, or not (control). Groups of mice were serially sacrificed over a period of 6 months and host responses analyzed.

Conclusions

All inoculated mice were Hp-positive when sacrificed, as verified with PCR and culture and displayed gastric histology typical for Hp colonization. All infected mice showed high levels of IgM/IgG to Hp but not to CagA. Nanostring® analysis, performed on stomach and lung, revealed a large number of immune genes in the stomach with increased expression. Immune gene expression was also altered in the lung and flow-cytometry confirmed significantly higher levels of Th17-cells in infected mice compared to controls. Extracted DNA from fecal pellets, gastric, ileal and cecal contents were subjected to 16S rRNA gene sequencing to determine changes in the microbiome due to Hp colonization. Overall, control and colonized mice displayed
different population structures in both their gastric and intestinal microbiota. These findings indicate that in this mouse model, *Hp* influences both the microbial population structure and local and distant host immune responses.
BACKGROUND

Although *L. pneumophila* serogroup(sg)1 is the common disease causing serogroup, rare serogroups can also cause legionellosis.

OBJECTIVES

We aim to present six cases of legionellosis, of which four cases presented as Pontiac fever and the other two cases presented as Legionnaires’ disease. And than emphasize the regional epidemic and pandemic threats of infectious diseases that are transmitted between neighboring countries.

METHODS

We reported 6 cases of legionellosis caused by rare sg of *L. pneumophila*. Their diagnosis was supported with clinical, Radiological and serological findings and serogrouping *L. pneumophila*. The analyses of IgG and IgM were performed in our unit using the Anti-Legionella Pneumophila Indirect Immunofluoresan IgM, IgG kit (Euroimmun AG, Leubeck, Germany). All six serum samples sent, Euroimmun AG Clinical Immunology Laboratory in Lubeck, Germany for serogrouping.

CONCLUSIONS

Two of them were diagnosed with Legionnaires’ disease caused by *L. pneumophila* sg12 (index case KY) and sg11 (his wife ZY) and 4 of the cases
were diagnosed with Pontiac fever caused by \textit{L. pneumophila} sg14 (ZY), sg4(AK), sg4 with sg6 (BT) whereas the sg of \textit{L. pneumophila} detected in ZC could not be identified. This study reemphasized that not only \textit{L. pneumophila} serogroup 1, but other rare serogroups might cause also legionellosis which may increase in frequency and cause regional epidemics. It should be noted that these epidemics can also become a threat and a severe public health problem for all countries. Global-scale solutions should be developed for epidemics that could threaten this region.
Background
Geographical variation in the frequency of various gastroduodenal pathologies was shown to be related to the geographical diversity of H. pylori cagA/EPIYA patterns.

Objectives
We sought to determine the EPIYA patterns of cagA-positive H. pylori strains isolated from patients with different endoscopic diagnoses and from patients with gastric cancer (GC) who lived in Istanbul and surroundings areas, a Western region of Turkey located both in Europe and Asia.

Methods
One hundred and fifty-eight cagA-positive strains were included in the study; 84 were isolated from patients with gastritis (G), 38 were from patients with GC, 22 were from patients with duodenal ulcers (DU) and 14 were from patients diagnosed endoscopically with having normal gastrointestinal systems (NGIS). Specific primers were used for the detection of EPIYA patterns and representative bands were also confirmed by DNA sequencing.

Conclusions
EPIYA A, B, C or D were found in 142 of the 158 strains (89.9%). EPIYA-ABC was detected in 83 H. pylori 50 (59.5%) were isolated from patients with G, 14 (63.7%) were isolated from patients with DU, 9 (23.7%) were isolated from patients with GC and 10 (71.4%) were isolated from patients with NGIS. EPIYA-C with ≥2 repeats was detected in 34 (21.5%) cagA-positive H. pylori cases and 22(64.7%) of these cases involved GC. The most common EPIYA pattern isolated from cases with different endoscopic diagnoses was the Western type EPIYA-C. EPIYA-C with ≥2 repeats was
found to be more common in cases with GC; in other cases with different endoscopic diagnoses, EPIYA-C with one repeat was the most frequent.
IDENTIFICATION AND CHARACTERIZATION OF STRESS FIBER INDUCING EFFECOR PROTEIN OF VIBRIO PARAHAEOMOLYTICUS T3SS2


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Background

Vibrio parahaemolyticus is an important pathogen that causes food-borne gastroenteritis in humans. The type III secretion system encoded on chromosome 2 (T3SS2) plays a critical role in the enterotoxic activity of this bacterium. Tissue culture analysis has shown that T3SS2 causes two dramatic changes in the actin cytoskeleton: the accumulation of F-actin beneath bacterial microcolonies and the induction of actin stress fibers. VopV, which is enterotoxic effector, exhibits F-actin binding activity and is responsible for the F-actin accumulation phenotype. However, the mechanisms that underlie T3SS2-dependent actin stress fiber formation and the main effector have not been elucidated.

Objectives

The aim of this study is to identify effector protein responsible for T3SS2-dependent stress fiber formation.

Methods

V. parahaemolyticus strain RIMD2210633 (KP-positive, serotype O3:K6) was used for parent strain. A four-primer polymerase chain reaction (PCR) technique was used to engineer an in-frame deletion mutation. Caco-2 cells were infected with V. parahaemolyticus for 3 h at a multiplicity of infection (MOI) of 10. Actin was detected with Alexa Fluor® 488-phalloidin. Cellular and bacterial DNAs were stained with Hoechst 33258.

Conclusions

After screening candidate ORFs encoded within the Vp-PAI region, a known pathogenicity island in pathogenic strains, we identified an effector candidate protein involved in stress fiber formation. A deletion of this gene did not affect to T3SS2-dependent enterotoxicity in rabbit ileal loop model, but this mutant caused a dramatic change in actin stress fiber formation, thereby suggesting that this protein is a T3SS2 effector involved in the induction of stress fiber formation.
STRUCTURE-FUNCTION ANALYSIS OF THE TYPE 3 SECRETION SYSTEM TRANSLOCATOR IPAB

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**Background**

The Type 3 Secretion System (T3SS) enables the invasion the human intestinal gut mucosa by *Shigella flexneri*. The T3SS consists of a membrane embedded basal body and an extracellular needle. At the distal end of the needle a tip complex senses and binds the host cell membrane. Upon binding secretion of effectors from the bacterial cytoplasm through the T3SS into the host cell is activated. The secretion of effectors follows a defined order and prior secretion unfolding of the effectors enables the transport through the needle. Early secreted effectors, known as translocators form pores in plasma membranes. The translocators such as the invasion plasmid antigen B (IpaB) are bound to chaperones (IpgC) in the bacterial cytoplasm. For successful infection the IpaB/IpgC complex disassembles and unfolded IpaB is secreted while IpgC remains in the bacterial cytoplasm. Thus, IpaB adopts different folding states before and after secretion which is studied by X-ray crystallography and other biophysical methods.

**Objectives**

Analysis and comparison of different IpaB conformations with the aim to gain insight into IpaB function.

**Methods**

Detergents for stabilizing the hydrophobic domains of full length IpaB were used for crystallization trials as well as full length IpaB in complex with IpgC without detergent. In another approach soluble fragments of IpaB were successfully crystallized and the structure determined. IpaB fragments were also cocrystallized with IpgC and the structural determination is in progress.

**Conclusions**

Based on this study, future design of small inhibitors preventing correct IpaB folding could be beneficial for therapeutic-clinical use against *Shigella* infections.

Barison (2012) *FASEB*26

Senerovic (2012) *CellDeathDis*3
MIXED INFECTION ENHANCES THE VIRULENCE OF AEROMONAS IN THE CAENORHABDITIS ELEGANS MODEL

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Background
Aeromonas virulence remains poorly understood, virulence of any given strain being hardly predictable. In addition, Aeromonas human infections involve mixed Aeromonas isolates (5-10% cases) with an unknown impact on virulence.

Objectives
To evaluate virulence of strains recovered from mixed aeromonad infections, alone and in association.

Methods
Twelve Aeromonas isolates recovered in pairs from 6 mixed infections were tested against the Caenorhabditis elegans virulence model. Dead worms were counted each day during 15 days. Median time for killing worms (TD50) was determined for single isolates and for natural and experimental pairs. Experiments were repeated 3 times, independently. Genomes of 7 isolates were sequenced using an Illumina Myseq; virulence-associated genes were sought within the draft genomes.

Conclusions
Eight isolates were weak killers (TD50≥7 days) when they were administered alone, two pairs showed an enhanced virulence: the TD50 was significantly lowered from 7.8 and 8.3 days to 3.3 days (couple 76c+77c) and from 6 and 9 days to 4.5 days (couple 25a+25b). Synergy was also observed for 5 of the 14 experimental pairs tested, each including one strain from the natural synergistic couples. Only couples involving strains from distinct species showed synergy. The genome content of virulence-associated genes failed to explain virulence synergy and to identify pathotypes, although some virulence-associated genes present in some strains were absent from the companion strain (e.g., T3SS).

Synergistic virulence observed between infectious Aeromonas isolates stresses to consider Aeromonas infection process at the community level and not only according to virulence content as in pathotype concept.
FEMS-2389
Bacterial pathogenicity

STAPHYLOCOCCUS AUREUS- DERIVED MEMBRANE VESICLES EXACERBATE SKIN INFLAMMATION IN ATOPIC DERMATITIS
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Background
Skin colonization or infection with Staphylococcus aureus is known to trigger aggravation of atopic dermatitis (AD). However, the exact mechanisms by which S. aureus can worsen AD are unknown.

Objectives
We investigated whether and how S. aureus-derived membrane vesicles (MVs) contribute to worsening of AD.

Methods
HaCaT cells were treated with S. aureus MVs and were analyzed for the expression of pro-inflammatory cytokine genes. Immunopathology and cytokine gene profiles were analyzed after topical application of S. aureus MVs to AD-like skin lesions in a mouse model.

Conclusions
Intact MVs from S. aureus delivered their components to keratinocytes and stimulated pro-inflammatory cytokine gene expression. However, MVs with a disrupted membrane neither delivered their components to keratinocytes nor resulted in cytokine gene expression. A knockdown of nucleotide-binding oligomerization domain 2 by using small interfering RNAs completely suppressed IL-8 gene expression. Topical application of S. aureus MVs to AD-like skin lesions in the mouse model induced massive infiltration of inflammatory cells and the resulting eczematous dermatitis. This inflammatory reaction was associated with a mixed Th1/Th2 immune response and enhanced expression of chemokine genes in AD-like skin lesions. S. aureus MVs delivered effector molecules to host cells and triggered an inflammatory response both in vitro and in vivo. MVs produced by S. aureus colonizing or infecting AD skin lesions may be responsible for worsening of AD. Thus, S. aureus MVs are a new therapeutic target for the management of AD aggravation.
THE INTIMIN PERIPLASMIC DOMAIN MEDIATES DIMERISATION AND BINDING TO PEPTIDOGLYCAN

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Background

Intimin and Invasin are prototypical inverse (Type Ve) autotransporters and important virulence factors of enteropathogenic Escherichia coli and Yersinia spp., respectively. In addition to a C-terminal extracellular domain and a β-barrel transmembrane domain, both proteins also contain a short N-terminal periplasmic domain that, in Intimin, includes a lysin motif (LysM), which is thought to mediate binding to peptidoglycan.

Objectives

We investigated the functions of the Intimin periplasmic domain, particularly to study its role in peptidoglycan binding and dimerisation.

Methods

We used pull-down, solid phase binding and in vivo binding assays to demonstrate that the Intimin LysM binds to peptidoglycan. Dimerisation of the periplasmic domain was shown by analytical size exclusion chromatography and in vitro cross linking experiments. We further solved the structure of the Intimin LysM using NMR.

Conclusions

We show that the periplasmic domain of Intimin does bind to peptidoglycan both in vitro and in vivo, but only under acidic conditions. We were able to determine a dissociation constant of 0.8 μM for this interaction, whereas the Invasin periplasmic domain, which lacks a LysM, bound only weakly in vitro and failed to bind peptidoglycan in vivo. Furthermore, in contrast to previous reports, we show that the periplasmic domain mediates dimerisation.

We further show that dimerisation and peptidoglycan binding are general features of LysM-containing inverse autotransporters. Peptidoglycan binding by the periplasmic domain in the infection process may aid in resisting mechanical and chemical stress during transit through the gastrointestinal tract.
Background
Brine shrimp is an aquatic crustaceans belonging to a genus of *Artemia*. This organism is widely used for testing the toxicity of chemicals and for live food in the larviculture of economically important fishes and crustaceans.

Objectives
In this study, a brine shrimp, *Artemia salina* was evaluated for the bacterial infection and symbiotic host model.

Methods
Brine shrimp nauplii were incubated in petri dish containing 5 ml of autoclaved artificial seawater. Various numbers of bacterial cells were added to the seawater and incubated at 28 °C for several days. The survival of shrimps was daily scored after the addition of the bacteria and the existence of bacteria in shrimp gut was observed by fluorescence microscope.

Conclusions
Pathogenic bacteria caused significant death of brine shrimps reflecting their virulence, but some shrimps surviving the infection were found to grow bigger and faster. Both *E. coli* and *P. aeruginosa* could survive in the brine shrimp gut, but *E. coli* was able to survive only for limited period whereas *P. aeruginosa* survived more and longer in the gut than *E. coli*. Furthermore, we found that the pre-infection of avirulent *P. aeruginosa* strain improves the survival of brine shrimp in the challenge of other pathogenic bacteria. These results strongly suggest that survival of *P. aeruginosa* as a symbiont has a beneficial effect on host and brine shrimps may be a valuable artificial model to study symbiosis.
DIRECT INVOLVEMENT OF CYCLIC AMP (CAMP) AND CAMP RECEPTOR PROTEIN (CRP) IN NATURAL COMPETENCE REGULATOR TFOX EXPRESSION

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Background
Vibrios are normal inhabitants in the estuarine and marine environments, where they exist both as free-living bacteria and in association with phytoplankton, zooplankton, and crustaceans. Chitin-induced competence found in several Vibrios represents a newly appreciated mode of horizontal gene transfer in these marine bacteria and undergoes tight regulation. Sxy (also called TfoX) and CRP are two important activator proteins in the competence development. Sxy functions synergistically with CRP to activate the competence genes regulon expression.

Objectives
Sxy/TfoX expression has been shown to be regulated by RNA second structure and sRNA tfoR at the post-transcriptional level. In this study, we investigated the transcriptional regulation of tfoX expression by cAMP-CRP complex.

Methods
qRT-PCR and complement assay were used to analyze the tfoX mRNA level. Promoter transcriptional fusion and site-directed mutagenesis of the putative CRP-binding sites were applied to determine the tfoX promoter activities. EMSA assay was employed to demonstrate the direct binding of CRP to the tfoX promoter. The transcriptional start site of tfoXVF was determined by 5’RACE.

Conclusions
CRP positively regulates the tfoX expression at the transcriptional level; there are two functional CRP binding-sites on tfoXVC promoter region and CRP directly binds to the promoter region to initiate its transcription; transcriptional start site of tfoXVF was mapped at -126 nucleotides upstream of translational start site; similar -10 motifs and putative CRP-binding sites on different species’ tfoX promoter indicate CRP regulation of tfoX is a conserved regulatory mechanism in Vibrio species.
Background
*Klebsiella pneumoniae* (Kp) is the predominant pathogen isolated from liver abscesses of diabetic patients in Asian countries. Although elevated blood glucose levels cause various immune problems, its effects on Kp virulence remains largely unknown. In bacteria, cyclic AMP (cAMP), a well-known second messenger, plays a fundamental role in global gene regulation in response to exogenous glucose levels. However, targets regulated by cAMP signaling pathway remain uncharacterized in Kp.

Objectives
To investigate the role of cAMP signaling pathway in Kp pathogenesis.

Methods
DNA microarray, promoter activity assay, qRT-PCR, western blotting assay, biofilm formation, electrophoresis mobility shift assay

Conclusions
Based on the analysis of DNA microarray results, we noted a serial of type 3 fimbrial genes was apparently increased in Δcrp strain. It indicates that CRP could repress the type 3 fimbriae expression. By using promoter activity assay, western blotting, and qRT-PCR to confirm that the MrkA expression, the major pilin of type 3 fimbriae, was regulated by cAMP signaling pathway. However, no typical CRP binding site was found in the sequence of P_{mrkA}, suggesting CRP regulates the mrkA transcription via other mediator(s). According to previous studies, multiple proteins, which include Fur, MrkH, MrkI, MrkJ, and YjcC have been shown to mediate the type 3 fimbriae expression. The underlying mechanism of cAMP signaling pathway and the mediators as described above in regulating the type 3 fimbriae expression was investigated in this study.
IDENTIFICATION OF FITNESS DETERMINANTS IN PSEUDOMONAS AERUGINOSA INFECTION

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Background

Pseudomonas aeruginosa is a clinically highly relevant opportunistic pathogen known to cause acute and chronic infections. This bacterium can adapt to the host by environmentally driven alterations in gene expression as well as genomic alterations in response to selective pressures by the environment.

Objectives

The aim of this study is to identify genetic adaptations that are key to fitness and successful establishment of acute and chronic infections by P. aeruginosa.

Methods

We have developed an in vivo model for P. aeruginosa biofilm formation in CT26 tumor-bearing mice. This murine model offers the unique opportunity to screen the contribution of individual genes to the fitness of P. aeruginosa during in vivo biofilm formation. Furthermore, the inclusion of an acute lung infection model allows the comparison of fitness parameters in chronic to acute infections. These parameters will be investigated using (i) a PA14 transposon mutant library (Skurnik et al. 2013) as forward genetic approach and (ii) a reverse genetic approach by passaging bacteria in the animal host. To evaluate the contribution of individual genes onto bacterial fitness, isolated bacteria are quantified using high-throughput insertion and whole-genome sequencing, respectively.

All animal experiments were approved by the local regulatory board LAVES (Niedersächsisches Landesamt für Verbraucherschutz und Lebensmittelsicherheit) and the EU Directive 86/609/EEC, OJ L 358, p 1-28; 18.12.1986.

Conclusions

Here we present a dual approach to gain insights into fitness determinants important during P. aeruginosa infection in different host environments.
Glasser's disease, caused by *Haemophilus parasuis* (*Hps*), produces annually pig significant economic losses. It is broadly known the relevance of transferrin binding proteins (Tbps) as virulence factor for developing this disease in the host.

**Objectives**

Subunit vaccines based on the native, wild-type TbpB and on a mutant TbpB (TbpBY167) (losing its ability for binding porcine transferrin) from *Hps* serovar 5 were designed.

**Methods**

Immunization and challenge (with an intratracheal administration of 10⁸ CFU/ml of the Nagasaki strain of *Hps* 5) experiment was implemented in colostrum deprived pigs for comparing the protective response developed for wild-type and mutant proteins. Clinical symptoms were monitored after challenge and pathological findings were determined upon necropsy. All the pigs immunized with adjuvant alone and challenged did not survive more than 72 hours. On the other hand, most of the pigs immunized with wild-type TbpB survived the full two weeks after challenge but clinical signs and severe chronic lesions were observed. All the pigs immunized with the mutant also survived until the end of the study, without signs of infection. Were confirmed the results by indirect ELISA test, by immunohistochemistry and real-time quantitative PCR was used to determine the relative expression of 27 immune-related genes in the lungs of the pigs belonging to experimental groups, at the necropsy time.

**Conclusions**

The highest protective response was that afforded by the mutant. Genes associated with the inflammation are highly overexpressed in the group of only infected animals and this does not happen in the other two experimental groups.
GENETIC DIVERSITY OF COLICIN E1 AND FIMBRIAE TYPE I IN ESCHERICHIA COLI STRAINS

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Background
Uropathogenic *E. coli* (UPEC) strains are causative agents in the majority of human urinary tract infections (UTIs). UPEC strains contain several virulence factors allowing them to colonize human urinary tract (e.g. fimbriae type I). Moreover, higher prevalence of colicin E1 like plasmids and a possible role of colicin E1 protein as a potential important virulence factor of UPEC strains was described in UPEC strains.

Objectives
The main aim of the study focused on the genetic variability of colicin E1 and *fimH* gene (encoding the mannose-sensitive FimH adhesin of fimbriae type I) between human intestinal and extraintestinal *E. coli* strains (UPEC strain).

Methods
In the set of 58 human fecal *E. coli* strains and 55 UPEC strains, *fimH* gene and colicin E1 gene was sequenced and analyzed. All clinical samples were collected after patients gave informed consent. The study was approved by the ethics committee of the Faculty of Medicine, Masaryk University, Brno, CZ.

Conclusions
Two different colicin E1 genetic variants were described. Colicin E1-A genetic variant that prevailed among human fecal *E. coli* had 1566 bp and colicin E1-B genetic variant (prevailed in UPEC strains) had 1569 bp. The main genetic difference identified between these variants encoded colicin receptor domain. A significant increase of the substitution of residue Arg166 - His166 in fimbriae-associated pilin domain of FimH was identified in UPEC strains. It is possible that genetic variability identified in genes encoding colicin E1 and FimH modify virulence of UPEC strains.
Background

Salmonella enterica is the causal agent of a widespread and neglected infectious disease in the world, that despite efforts, it lacks completely effective vaccine formulations. The non-typhoidal serovars Typhimurium and Enteritidis are frequently responsible for salmonellosis outbreaks, usually associated with gastrointestinal infections, but they can spread systemically, particularly in immunocompromised patients or those who suffering from malaria. In Africa, non-Typhi Salmonella comprises around 50% of reported cases of bacteremia and are associated with nearly 20% of the fatal cases of salmonellosis. It's crucial to test the potential of new mutants and new serovars for use as live attenuated vaccines to contain the disease.

Objectives

We used recent clinical isolates of S. Typhimurium and S. Enteritidis to develop the strains LGBM01∆fis and SEnPT4∆fis, respectively, and evaluated their phenotype under in vitro conditions.

Methods

The phenotypic tests performed included analysis of growth in Luria Broth with measurements taken every hour, motility test on semi-solid agar plates and invasiveness in primary macrophages obtained from BALB/c mice.

Conclusions

The results showed no significant differences in growth rate in Luria Broth between the mutant strains and the respective wild strain, but the motility in semi-solid agar plates was significantly decreased, at least 50%, in the mutant strains. Interestingly, the ability in invade primary macrophage cells was significantly affected only in the Typhimurium mutant strain. Those results showed an important difference for the role of the same DNA binding protein in these serovars that needs to be considered for future use in vaccine development.
PATHOGENIC FEATURES OF BACTERIAL STRAINS ISOLATED FROM SURGICAL RINSE WATER AND MEDICAL INSTRUMENTS

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Background

One of the potential sources for bacterial contamination in hospital environments is represented by the inadequate manual cleaning of medical instruments.

Objectives

The purpose of this study was to investigate the pathogenic features of microbial strains isolated from sterile water use for rinsing the surgeons hands and surgical medical instruments, in order to establish potential contamination risks for patients.

Methods

A number of 200 water samples and 400 surgical instruments have been processed for routine microbiological analyses, following the existent ISO guidelines. The isolated strains have been identified using miniAPI galleries and investigated for antibiotic susceptibility profiles and for the production of cell-associated (adherence to inert and cellular substratum, biofilm development) and soluble, enzymatic (hemolysins, lecithinase, lipase, caseinase, gelatinase, amylase, esculin hydrolysis, DN-ase) virulence factors, using phenotypic (disk diffusion method, selective media for enzymatic factors production) and PCR-based methods.

Conclusions

Bacteria were isolated in 10% of the analyzed water samples and from 5% of the investigated instruments. The following bacteria were isolated in the frequency decreasing order: \textit{Pseudomonas aeruginosa}, \textit{Brevundimonas sp.}, \textit{Micrococcus luteus}, \textit{Staphylococcus haemolyticus}, \textit{S. warneri}, \textit{P. putida}, \textit{P. stutzeri}, \textit{Stenotrophomonas maltophilia}, \textit{Moraxella sp.} Pore-forming toxins and enzymes have been the most frequently produced, followed by caseinase and esculin hydrolysis. The genetic support of these virulence factors has been confirmed by PCR. The analyzed strains exhibited only natural resistance profiles, demonstrating the
environmental origin of the isolated strains. The highest frequency of pseudomonades suggests that biofilm formation within piping is the source of contamination of surgical rinse water.
UNUSUAL INCREASE OF TYPHOID FEVER CASES IN JAPAN AND MOLECULAR EPIDEMIOLOGICAL ANALYSIS OF THE CAUSATIVE AGENT, SALMONELLA TYPHI

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Background
Typhoid fever is a systemic infection caused by Salmonella enterica serovar Typhi and transmitted from human to human via food or drinking water; therefore, hygiene and sanitary conditions mainly determine its spread. As a result of improved public sanitation, most of the cases in Japan have been sporadic and have come from abroad in recent years. In 2013, however, we observed an unusual increase of typhoid fever cases which seemed to be domestic infection.

Objectives
In this study, we assessed increase of the typhoid fever patients without histories traveling abroad and examined molecular epidemiological analysis of S. Typhi.

Methods
A total of 27 typhoid fever cases without histories traveling abroad were identified in 2013 and 26 S. Typhi isolates were collected. All isolates were examined with phage type and MLVA.

Conclusions
Of the 26 isolates, 15 strains were classified as phage type A or B1 and formed major cluster with 5 types of single locus variants by cluster analysis of the MLVA profiles. It seemed that closely related strains were causative agents of increased typhoid fever cases in Japan, 2013. These 15 patients were reported during 5 months (July to November) peaked at September. They ranged in age from 6 to 83 years (median age 32 years) and the male:female ratio was 1:1.5. Interviews by physicians or public health officers could not reveal specific information which leads to epidemiological link; therefore we have no identifiable source of infection.
FEMS-1708
Bacterial pathogenicity

COMPARATIVE TRANSCRIPTOMICS OF BURKHOLDERIA CONTAMINANS ISOLATES FROM DIFFERENT STAGES OF INFECTION IN CYSTIC FIBROSIS PATIENT

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Background
Burkholderia cepacia complex (Bcc) bacteria pose major threat to cystic fibrosis (CF) patients. Bcc infections can result in untreatable and fatal cepacia syndrome (CS) which is manifested as rapid deterioration of lung functions and sepsicaemia. The mechanisms behind transition from Bcc chronic infection to CS remain unknown.

Objectives
To gain insight into processes involved in development of CS, we compared transcriptomes of a lung isolate (chronic infection) and a bloodstream isolate (CS) from the same patient. Both isolates were of the same sequence type (ST872) and belonged to the Bcc species B. contaminans which is predominant among CF infections in Argentina.

Methods
Both strains were cultivated in three growth media: sputum (natural habitat of lung isolate), heat-inactivated human serum (natural habitat of bloodstream isolate) and control mineral medium, each in biological triplicates. RNA was extracted in mid-log growth phase, sequenced using RNA-Seq technology (Illumina) and converted to normalized transcript level values.

Conclusions
The expression of approximately 1,300 (18%) genes differed more than 3-fold between lung and sputum isolate. The bloodstream isolate showed markedly increased expression of quorum sensing-regulated pathogenicity determinants (motility, extracellular proteases, AidA, lectins, Flp pilus), hypoxia-activated genes and two antifungal compound synthetic clusters (occidiofungin and pyrrolnitrin). Agar plate assays confirmed rapidly increased motility and proteolytic, hemolytic and antifungal activities of bloodstream isolate. Assessing these phenotypes might help to monitor the progress of infections by B. contaminans.

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Background

H. pylori is the main cause of chronic gastritis, ulcer and gastric cancer. The balance between the bacterial virulence factors and the host immune response in the infection determines the different clinical outcomes. The association between Th1, Th17, Treg cells and H. pylori infection has been identified, but the effect of the nine major H. pylori specific virulence factors; cag A, vacA, oipA, babA, hpaA, napA, dupA, ureA, ureB on Th1, Th17 and Treg cells response in H. pylori infected patients has not been fully elucidated.

Objectives

To investigate the correlation of the H. pylori virulence factors with the manifestations of gastric disease by developing a multiplex–PCR and the relationship between these virulence factors with Th1, Th17 and Treg cells.

Methods

Multiplex and qRT-PCR were carried out to detect the H.pylori specific virulence factors and relationship between these virulence factors with T cells.

Conclusions

The multiplex-PCR was developed to rapid characterisation of nine H. pylori virulence genes with in a three PCR reactions. Due to the multiplex-PCR results there was no significant difference in prevalence of virulence factors in patients with gastritis and ulcer. However, prevalence of napA virulence factor was significantly higher in
patients with ulcer than gastritis. A positive correlation between the *dupA* virulence factor and IFN-g was observed in gastritis patients. Additionally, IL-17 expression was found significantly positively correlated with the *babA* virulence factor in ulcer patients. Furthermore, a novel expert derived model is developed to identify set of factors and rules distinguishing the ulcer patients from gastritis patients.
Background

*Legionella pneumophila*, the causative agent of the pneumonia-like Legionnaires’ disease, is commonly found in aquatic habitats worldwide where it multiplies within protozoa. To adapt between intra- and extracellular environments, *L. pneumophila* evolved a biphasic lifecycle wherein it alternates between a replicative (non-virulent) and a transmissive (virulent) phase. This switch is governed by a complex regulatory network. Expression of the Host Factor Q protein (Hfq), a hexameric, RNA-binding protein and chaperon of small RNAs (sRNA) is life cycle regulated and implicated in virulence. How Hfq expression is regulated in *L. pneumophila* is not known.

Objectives

Our aim was to understand how the growth-phase dependent expression of Hfq is regulated and whether a newly identified sRNA is implicated

Methods

By Transcriptional Starting Site mapping of the *L. pneumophila* genome, a sRNA that is transcribed antisense to the *hfq* gene and is overlapping its 5’UTR region was identified. Thus, we postulate that this antisense RNA, named *anti-hfq*, might regulate the life cycle dependent expression of Hfq. We constructed and characterized the *L. pneumophila hfq* deletion mutant and *anti-hfq* overexpressing strains *in vitro* and *in vivo*.

Conclusions

Our results show that Hfq expression is growth-phase dependently regulated by this newly identified antisense RNA. Infection assays revealed that Hfq is a virulence factor necessary for efficient replication in amoeba. Overexpression of *anti-hfq* led to a similar virulence phenotype as *hfq* deletion. This suggests an important role of Hfq and *anti-hfq* in virulence and in the regulatory network governing the biphasic life cycle of *L. pneumophila*. 
Background

Piscirickettsia salmonis is a Gram-negative intracellular bacterium that causes Piscirickettsiosis in salmonids farms in Chile. It was recently reported that P. salmonis produces exotoxins that play a role in the pathogenesis. However, a delivery system has not yet been identified. Outer membrane vesicles (OMVs) are 10 to 300 nm spherical-bilayer structures discharged from the surface of many Gram-negative bacteria which are able to deliver toxins and virulence factors. However, the production of OMVs by P. salmonis has not been described.

Objectives

The aim of this study was to investigate if P. salmonis is able to produce OMVs and to realize a microscopic characterization of these vesicles.

Methods

P. salmonis was grown in basal broth supplemented with Cysteine (3.18 mM) and ferric chloride (0.05 mM) at 18°C until early stationary phase. Bacteria were removed by centrifugation (5000 x g, 10 min at 4°C) and the supernatant were filtrated through a 0.22-μm-pore-size filter. Then, vesicles present in the bacterial free supernatant were isolated by ultracentrifugation (125,000 x g, 2h at 4°C) and analyzed by SDS-PAGE. Finally, CHSE-214 cells infected with P. salmonis and negative stained OMVs were visualized by TEM.

Conclusions
*P. salmonis* is able to produce OMVs. The purified OMVs appeared as spherical vesicles of different sizes between 27.3 and 145.5 nm. SDS-PAGE analysis showed similar protein profile between OMVs and outer membrane extracts. Finally, OMVs were found into *P. salmonis*-containing vacuoles in infected CHSE-214 cells, suggesting that OMVs may contribute to the pathogenesis of *P. salmonis*. 
MULTIPLE COLONIZATION OF HELICOBACTER PYLORI IN PATIENTS WITH CHRONIC GASTRITIS OF REGIONS OF HIGH AND LOW RISK OF GASTRIC CANCER IN COLOMBIA

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Background
Infection with virulent strains of Helicobacter pylori is necessary but not enough for the development of gastric cancer (GC) and its precursor lesions. In Nariño-Colombia, there are populations with high prevalence of H. pylori infection, but different GC risk. These results suggest different genetic characteristics of the circulating strains with varying degrees of pathogenicity.

Objectives
To evaluate multiple colonization of H. pylori isolated from patients with chronic gastritis from two populations with contrast in the risk of developing GC: Tumaco, low risk; Túquerres, high risk. Our hypothesis: there is a greater probability of multiple colonization of H. pylori in the population at highest risk for GC.

Methods
409 adult patients with dyspeptic symptoms were studied, of which gastric mucosa fragments from the antrum and body were used for the histopathological diagnosis, culture and genotyping (cagA, vacA and PCR-RAPD). 72 individuals were included, in whom H. pylori isolates was achieved in the three biopsies of gastric mucosa, (41/203) from Tumaco and (31/206) Tuquerres. Genetic similarity was calculated using the Nei’s coefficient.

Conclusions
Genetic diversity was higher among isolates from Tuquerres (0.13) than Tumaco (0.07). After adjusting for age, sex and diagnosis, multiple colonization was 1.7 times more frequent in Tuquerres than Tumaco, p = 0.05. In Túquerres: high risk of gastric cancer, there was more likely to have multiple colonization. Based on the analysis of the results of the PCR - RAPD, we found greater genetic similarity from isolates of H. pylori in the population with low risk for developing GC.
Background
pUM505 is a conjugative plasmid of 123 kilobases, originally isolated from a clinical strain of *Pseudomonas aeruginosa*. pUM505 possesses a pathogenicity island (PAI) of 78 genes, 64 of them have been found in the chromosomal PAPI-1 and PAPI-2 PAIs of *P. aeruginosa* PA14, a virulent clinical isolate, however it is unknown whether pUM505 is involved in virulence.

Objectives
The aim of this study is to determine if the plasmid increases the virulence of the *P. aeruginosa* PAO1 standard strain and to identify the genes responsible for this property.

Methods
Using as models of virulence lettuce leaves and *Dictyostelium discoideum* cultures pUM505 showed increased virulence of the PAO1 strain. To identify genes involved in virulence a gene bank was constructed by digesting the pUM505 plasmid and ligating the fragments to the vector pUCP20. A library of 120 clones was obtained in the Top10 strain of *E. coli* and PAO1 of *P. aeruginosa*. Four clones of this library were found to increase virulence in both *E. coli* and *P. aeruginosa*. The cloned inserts of these transformants was sequenced, and several ORF’s of pUM505 were identified. The ORFs 2, 17 and 42 (hop) have homologues that have been reported involved in virulence.

Conclusions
In conclusion, pUM505 plasmid increases the virulence of its hosts due to some ORF’s outside the pathogenicity island. The ORF’s identified are currently under study.
ANALYSIS OF THE SALMONELLA TYPHIMURIUM SPI-1 TYPE 3 SECRETION SYSTEM SORTING PLATFORM  

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Background

Many Gram-negative bacterial pathogens use a type III secretion system (T3SS) to deliver virulence factors into the cytosol of host cells in order to establish and maintain an infection. This system relies on the sequential secretion of first structural and later effector proteins for its correct assembly and function. The hierarchy of substrate secretion has been proposed to be ensured by a cytoplasmic protein complex, called the "sorting platform".

Objectives

We aimed to investigate and characterize the Salmonella Typhimurium "sorting platform" and its components, particularly SpaO, with regard to both their structure and function.

Methods

The two expression products of the spaO gene were analyzed and the gene mutated to investigate the mechanism by which the second, shorter product is generated. The two proteins were analyzed by SDS-PAGE, mass-spectrometry, size-exclusion chromatography and multi-angle light scattering. The SpaO protein was further co-purified with other "sorting platform" components and the ability of the complex to bind T3SS substrates tested by surface plasmon resonance.

Conclusions

The gene for the component SpaO gives rise to both the full-length protein (SpaO) and a shorter product SpaO'. SpaO' is produced from an internal translation initiation site and is required for the stability of full-length SpaO. Together these two proteins associate with other "sorting platform" components to form a complex that is capable of binding different T3SS substrates.

References
Background
Recent studies have been shown that Helicobacter pylori has an effective role in the migration of bone marrow derived mesenchymal stem cells (BMD-MSCs) throw to gastric tissue.

Objectives
This study aimed to find the effect of H.pylori on as bacterial microenvironment on BMD-MSCs transforming into cancer stem cells and metastasis of the tumor cells.

Methods
BMD-MSCs were followed for evaluation by flow cytometric analysis with the hAbs for positive and negative of surface markers and treated under the osteogenic and adipogenic differentiation medium. BMD-MSCs were co-cultured with H. pylori and Gastric epithelial cell line (AGS). The expressions of MMP-2, MMP-9, p53 and bcl2 were examined by qRT-PCR

Conclusions
When mesenchymal stem cells are attracted to chronic H.pylori infection tissue and gastric ulcer for their tissue healing function, they will be trapped under special microenvironment. This study demonstrated that H. pylori increased the anti-apoptosis factor bcl-2 to keep BMD-MSCs alive and lead them to cancer stem cells by changing the regulation of p53. H. pylori increased the metastatic proteins MMP2 and MMP9 that shows the role of H.pylori on metastasis action. Focusing on H.pylori-induced molecular pathogenesis and the impact of microenvironment in gastric progenitor cells or BMD-MSCs will be crucial to identify the molecular targets in tumor initiation and the origin of gastric cancer.
A NOVEL, UNSUSPECTED FEATURE OF SALMONELLA ENTERICA SPI-1 BISTABILITY

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Background
Salmonella enterica pathogenicity island 1 (SPI-1) is a gene cluster that encodes a type 3 secretion apparatus and effectors involved in invasion of epithelial cells. A well-known trait of SPI-1 is bistable expression, which generates SPI-1 ON and SPI-1 OFF subpopulations. The biological significance of SPI-1 bistability has been addressed by previous, insightful studies. Bistability has been viewed, for instance, as a division of labour involving self-destructive altruism by the SPI-1 OFF subpopulation (Ackermann et al. Nature 454, 987-90, 2008). Another study, however, has envisaged that the SPI-1 OFF subpopulation might benefit from inflammation triggered by the SPI-1 subpopulation (Stecher et al. PLOS Biology 5:2177-89, 2007). Furthermore, enhanced tolerance to antibiotics has been detected in slow-growing SPI-1 ON cells (Arnoldini et al. PLOS Biology e1001928, 2014).

Objectives
In this communication we describe an additional, unsuspected feature of SPI-1 bistability.

Methods
Single-cell analyses, such as flow cytometry and cell sorting were used to study the expression of SPI-1 in Salmonella enterica and its ability to invade epithelial cells.

Conclusions
We show that a pure SPI-1 ON population obtained by bacterial cell sorting is non-invasive, suggesting that the SPI-1 OFF subpopulation plays an active role in invasion. In support of this view, we also show that the invasion defect associated to unimodal expression of SPI-1 can be suppressed by mutations that permit formation of a SPI-1 OFF subpopulation.
THE INVOLVEMENT OF STREPTOCOCCUS ANGINOSUS INFECTION AND THE ABERRANT ACTIVATION-INDUCED CYTIDINE DEAMINASE EXPRESSION IN HUMAN ORAL SQUAMOUS CELL CARCINOMA

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Background

Streptococcus anginosus infection could be closely related with oral squamous cell carcinoma, although the mechanism underlying carcinogenesis is still unknown. Recent studies suggested that the aberrant expression of activation-induced cytidine deaminase (AID) in the epithelial cells could result in the generation of nucleotide alterations in tumor-related genes and possible malignant transformation of the AID-expressing cells.

Objectives

Using surgical tissue samples and cultured epithelial cells, a possible involvement of the aberrant AID expression in oral squamous cell carcinoma and S. anginosus infection was assessed.

Methods

The tissue specimens were obtained from the 17 patients with oral cancer after giving informed consent. S. anginosus infection and the aberrant AID expression were assessed by the species-specific PCR and RT-qPCR, respectively. Further, the aberrant AID expression and NF-κB activation were examined by a dual luciferase assay and RT-qPCR in three epithelial cell lines and the primary human gingival epithelial cells after stimulation with an S. anginosus antigen, SAA.

Conclusions

Both S. anginosus infection and the aberrant AID expressions were frequently observed in the tissue specimens (47% and 41%, respectively), and the infection was significantly correlated with the aberrant AID expression. The stimulation of the cultured cells with SAA could induce the NF-κB activation and aberrant AID expression in all the epithelial cells tested, and the addition of an inhibitor of NF-κB activation abrogated the aberrant AID expression. Thus, S. anginosus infection could be closely related with oral squamous cell carcinoma through the induction of the aberrant AID expression by S. anginosus antigen(s).
IDENTIFICATION OF VIRULENCE FACTORS OF CRONOBACTER SPP.

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Background

*Cronobacter* spp. are Gram-negative, motile, non-sporeforming, facultative anaerobic bacteria that have been implicated in rare but severe cases of illnesses predominantly in premature and newborn infants. These organisms have been isolated from a wide range of environments, including humans, different animals as well as (raw and processed) food and food production facilities.

Objectives

Although progress has been made during the last years, there is still a lack of knowledge on the virulence-associated factors and processes involved during pathogenesis.

Methods

To survey the pathogenesis of *Cronobacter*, a *C. sakazakii* ES5 transposon mutant library was screened in several cell lines for genes that are involved in adhesion to and invasion into host cells as well as subsequent intracellular survival.

Conclusions

The majority of attenuated mutants carried insertions in genes involved in energy production and conservation, cell membrane biogenesis, biofilm formation, motility and metabolism. Several selected genes are being functionally analyzed such as genes for flagellar biosynthesis. Preliminary analyses showed that *C. sakazakii* flagella are necessary for adherence and efficient invasion of host cells. Using a specific monoclonal antibody against a living strain of *C. turicensis* 3032, flagellar motility could be inhibited and the ability to invade Caco-2 cells was reduced. Therefore, we present evidence that *Cronobacter* flagella play a crucial role in colonization of host cells.
Background

Burkholderia glumae is a causal agent in grain and sheath rot, leading to severe damages in many rice-growing countries under the favorable conditions such as high temperature and humidity. However, its molecular mechanisms are not yet fully understood. For understating of infection mechanism, we analyzed B. glumae transcriptome from different infected rice tissues.

Objectives

We investigated molecular and physiological changes of B. glumae through comparative analyses of differentially expressed genes (DEGs) between in infected grains and in stems.

Methods

To gain a genome-wide gene expression profiling, we compared B. glumae transcriptome in stem and grain infection sites using the RNA sequencing. We then performed KEGG pathway and module enrichment of differentially expressed genes.

Conclusions

Most enriched metabolic and signaling pathways of B. glumae in infected stem tissues were bacterial chemotaxis-mediated motility, ascorbate metabolism, and sugar transporters including arabinose and xylose. However, we have confirmed different expression levels of genes involved in those pathways from the infected grains. For example, genes involved in flagellar assembly pathway were strongly down-regulated compared to those of stem tissues. Starch metabolisms were found to be highly enriched in infected grain tissues. Our study provides in vivo transcriptional profiling of B. glumae in two different tissues. Additionally, comparative analysis of B. glumae transcriptome obtained from the stem and grain will give clues on common and unique infection mechanisms in different tissues.
PREVALENCE OF COLIBACTIN GENES IN ESCHERICHIA COLI RECOVERED FROM URINARY TRACT INFECTION FROM PATIENT WITH GYNECOLOGICAL CANCER

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Background
Patients with gynecological cancer have increased risk for urinary tract infection (UTI) in which *Escherichia coli* is the main bacteria. The rates of antimicrobial resistance are increasing among these bacteria. Moreover, *E. coli* exhibits multiple accessory traits, including yersiniabactin. This virulence factor is associated with a peptide-polyketide compound, colibactin. This genotoxin, which damages DNA in eukaryotic cells, is related to the formation of colorectal cancer. However, little is known about colibactin production ratio in *E. coli* and its relation with antimicrobial resistance.

Objectives
To assess the prevalence of yersiniabactin and colibactin genes in a culture collection of *E. coli* isolates from patients with gynecological cancer with UTI and its relation with antibiotic resistance profile.

Methods
Two hundred eighteen *E. coli* UTI isolates were analyzed by PCR for detecting the phylogenetic group, *fyuA*, *clbB* and *clbN* genes. The isolate’s antibiotic resistance profile was performed previously. Statistical analysis was performed using Fisher’s test.

Conclusions
Virulence genes *clbB* and *clbN* were detected in 14.7% (32/218) of *E. coli* isolates. All strains in which these genes were detected belonged to the phylogenetic group B2 and had *fyuA* gene. Interestingly, multidrug resistance, defined as resistance to three or more antibiotic classes, showed to be a negative factor for the presence of colibactin production-related genes (p=0.0008). Colibactin genes were not detected in aminoglycosides, gentamicin and tobramycin, resistant strains (p=0.05) neither in fluoroquinolones, ciprofloxacin and norfloxacin, resistant strains (p<0.0001). Finally, 50.8% (32/63) of fluoroquinolone-sensitive strains belonging to the phylogenetic group B2 were positive for *clb* genes (p<0.0001).
Background
Moraxella catarrhalis is a human respiratory pathogen that causes acute otitis media in children and is a common cause of exacerbations in patients with chronic obstructive pulmonary disease. The first step for M. catarrhalis colonization is adherence to host tissues that is achieved by interaction with host extracellular matrix (ECM) proteins.

Objectives
Characterization of the interaction between M. catarrhalis with host collagens present in respiratory system.

Methods
Collagen I – VI were analysed for their interaction with M. catarrhalis clinical isolates by using ELISA and a radioactive direct binding assay. Collagen binding capacity of M. catarrhalis was also verified by coating collagens on the glass surface, followed by subsequent bacterial binding and detection by Gram staining. Molecular interaction between different human tissues/collagens and bacterial proteins were performed by EM.

Conclusions
Ubiquitous surface protein (Usp) A2 and UspA2 hybrid (UspA2H) of M. catarrhalis were identified as major collagen interacting proteins. The mutants of UspA2 and UspA2H exhibited defective collagen interaction and did not recognize collagen coated on glass surface. All clinical isolates showed binding capacity to fibrillar collagens type I, II and III, and network forming collagen IV and VI. Only 20% clinical isolates showed collagen IV binding capacity. Our data suggests that UspA2/A2H based interaction to collagens could be critical for adhesion of M. catarrhalis to host tissues and plays an important role in pathogenesis.
Background

Cronobacter spp. are opportunistic pathogens that cause severe infantile meningitis, septicemia, or necrotizing enterocolitis. Contaminated powdered infant formula has been implicated as the source of this pathogen in most cases, but questions still remain regarding the likely habitat and virulence potential for this strain.

Objectives

The motive of this study was to determine the occurrence of putative plasmid-harbouring virulence traits in C. sakazakii.

Methods

The isolation was done as per ISO 22964:2006 on chromogenic media followed by further confirmation by phenotypic and genotypic characterization. Two putative virulence genes Cronobacter plasminogen activator (cpa) and ferric-iron transporter eitABCD gene (eitA) were detected by PCR. The cpa gene is reported to provide serum resistance to C. sakazakii whereas eitA gene is generally associated with iron uptake mechanism especially from breast milk and infant formula by Cronobacter spp. which may enhance its spread and invasion in a host.

Conclusions

In our investigation, 15 C. sakazakii isolates were identified from 154 food and environmental samples based on phenotypic and genotypic characterization. PCR screening using eitA-specific primers revealed that the majority (67%) of the C. sakazakii isolates harbor the eitA gene while in contrast only 27% of the isolates were positive for the gene cpa. Taken together, these properties may contribute to the systemic survival of C. sakazakii and subsequent invasion of the central nervous system to cause disease. The current study clearly indicates that foods of plant origin are one of the most possible natural reservoirs of this pathogen and also provide important insight into this virulence plasmid from an emerging pathogen.
INHIBITION OF THE LYTIC ACTIVITY OF THE ALTERNATIVE AND CLASSICAL COMPLEMENT PATHWAYS BY A SURFACE LEPTOSPIRAL ADHESIN OF LEPTOSPIRA INTERROGANS

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Background
Pathogenic Leptospira are the etiological agent of leptospirosis, a disease of human and veterinary concern. The ability of pathogenic leptospires to survive the bactericidal activity of human sera was demonstrated. Recently, it has been shown that leptospires acquire soluble regulators to evade the immune system.

Objectives
To assess the effect of the leptospiral adhesin Lsa23 on the activity of complement system.

Methods
The gene LIC11360 was cloned and the protein Lsa23 expressed in Escherichia coli. The effect of Lsa23 on classical pathway of complement was assessed by hemolytic assay using antibody-coated sheep erythrocytes, while the effect on alternative pathway was evaluated measuring the deposition of C5b9 on zymosan in presence of MgEGTA buffer. Interaction with factor H (FH), C4BP and plasminogen was evaluated by ELISA. Cofactor activity of complement regulators and proteolytic activity of plasmin were assessed by immunoblotting.

Conclusions
Lsa23 was expressed and purified successfully, as visualized by SDS-PAGE. It has been able to inhibit classical pathway-mediated hemolysis by 83%, and also blocked the alternative pathway reducing C5b-9 deposition by 90%. The interaction of Lsa23 with soluble C4BP, FH and plasminogen was dose-dependent. Competition assays suggest these components have distinct binding sites on Lsa23. C4BP and FH preserved their cofactor activity for factor I when bound to Lsa23, as demonstrated by cleavage of C4b and C3b, respectively. Plasminogen bound to Lsa23 could be converted into plasmin and degraded C3b and C4b. Thus Lsa23, a surface receptor for human plasma components, prevents the lytic activity of complement system and could contribute to leptospiral immune evasion process.
THE VIRULENCE EFFECT ON A HIGHLY CONSERVED REGION OF GENES RESPONSIBLE FOR CAPSULAR POLYSACCHARIDE SYNTHESIS IN KLEBSIELLA PNEUMONIAE
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Background
Capsular polysaccharides (CPS) are one of the major virulent determinant in Klebsiella pneumoniae (KP). Among different serotype of KP, CPS gene cluster could be distinguished into two regions, a conserved and a hyper-variable region.

Objectives
Virulence's study by genes' knockout, phagocytosis, serum resistance and mice lethality on highly conserved region of genes in CPS cluster was performed.

Methods
Serotype K20 KP was selected. Six genes including galF, acidPPc, wzi, wza, wzb or wzc in CPS conserved region were knocked out and assessed their effect on virulence. In comparing to parental K20 isolate, mutants showed a varied decline in mice lethality (LD\textsubscript{50}) from 10 fold to > 10\textsuperscript{5} fold and could be categorized into low (L), moderator (M), and High (H) effect on virulence. For polycistronic mRNAs driven by P1 promoter consisted galF and acidPPc, low effect on serum resistance and anti-phagocytosis was observed. Only 10 fold of decreasing in mice lethality was achieved in the DgalF and DadicPPc mutants. For the rest of wzi, wza, Dwzb or Dwzc that were driven by P2 promoter, disruption of capsule surface assembly, Dwzi, had moderate effect with reduced mice lethality for 100 fold. The deletion of genes, Dwza, Dwzb or Dwzc which were involved in CPS polymerization, caused a significant decrease on virulence (LD\textsubscript{50} >10\textsuperscript{7}) and became phagocytic susceptible indicating the importance of these genes in synthesis of capsule.

Conclusions
Although conserved genes in CPS cluster were all involved in KP-CPS synthesis, they contributed differently in virulence.
UNRAVELING THE FUNCTION OF A LEE-ENCODED ORF: ESCK (ORF4) IS A STRUCTURAL COMPONENT OF THE TYPE III SECRETION SYSTEM OF ENTEROPATHOGENIC ESCHERICHIA COLI

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Background
The type III secretion system (T3SS) is a molecular device employed by many pathogenic bacteria to translocate a set of proteins, called effectors, directly into the cytoplasm of eukaryotic cells in order to manipulate normal cellular processes to the benefit of the bacterium.

Enteropathogenic Escherichia coli (EPEC), an important causative agent of infant diarrhea, relies on a T3SS to deliver effectors into intestinal enterocytes allowing it to colonize the host gastrointestinal tract. Structural components assembling this biological machinery are encoded on a chromosomal pathogenicity island named the locus of enterocyte effacement (LEE).

Although the vast majority of LEE-encoded proteins have an assigned function, there are still few of them uncharacterized. One such example is the protein encoded by the escK gene (formerly orf4).

Objectives
To elucidate the role of the LEE-encoded protein EscK in T3SS biogenesis and function.

Methods
An EPEC ΔescK null mutant was generated to assess its type III secretion phenotype. An extensive search of EscK protein-protein interactions with components of EPEC T3SS was performed using the yeast two-hybrid system. Novel protein interactions were corroborated by pull-down experiments. Bacterial fractionation experiments and site directed mutagenesis were carried out in order to dissect the molecular function of this protein.

Conclusions
EscK is a critical component of the EPEC T3SS, it is localized to the inner membrane and its localization does not depend on other T3SS components. EscK interacts directly with the C-ring/sorting platform component EscQ. Essential residues for EscK function were identified.
Background

*Escherichia coli* is a commensal bacterium that can be found in the intestinal microbiota. However, pathogenic *E. coli* strains that are associated with numerous infections, possess virulence factor (VF) genes. *E. coli* is known to be an important cause of skin and soft-tissue infections (SSTI) as it is the 3rd most prevalent isolated species from SSTI nevertheless, *E. coli* from such infections are rarely investigated.

Objectives

The main goal of our research was to shed more light on certain iron uptake systems among SSTI *E. coli* strains. We determined their prevalence and analysed possible correlations between their genes and phylogenetic groups and some other VFs as well as bacteriocin genes.

Methods

A collection of 102 previously described SSTI *E. coli* strains was used. The methods employed were cultivation in LB medium, preparation of lysates, and polymerase chain reaction (PCR) with primers specific for the investigated target genes (*fyuA*, *iroCD*, *iucD*, *iha*, *ireA*, *picU* and *hbp*). The PCR products were detected and visualised with agarose gel electrophoresis. All results were statistically processed using Fischer’s exact test and Bonferroni correction.

Conclusions

The prevalence of iron up-take systems among the studied collection was 76%, 60%, 47%, 30%, 19%, 10% and 5% for *fyuA*, *iroCD*, *iucD*, *iha*, *ireA*, *picU* and *hbp*, respectively. The following correlations were observed: *fyuA* with B2 phylogenetic group and *cnf1*, *hlyA*, *kpsMTII*, *ompT* and *usp* genes; *hbp* with *ompT*_{APEC} and microcin V; *ireA* with *papGII*; *iha* with *papGII* and *iucD*; and *iroCD* with microcinH and *sfa*.
Background

Bacteria from the *Brucella* genus are gram negative intracellular pathogens responsible for Brucellosis, one of the most widespread anthropo-zoonosis worldwide. *Brucella* spp. are intracellular pathogens and despite their tremendous impact on world health and economics no human vaccine is currently available and little is known about molecular mechanisms underlying the infection process.

*Brucella* infection of cultured host cells (*i.e.* HeLa cells and RAW 264.7 macrophages) is biphasic. It is first characterized by a « lag phase » consisting in a very low but constant number of colony-forming units during the first hours of infection, and followed by a second « duplication phase » during which bacteria proliferate massively. This defines adhesion/invasion of host cells as a critical step for successful infection.

Recent data showed that infection is mainly carried out by a bacterial subpopulation composed of newly generated bacteria called « newborns », characterized by a single genome copy (Deghelt, Mullier *et al.* 2014)

Objectives

Identification of bacterial components mediating preferential adhesion to and/or invasion of cultured host cells by newborn *Brucella*.

Methods

'With a priori' gene deletion, 'without a priori' transpositional mutagenesis screen (Tn-seq), lectin staining.

Conclusions

So far, three adhesins (BmaC, BtaE, BtaF) have been shown not to act in the newborn selection when infecting HeLa cells and RAW 264.7 macrophages. Tn-seq is still ongoing. We have detected specific binding of the wheat germ agglutinin to the new pole of *B. abortus* when grown until stationary phase in rich medium.
INTERACTION WITH VITRONECTIN, LAMININ AND FIBRONECTIN: A NOVEL ROLE OF Haemophilus influenzae LIPOPROTEIN P4 IN SERUM RESISTANCE AND ADHERENCE TO PULMONARY EPITHELIAL CELLS

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Background Nontypeable Haemophilus influenzae (NTHi) is a human-specific Gram-negative species that primarily colonizes the nasopharyngeal and respiratory tract. Interaction with extracellular matrix (ECM) proteins is one of the colonization strategies described for NTHi, and involves several bacterial adhesins.

Objective We describe herein the identification and functionality characterization of a NTHi multifunctional ECM protein receptor.

Methods Outer membrane proteomic analysis revealed that Haemophilus outer membrane protein 4 (P4; an acid phosphatase involved in the uptake of nicotinamide adenine dinucleotide) interacted with multiple ECM proteins including vitronectin, laminin and fibronectin. This was further validated when an NTHi 3655 mutant devoid of P4 (NTHi3655Δhel) significantly lost binding to the ECM proteins. Binding was restored by a hel gene trans-complemented mutant. Analyses of protein-protein interactions by ELISA revealed that purified recombinant P4 has a high binding affinity for laminin (dissociation constant, KD=9.26 nM) and fibronectin (KD=10.19 nM), but slightly less to vitronectin (KD=16.51 nM). Interestingly, binding to the ECM proteins was optimal with dimeric P4, a structure that is dependent on the α-domain. Vitronectin acquisition conferred serum resistance to both P4-expressing NTHi 3655 and Escherichia coli transformants, but not to the P4-deficient strains. Importantly, NTHi3655Δhel also displayed decreased adherence to both type II alveolar epithelial and pharynx epithelial cells, and blocking with antibodies suggested the P4-mediated cell adherence was mainly attributed to fibronectin.

Conclusions In conclusion, our data provides new insight into the potential role of P4 as a multiple ECM protein receptor important for colonization and establishment of NTHi infection.
Background
Pasteurella pneumotropica (PP) is a small gram (-) coccobacillus which is a normal inhabitant of the oropharynx of small animals. Human infections due to PP rarely occur. We saw an elderly woman with septicemia due to PP, and describe herein.

Objectives
Human infections due to PP are rarely seen, and it is our intention to share the case experience with others.

Methods
A 76 yo woman with comorbidities including ESRD on hemodialysis, DM, CVA, and hypertension was admitted for evaluation of fever, chills, sore throat and fatigue. She also complained of increased cough with yellowish sputum production. She denied myalgia, arthralgia, and dyspnea. She owns a dog. On exam. temp., 38.9°C, RR, 23/min, and PR, 112/min. Other positive findings were: erythematous pharynx without exudate, lung auscultation showed diffuse bilateral rhonchi. Lab. findings: leukocytes, WBC at 31.7K/cmm; BUN 43 mg/dl; and Cr 8.69 mg/dl. Sputum culture grew normal oral flora. Blood cultures (B/C) obtained on admission revealed positive (4/4) for small gram-negative coccobacilli which was confirmed to be PP. Antibiotic susceptibility testing: sensitive to penicillin containing preparations (i.e., ampicillin, amoxicillin/clavulanate), doxycycline, TPM/SMX, and moxifloxacin, while resistant to ceftriaxone and azithromycin. She was initially begun on amp./sulb. and she became afebrile and abacteremic on day 3. She remained abacteremic afterward and was discharged on amox/clav 875 mg po BID to complete a 2-wk course.

Conclusions
A patient with no known history of definite animal bite or scratch developed PP septicemia and she responded to penicillin preparation nicely without further complication.
Background

Salmonella infection is a major health concern and continues to have a serious economic impact worldwide. It is estimated that Salmonella serotypes cause 93.8 million human infections and 155,000 deaths annually through the world.

Objectives

The main objective of this study was to update the prevalence and antimicrobial resistance characteristics of Salmonella isolated from poultry and from humans experiencing gastroenteritis in N’Djamena and also to evaluate the phylogenetic relationships and to find epidemiological links between human and avian strains isolated in the same period of time and in the same region.

Methods

All samples (dropping, sterile Cloths, food and water) collected were analysed according to French Norm for Salmonella Spp. NF ISO 6579/2002.

A total of Salmonella isolates found in humans and poultry in this study were characterised by ERIC-PCR and IS200-PCR. The PCR analysis was followed by macrorestriction analysis (PFGE).

Conclusions

Diagnostic methods carried out during this study led to the isolation of one hundred and twenty seven Salmonella strains, belonging to forty two different serotypes. Salmonella Colindale was the most prevalent serovar (13.6 %), followed by Salmonella Minnesota (10.8 %), Salmonella Stanleyville (5.8 %).

PCR results demonstrated that, in the context of this epidemiological study, ERIC- and IS200-PCR methods can be used effectively to limit the number of isolates that have to be serotyped. The comparison of ERIC-PCR, IS200-PCR, PFGE and antimicrobial susceptibility profiles among isolates from human and avian origins has highlighted, the indirect evidence of human contamination sources by Salmonella serovars from poultry farms.
Bacterial pathogenicity

QUANTIFICATION OF LIPOTEICHOIC ACID (LTA) IN ROOT CANALS WITH NECROTIC PULP SUBJECTED TO ENDODONTIC TREATMENT

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Background

Gram-positive bacteria release lipoteichoic acid (LTA) as an important virulence factor, which is structurally and immunologically similar to lipopolysaccharide (LPS). The knowledge about LTA actions in vivo remains unclear, as well as their participation in the etiology of pulp and periapical diseases, with no reported studies on the quantification of LTA in root canals.

Objectives

a) quantifying the levels of LTA in primary infections of root canals with necrotic pulp and apical periodontitis; b) verifying the effects of biomechanical preparation using sodium hypochlorite 2.5% in LTA reduction in root canals.

Methods

Ten single-rooted teeth of patients needing endodontic treatment were prepared with Reciproc rotation system and irrigated with 2.5% NaOCl. Root canals samples were obtained immediately after coronary opening (S1) and after biomechanical preparation (S2). LTA quantification was performed by enzyme immunoassay (ELISA), with specific anti-LTA antibodies. The optical density values were converted to µg/mL and analyzed by Student’s t test (significance level of 5%).

Conclusions

Results: All root canals showed LTA in the collection S1 with an average of 119.4 ± 73.2 µg/mL. After instrumentation, it was observed a reduction in the levels of LTA, with an average in the collection S2: 77.6 ± 22.4 µg/mL.

Conclusion: The root canals with necrotic pulp showed high levels of LTA and only instrumentation with sodium hypochlorite 2.5% was not enough for its entirely
elimination of the root canals. New studies with other endodontic treatment protocols should be performed for new findings.
CHARACTERIZATION OF TWO PROTEINS OF LEPTOSPIRA INTERROGANS WITH POTENTIAL ROLE IN HOST-PATHOGEN INTERACTIONS

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Background
Leptospirosis is a worldwide zoonosis regarded as a major public health problem. Measures to control the disease are difficult to implement. The development of new strategies to prevent and control the spread of disease is urgently needed. Accordingly, vaccines emerge as strong candidates to solve the problem. For this reason, currently research has focused to identify conserved antigens that are involved in host-pathogen interactions.

Objectives
Evaluate the functional properties of the coding sequence LIC13479 and LIC10050 of L. interrogans serovar Copenhageni, identified by bioinformatics as putative outer membrane proteins.

Methods
The gene sequences were cloned into the expression vector pAE. Plasmids containing cloned DNA were introduced in E. coli strains for protein expression. After purification of the recombinant proteins, mice were immunized for polyclonal antibodies production. Reactivity of the recombinant proteins was evaluated in serum samples of leptospirosis patients and of febrile unrelated diseases. The ability of recombinant proteins to interact with the host by adhering to extracellular matrix proteins or serum components was examined.

Conclusions
The coding sequences LIC13479 and LIC10050 were cloned and expressed successfully. Evaluation of the purified recombinant proteins showed that they are capable to stimulate antibody immune response in mice and, in addition, they are recognized by infected human serum samples. Both recombinant proteins exhibited adhesin properties and, in addition, interacted with plasminogen and can generate plasmin in the presence of activator. Our data indicate that these proteins could promote the attachment and contribute to the invasion processes within the hosts.
MOLECULAR CHARACTERIZATION OF SHIGA TOXIN-PRODUCING
ESCHERICHIA COLI (STEC) ISOLATED IN 2012 IN THE COUNTRY OF GEORGIA

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Background

STEC-associated HUS was first recognized in Georgia in 2009 following the diagnosis of a cluster of HUS cases in Georgia.

Objectives

In this study we genetically characterized two sporadic cases (Case A and B) of HUS syndrome occurred in 2012. Among them was one family case reported (Case B), where four individuals revealed positive on STEC, but only one developed HUS.

Methods

Clinical samples were enriched in broth and simultaneously plated on selective agar media. DNA was extracted and tested by two different conventional Multiplex PCR assays for final confirmation of STEC (stx1, stx2, eae, Ehly) and detection O104 strain markers (stx2, terD, rfbO104, fliC H4). Furthermore, for molecular genotyping Pulsed Field Gel Electrophoresis (PFGE) was applied. Five STEC strains were isolated from both described cases. All revealed positive results by molecular tests on STEC but showed different genetic profiles based on combination of existing
toxigenic markers. Interestingly, it was observed that case A tested positive on all four O104 specific genes. Besides, PFGE typing showed two different genetic profiles as well.

Conclusions

Here described in both HUS cases, a diversity of STEC isolates was detected. However, one of the causative agents was considered to be STEC O104. It is remarkable that two O104 strains were isolated during STEC outbreak in Georgia in 2009 which are genetically related with the strains isolated during STEC outbreak in Germany in 2011. This finding suggests that O104 strain has spread and been circulating for several years in the country.
ROLE OF IRON IN THE CROSS-TALK BETWEEN THE BIOSYNTHETIC PATHWAYS LEADING TO THE PRODUCTION OF SIDEROPHORES AND COLIBACTIN.
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Background

The biosynthetic pathways of several siderophores and the genotoxin colibactin require the enzymatic activity of a phosphopantetheinyl transferase (PPTase). We have shown that ClbA, the PPTase associated with the production of colibactin, can also contribute to the production of siderophores, whose production is dependent on the bioavailability of iron in the medium, through the regulation of the expression of EntD, the PPTase previously thought to be the unique PPTase involved in the production of siderophores in E. coli.

Objectives

We hypothesized that iron could also modulate the production of colibactin through the transcriptional regulation of clbA.

Methods

Mutant derivatives of E. coli were engineered. We investigated transcription of genes involved in the production of colibactin in iron depleted or repleted conditions using qRT-PCR and luciferase reporter gene assay. Quantification of total siderophores was assessed.

Conclusions

This study revealed an increased transcription of clbA in iron limiting conditions, and a decrease of clbA expression in iron supplemented media. Quantification of total siderophores revealed that the increased expression of clbA in iron-depleted conditions was associated to an increased synthesis of siderophores. Mutation of entD leads to an exacerbation of the induction of clbA expression, whereas overexpression of entD or clbA leads to a repression of clbA transcription. Analysis of the region upstream clbA shows the presence of two putative Fur-boxes, which could explain its regulation by iron. In conclusion clbA is tightly regulated by iron.
bioavailability leading to an increased production of siderophores that could explain the high pathogenicity of the strains synthetizing colibactin.
Background

*Streptococcus pyogenes* is an important bacterial pathogen that colonizes the throat and skin of a host and produces a wide variety of virulence factors such as toxins, proteases or DNases.

Objectives

The aim of this study was to investigate the antibiotic resistance and virulence factors (speA, B, C, G, H, I, J, K, L, M, smeZ, ssa, spd3, sdc, sdaB, sdaD, spyCEP, scpA, mac and sic) of *S. pyogenes* strains isolated from throat cultures of patients with symptomatic tonsillopharyngitis.

Methods

One hundred and fifty *S. pyogenes* isolates were identified by conventional methods and VITEK 2 automated system. Antibiotic susceptibility tests were performed by Kirby-Bauer disk diffusion method as recommended by Clinical and Laboratory Standards Institute. The virulence factors were determined by multiplex PCR.

Conclusions

All of the *S. pyogenes* isolates were susceptible to penicillin G, cefotaxime, ceftriaxone, chloramphenicol, clindamycin, erythromycin, levofloxacin, vancomycin and linezolid. Among streptococcal pyrogenic exotoxin genes the most frequent gene was speG (88.0%) following speC (59.3%), smeZ (46.7%), ssa (42.7%), speA (33.3%), speJ (24.0%), speK (18.7%), speH (14.0%), speI (13.3%), speL and speM (9.3%). From DNases, proteases and inhibitors sdaB, speB, spyCEP, scpA and mac were positive in all strains, and spd3, sdc, sdaD and sic were carried 64.7%, 36.0%, 24.7%, 2.0% of the isolates respectively. In conclusion, *S. pyogenes* isolates collected from throat cultures of patients with symptomatic tonsillopharyngitis in Konya/Turkey possess high virulence factors, and susceptible to antibiotics.
COMBINATION BETWEEN A FEW T3SS INJECTISOME AND A LOT EFFECTOR FOR KILLING HOST CELLS ON VIBRIO PARAHAEOMLYTICUS

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Background
Type 3 secretion system (T3SS), is major virulent factor in over twenty species of Gram-negative bacteria which export bacterial protein (effectors) to host cell to manipulate its cellular function for owns hope. Moreover, T3SS is a prospective useful tool with both experimental and therapeutic applications, including vaccine development.

Objectives
Upon analysis of the contribution of the T3SS injectisome and effector involved in this paradigm, it is still unknown that the number and timing of expression of the T3SS injectisone and effectors, and which factor have dominant role during infection are unclear. In the present study, we used fluorescence protein combined with single cell analysis to solve dynamics of the T3SS and effector during infection in Vibrio parahaemolyticus.

Methods
During infection, Vp1671 (T3SS component) but not the Vp1680 (effector) proteins showed to make spot like localization along the membrane of the cell. Although both percentages of spot positive cells and number of T3SS spots were increased depend on a time of infection, assemblies of the Vp1671 to the T3SS occur prior to increase fluorescence levels of bacterium in early time of infection. Cytotoxicity against the host cells were increased depend on the expression levels of Vp1680 and number of T3SS spot, if it is only one spot.

Conclusions
V. parahaemolyticus use a combination between a few T3SS injectisome regulated by localization and a lot effector regulated by expression for killing host cells.
ASYMPTOMATIC BACTERIURIAS STREPTOCOCCUS AGALACTIAE: MOLECULAR CHARACTERIZATION AND IMPLICATIONS FOR PATHOGENESIS

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Background

Streptococcus agalactiae causes urinary tract infection (UTI) including asymptomatic bacteriuria (ABU); however, growth of S. agalactiae in urine has not been reported and the role of this growth phenotype in human infection is unknown.

Objectives

We characterised the ability of different S. agalactiae UTI isolates to grow in urine, and defined the molecular mechanism(s) of growth and implications for human ABU.

Methods

We used growth assays, competition assays, metabolic phenotype arrays, whole-genome sequencing, and mutation analysis in S. agalactiae. We report robust growth of ABU S. agalactiae (ABSA) in human urine that was not seen among uropathogenic S. agalactiae (UPSA) isolated from patients with acute UTI. In competition assays using a prototype ABSA strain, designated ABSA 1014, and any one of several UPSA strains, we observed markedly superior fitness of ABSA 1014 for urine growth. Phenotype profiling of ABSA 1014 and UPSA 807, isolated from a patient with cystitis, using metabolic arrays revealed specific L-Malic acid catabolism in ABSA 1014 that was absent in UPSA 807. Whole-genome sequencing revealed divergence in malic enzyme-encoding genes between the strains predicted to impact the activity of malate metabolism. Urine growth assays comparing wild-type ABSA and mutants that were functionally inactivated for malate metabolism by disruption of maeE or maeK demonstrated attenuated growth of the mutants in urine and synthetic human urine containing malic acid.

Conclusions

We conclude that some ABU S. agalactiae can grow in urine, and this relates in part to malic acid metabolism, which may affect S. agalactiae UTI.
THE ROLE OF THE MYCOSIN PROTEASE IN TYPE VII SECRETION OF PATHOGENIC MYCOBACTERIA.

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Background

A promising new group of novel drug targets for *Mycobacterium tuberculosis* are the type VII secretion (T7S) systems. *M. tuberculosis* has five of these systems, ESX-1 to ESX-5, of which at least three are crucial for the virulence and/or viability of this pathogen. One of the conserved components of T7S systems is mycosin (MycP), a membrane-associated protease that is an essential part of the T7S system. Due to the proteolytic activity of the mycosins they are promising targets for novel drugs against TB.

Objectives

We set out to functionally dissect the role of the mycosin proteases in T7S, by analyzing the phenotype of MycP1 and MycP5 mutants.

Methods

Deletion strains of the mycosins of the ESX-1 and ESX-5 secretion systems were created in *Mycobacterium marinum*. Subsequently, proteolytic inactive versions of MycP1 or MycP5 were introduced in the deletion strains. Functional complementation by these variants was verified by the effect on the secretion by the ESX-1 and ESX-5 systems.

Conclusions

While deletion strains of MycP1 or MycP5 were defective in respectively ESX-1 or ESX-5 dependent secretion, the protease inactive variants were able to mediate secretion by ESX-1 or ESX-5. Thus the proteolytic activity of MycP is not essential for its respective ESX-system dependent secretion. These results indicate a dual function for mycosins, with a proteolytic role in substrate processing and a second, so-far unknown, role in the regulation of the secretion process. We are currently
unravelling this second function in T7S and pinpointing which domain of MycP is involved in this process.
FEMS-1868
Bacterial pathogenicity

EFFECT OF DIFFERENT FACTORS ON THE (TWITCHING) MOTILITY OF PHOTOBACTERIUM DAMSELAE SUBSP. PISCICIDA.
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Background

Photobacterium damselae subsp. piscicida, which is the etiological agent of photobacteriosis and affects different species of sea fish, is a non-motile Gram-negative bacterium. Notwithstanding, it is able to move on solid surfaces. Because of the presence of pili-like structures on the bacterial surface, discovered the last year by Remuzgo-Martínez \textit{et al.}, and being associated with twitching motility,

Objectives

the objective of this study was to modify some conditions (temperature, pH, concentration of nutrients, salinity, nature of the medium and others) to determine which ones promote motility.

Methods

A total of five bacterial strains were tested: C2, 94/99, DI21, PP3 and ATCC 17911. The results of motility assays were obtained after two and three days of inoculation in “twitching motility” medium and statistically analyzed with IBM SPSS Statistics 22.0 program.

Conclusions

Lower temperatures, alkaline and acidic environments and lacking salinity had a negative effect on cell motility, while nutrient limitation did not affect the bacterial response. The best twitching motility was observed when \textit{Phdp} was inoculated in medium with 0.2\% agar and pH 7.0. Also, interesting results were achieved after scraping petri dishes (surface where the bacteria moved) or the addition of cellular debris of SAF-1 cell line to the medium.
IDENTIFICATION AND DISTRIBUTION OF A NOVEL 22-KDA OUTER MEMBRANE PROTEIN RELATED TO SHIGA TOXIN–PRODUCING ESCHERICHIA COLI (STEC) STRAINS NEGATIVE FOR LOCUS ENTEROCYTE EFFACEMENT.

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Background
STEC strains are etiologic agents of acute diarrhea, bloody diarrhea and hemolytic uremic syndrome (HUS). Associated with STEC outbreaks and HUS, we may observe two main groups of strains, those that in their genomes is encoded the Locus Enterocyte Effacement (LEE-positive), including the serogroups, O157:H7, O26, O103 and O111, and bacteria without this Locus (LEE-negative) which include the serogroups, O113 and O91: H2.

Objectives
Describe a novel antigen present in the outer membrane (OM) of a LEE-negative STEC strain.

Methods
By western blot and immunoproteomic analysis (2D electrophoresis - MALDI-TOF/TOF) using sera from patients who developed HUS, we were able to identify an immunoreactive protein of 22 kDa with an isoelectric point of 5.0. By mean of bioinformatics tools, we found the encoded gene in the genome of STEC strain O91: H21 B2F1 LEE-negative (Accession: AFDQ01000026.1). Using specific primers, the gene was amplified and its presence was studied in a collection of 170 STEC strains and 11 commensal E.coli. Interestingly, the gene was detected only in LEE-negative STEC strains (63%; 32/51). Sequencing of PCR products showed that this gene is highly conserved. Preliminary results suggest that this protein could be involved in bacterial adhesion and hemagglutination.

Conclusions
Our results suggest that the identified antigen might be associated only with a subset of LEE-negative STEC strains. Additional studies are needed to determine the role of this protein in STEC pathogenicity.

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LEPTOSPIRA INTERROGANS STIMULATES PLASMINOGEN ACTIVATORS IN VIVO IN EXPERIMENTALLY INFECTED ANIMALS AND NATURALLY ACQUIRED HUMAN LEPTOSPIROSIS.

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Background
Leptospirosis is a globally important zoonosis, caused by pathogenic bacteria of genus Leptospira. The molecular mechanisms for leptospiral pathogenesis and virulence remain poorly understood. A pathogen invasion, dissemination and host tissue damaging depend on proteolytic enzymes both of the invading organism and of the host. Many pathogens express their own proteases or exploit hosts proteases to activate other protease-dependent cascade systems. We have described that leptospires capture plasminogen (PLG) on the outer surface, which is converted to plasmin (PLA) by exogenous activators. We also showed that leptospires induce the expression of PLG activators by human endothelial cells in vitro.

Objectives
Here, we further characterized the interactions of L. interrogans with human PLG/PLA system in vivo.

Methods
We studied the stimulation of PLG activators in different time points during leptospirosis in hamsters infected with lethal doses, and in human sera samples from the initial and convalescent phase of the disease.

Conclusions
Our data indicate that leptospirosis human patients have increased levels of circulating urokinase-type and tissue-type PLG activators when compared to normal sera, being especially augmented at the early phase of the disease. In the sera of experimentally infected hamsters, increasing levels of PLG activators were observed during the progression of the infection, until euthanasia. Additionally, hamsters' organs extracts similarly showed increased PLG activators activity.

The results presented here further characterize the host response to the Leptospira infection, in the light of PLG/PLA system stimulation. Our data strengthen the importance of the fibrinolytic system to the leptospirosis infectious process.

Support: FAPESP, CNPq, Fundação Butantan.
Bacterial pathogenicity

BINDING OF LEPTOSPIRA INTERROGANS TO HUMAN THROMBIN, BIOLOGICAL IMPLICATIONS AND IDENTIFICATION OF POSSIBLE BACTERIAL LIGANDS


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Background
Leptospirosis is a zoonosis caused by pathogenic spirochete of genus Leptospira. The symptoms range from a mild fever to a more complicated, severe form of the disease, called Weil’s syndrome. Since the 1980s, the incidence of severe pulmonary hemorrhage caused by Leptospira spp. infection has increased. The reasons behind the emergence of this syndrome are not known. Many pathogens can interfere in the coagulation cascade by binding to fibrinogen or by interaction with the enzyme thrombin.

Objectives
To characterize the interaction of leptospiral strains to thrombin, analyze the possible biological relevance and select possible proteins that could mediate these interactions.

Methods
The interaction of pathogenic (virulent and attenuated) and saprophytic strains with thrombin was performed by ELISA and western blotting. Characterization of the binding sites in thrombin molecule was accessed by co-incubation with different competitors. Reduction in thrombin activity was evaluated by fibrin clot formation assay. Identification of ligands was performed by ELISA by employing a set of recombinant leptospiral proteins.

Conclusions
We demonstrated that the pathogenic strains of Leptospira display a prominent binding to thrombin, particularly the virulent one. Competition assays indicate that virulent strain binds thrombin via exosite I. Virulent strain could reduce thrombin activity. Although we have identified some proteins that displayed binding to thrombin, none was capable to reduce the enzymatic activity. The interaction of virulent strains of Leptospira with the key enzyme of the coagulation cascade, thrombin, might constitute a novel mechanism of virulence, that could help understand the hemorrhagic features of leptospirosis.
FEMS-1917
Bacterial pathogenicity

RELATION BETWEEN THE SEQUENCE TYPE AND THE PROSPECTIVE VIRULENCE OF CRONOBACTER STRAINS

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Background

Bacteria from the genus Cronobacter are pathogens endangering all age groups, with severe clinical complications especially in neonates and infants. A multilocus sequence typing (MLST) scheme based on 7 housekeeping genes was constructed for Cronobacter spp. identification. The method also enables the recognition of sequence types (STs) and clonal complexes (CCs). Although 7 housekeeping genes (atpD, fusA, glnS, gltB, gyrB, infB, and ppsA) used for MLST analysis are not virulence related, connection between infections and particular sequence types were described in the literature.

Objectives

The aim of this work was to evaluate the relation between sources of tested strains and their characteristics particularly their sequence type.

Methods

MLST aimed to 7 genes (atpD, fusA, glnS, gltB, gyrB, infB, and ppsA) was performed on bacterial collection (over 80 strains). The amplification of genes gltB and gyrB showed to be difficult, and therefore new sets of primers were designed and tested.

Conclusions

The target genes were sequenced in the majority of tested strains; in the case of incomplete information putative sequence type was determined. The obtained results showed widespread distribution of STs within tested strains. The significant predominance of particular STs was not determined in tested strains. Perhaps, the amount of tested strains was not representative enough; the ST relation to virulence will be further studied together with other possible factors.
This work was supported by the Czech Grant Agency (13-23509S) and Specific University Research (MSMT No. /2015).
Background

*Staphylococcus aureus* is an opportunistic pathogen of humans and animals. Our studies have revealed a link between the genotype of poultry-isolated *S. aureus* strains and their virulence in chicken embryo but not nematode model, what may suggest the existence of factors responsible for host preference and virulence.

Objectives

Here we compare genomes and extracellular proteomes of four virulent and four non-virulent *S. aureus* strains in chicken embryo model. The open reading frames translation products for all strains were taken together and clustered according to the similarity level of 80%. Over three thousands clusters were obtained with only around 40% of clusters containing proteins with identical sequence across all strains. However, within the virulent and non-virulent group around 80% and 54% of clusters ORFs encoded identical proteins, respectively. The exoproteomes were highly heterogeneous irrespective virulence, however we were able to point alpha-hemolysin and bifunctional autolysin as indicators of virulence whereas glutamylendopeptidase as potential virulence attenuator.

Methods

The genomes were obtained using MiSeq Illumina sequencer and assembled with MIRA and CLC Main Workbench software. Extracellular proteins were precipitated, labeled with fluorescent dyes and subjected to two-dimensional difference gel electrophoresis in pairs virulent versus non-virulent. Differentiating protein spots were subjected to mass spectrometry analysis.

Conclusions

Deep sequencing confirmed genetic dissimilarity of virulent and non-virulent strains which finds its reflection in extracellular proteome. However, proteomics also points transcriptional and translational events as modulators of *S. aureus* virulence.

The study was supported by the grant UMO-2012/07/D/NZ2/04282 (to B.W.) from National Science Centre, Poland.
Background

_Yersinia ruckeri_ is a Gram-negative bacterium pathogen responsible for enteric redmouth disease (ERM). _Yersinia ruckeri_ infections cause significant economic losses in salmonid aquaculture. ERM can be effectively controlled by antibiotic treatment and by application of whole cell based vaccines. Nevertheless, ERM outbreaks are still observed. More recently, vaccine resistant isolates caused disease throughout Europe. Research into the pathogenicity of _Yersinia ruckeri_ is limited. Much more is known about the host-pathogen interaction of the three _Yersinia_ species pathogenic to humans. The group of proteins, that mediate the host-pathogen interaction comprise adhesins anchored in the bacterial outer membrane. They are essential for infection as they possess the ability to bind a variety of host molecules such as collagen, fibronectin, laminin, β1 integrins.

Objectives

The aim of my research is to establish a fish infection model – zebrafish to understand the molecular mechanisms of adhesion and to identify adhesins of interest and their cellular receptors in order to assess their function in pathogenesis.

Methods

To examine whether adhesins of interest are involved in _Yersinia ruckeri_ virulence, fish cell line and in vivo system (zebrafish embryos) will be used and optimise. Fluorescence microscopy will allow to visualize infections to assess the role of virulence genes. Infection of colour-fluorescence bacteria will be essential to follow the infection.

Conclusions
Understanding the molecular mechanisms of adhesion in fish, might prevent progression of infections. Moreover, the knowledge gained could help in developing new antimicrobials that could directly act as vaccines against bacterial fish diseases.
Background
Brucella abortus is a well-known intracellular pathogen. Underlying mechanisms of the bacterial infection is very complicated to understand the pathogenesis even though most of clues have been solved. Therefore, prevention and control of the B. abortus infection is still problematic in animal and human. So, several methods including random mutation have been used to understand the mechanisms and find out the solution in control and prevention of the infection. Regardless the trials, the doors to be opened are still locked in understanding of the mechanism.

Objectives
To understand the underlying mechanism of B. abortus infection, mutants were generated with a transposon and function of the disrupted genes were revealed.

Methods
Mutants of B. abortus were generated by random insertion of a transposon, Ez-Tn5™ pMOD™-3 <R6K<ori/MCS> into chromosome. Molecular characteristics of the mutants were investigated using PFGE, Southern blot and sequencing of the genes.

Conclusions
B. abortus mutants were generated and insertion of the transposon was confirmed by Southern blot analysis with the transposon as a probe after PFGE of chromosome. Both sides of insert in the chromosome were sequenced and the location of interrupted genes were identified in chromosome. Using sequencing information, twenty-eight genes were identified from the mutants by comparison with wild type of the bacteria. Also, function of the genes were revealed. This work was supported by NRF (No. 2014R1A2A2A01007291).
CYTOKINE EXPRESSION PROFILE IN RAW 264.7 CELLS STIMULATED WITH BRUCELLA ABORTUS MUTANTS

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Background

Brucella abortus have been known as an important intracellular, zoonotic pathogen. Intesne immune responses are induced in animals by the bacterial infection. Cytokine has key roles in activation of innate immunity and acquired immunity. Precious roles of cellular components of B. abortus infection have been remaining to be solved even though involvements of immune cells in activates of acquired immunity are already known. On the basis of current understanding, production profiles of inflammation cytokines such as NO, IL-1β, IL-4, IL-12 and IFN-γ, were compared with Brucella mutants. Moreover, it might be given a key to find immune system, pathological pathways, and mechanism.

Objectives

To understand the roles of cellular components of B. abortus in immune cells, production profiles of cytokines were investigated in a mouse macrophage cells, RAW264.7 cells after stimulation with B. abortus mutants.

Methods

RAW 264.7, murine macrophages, cells were stimulated with mutants of B. abortus. Culture supernatants were collected from RAW 264.7 cells after stimulation with B. abortus mutants at different time intervals. Amounts of cytokine such as NO, IL-1β, IL-4, IL-12 and IFN-γ, were quantified with ELISA.

Conclusions

Inflammatory cytokines were produced from Raw 264.7, murine macrophage, cells after stimulation with the B. abortus mutants. After then, production levels of inflammatory cytokines such as NO, IL-1β, IL-4, IL-12 and IFN-γ, were measured. The patterns of the cytokine productions will be presented. This work supported by NRF (No.2014R1A2A201007291) and IPET112012-03-1-HD020.
Background

Brucella pathogens proliferate within several eukaryotic cells. Cumulative evidence indicates that adhesion of Brucella to host cells is an important step to establish infection. We have shown that the unipolar monomeric autotransporter BmaC is involved in the adhesion of Brucella suis to host cells through binding to cell-associated fibronectin.

Objectives

Genome analysis shows that the B. suis genome encodes two trimeric autotransporters (BtaE and BtaF) and two additional monomeric autotransporters (BmaA and BmaB). The aim of this work was to explore the roles of these proteins in the interaction of Brucella with the host.

Methods

Clean deletion mutants were analyzed by in vitro binding assays and in vivo using the mouse model. Adhesin localization was analyzed by immunofluorescence.

Conclusions

BtaE was involved in the binding of B. suis to hyaluronic acid and fibronectin while BtaF may contribute to the binding to several ligands. Both the ΔbtaE and ΔbtaF mutants showed a reduction in the attachment to epithelial cells and were attenuated in mice. Similar to BmaC, BtaE and BtaF were detected in a low proportion of bacteria but in all cases at one particular pole. A remarkable variability in the number of motifs of autotransporter orthologues from different Brucella species was observed.
Furthermore, while both trimeric autotransporters might be functional in all *Brucella* species, pseudogenization occurred in *bmaA* and *bmaB* orthologues. In particular, BmaA would not be functional in *Brucella abortus* while it is predicted to be functional in *B. suis* and *B. melitensis*. Thus, functional autotransporters may contribute to host preference.
Background

*Bifidobacterium breve* is a common inhabitant of the infant gut and its presence has been correlated with particular health-promoting effects, such as enforcement of the intestinal barrier, activation/modulation of the host’s immune response and protection against particular infections.

Objectives

Genome sequencing of *B. breve* JCM 7017 revealed the presence of the first verified bifidobacterial megaplasmid pMP7017 (190 kb of size). The objective of this study was to provide evidence of its horizontal transmission between strains of the same and/or different (bifido)bacterial species by means of a predicted conjugative machinery.

Methods

DNA sequencing was performed using Next Generation Sequencing platforms. The *in silico* predictions were performed using BLAST and PFAM databases as well as additional bioinformatics tools (e.g prodigal, tRNAscan, CRISPR and GCUA). Proof of conjugal transfer was obtained by demonstrating the presence of the megaplasmid using Pulsed Field Gel Electrophoresis in combination with S1 nuclease treatment.

Conclusions

*In silico* characterization of the megaplasmid revealed several genomic features supporting a stable establishment in its host, illustrated by predicted CRISPR-Cas functions that are known to protect the host against intrusion of foreign DNA. Interestingly, pMP7017 is also predicted to encode a conjugative DNA transfer apparatus and consistent with this notion we demonstrate conjugal transfer of pMP7017 to representative strains of *B. breve* and *B. longum* subsp. *longum*. We furthermore demonstrate the presence of a megaplasmid with homology to pMP7017 in three *B. longum* subsp. *longum* strains, indicating that similar elements are also naturally present in other bifidobacterial species, thereby supporting our proof of cross-species transfer.
Background
Competition for substrate is a common mode of microbial interaction in natural environments. Both, growth and motility phenotype influence competition, with the former having been studied well, while the latter has received less attention.

Objectives
To elucidate the impact of microbial motility on competition, we focus on microbial communities in which two strains populating a homogeneous environment compete for a substrate. Strains share identical growth phenotypes but differ in their chemotactic preference, either responding more sensitively to substrate or to a chemoattractant that is excreted by the cells themselves. By systematically varying chemotactic preferences of the competing strains, we assess the impact of each chemotactic preference on competition and on overall community fate.

Methods
Microbial communities are simulated using an individual-based modelling approach. Competing strains are initially homogeneously populating a two-dimensional environment. Microbial growth and motility including random movement and chemotaxis towards substrate and microbial cells, and substrate diffusion are simulated, and the resulting spatio-temporal microbial distribution patterns are observed and analyzed.

Conclusions
Results show that, depending on the combination of chemotactic preferences, microbial distributions remained homogeneous or transient or permanent spatial patterns emerged. The presence of a competing strain is able either to suppress or to induce aggregate formation, indicating more complex interactions beyond simple substrate competition. Strains being more attracted to self-excreted compounds tend to form aggregates of high densities where starvation conditions prevail, leading communities to become dominated by the competing strain. The model indicates a mechanism by which aggregate formation can evolve, even if it is initially disadvantageous.
INDIVIDUAL-BASED MODELLING OF THE IMPACT OF INITIAL EVENNESS ON
THE PRESERVATION OF BIODIVERSITY FOR IN SILICO BACTERIAL
COMMUNITIES
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Background

One of the most important aims in ecology is to identify and comprehend the
mechanisms that sustain biodiversity - often critically important for ecosystem
viability. Many theoretical models have shown that species in competition can coexist
− thus maintaining the ecosystem's biodiversity - if ecological processes such as
competition and mobility occur on a local scale [1]. This is also true in the case of
communities with non-transitive competition between species, the classical example
being the rock-paper-scissors game.

Objectives

Initial community evenness has been shown to be a key factor in preserving
ecosystem functional stability [2], but has not been accounted for in previous
modelling studies. We formulate a model that allows initial community evenness to be
varied in order to investigate the consequent impact on ecosystem biodiversity.

Methods

We consider an ecosystem of four interacting bacterial species, and present a
stochastic, spatial individual-based model simulating the ecosystem dynamics.
Interactions take place on a two-dimensional lattice. Three processes are
incorporated: reproduction, competition and mobility. In addition to variable initial
evenness, multiple competition schemes are implemented, modelling various
possible communities, resulting in diverse coexistence and extinction scenarios.

Conclusions

Simulations show that long-term ecosystem behaviour is strongly dependent on initial
evenness and competition structure. The ecosystem is generally unstable; higher
initial evenness has a small stabilizing effect on ecosystem dynamics by extending
the time until the first extinction.

Background
Antibiotic activity is assessed in vitro by quantifying the reduction of growth of a bacterial culture. Growth can be described as change in cell number on population level. On the single-cell level, physiological properties like cell size before division or translational capacity vary with growth rates: Cells adapt to their environment. Typical drug-effect models link population growth directly to the exposure of antibiotics. They rarely account for known mechanisms of action of the drug - which are particularly relevant for the analysis of synergistic or antagonistic effects of drug combinations.

Objectives
Development of a pharmacodynamic model which allows for mechanistic integration of antimicrobial effects on the cellular level to predict bacterial growth.

Methods
Control experiments without drug resulted in baseline values for population growth. An established single cell model predicted cell level parameters from this growth rate. Induction of cell killing and growth inhibition were linked to known drug characteristics (minimal bactericidal / inhibitory concentration). A transit compartment cell-cycle model integrated time dependent cellular adaptation processes. Two bacterial subpopulations were considered (resting and growing). We validated our model for predicting time-kill curves with E. coli exposed to tetracycline. Furthermore we predicted septation dynamics of B. subtilis during a shiftdown from exponential to stationary phase.

Conclusions
Since both scenarios show good agreement between predicted and experimental data, these promising results are a first step to mechanistically model bacterial growth during exposure to multiple antibiotics.
INFLUENCE OF ACTIVE SITE GROOVE VOLUME ON DD-CARBOXYPEPTIDASE ACTIVITY OF E. COLI DACD

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Background

\textit{E. coli} DacD is a DD-carboxypeptidase, having much weaker DD-CPase activity than PBP5. The reason for such a weak DD-CPase activity of DacD and its importance within an \textit{E. coli} cell is unknown.

Objectives

Deciphering the role of active-site groove volume (AGV) on DD-CPase activity of \textit{E. coli} DacD.

Methods

Here, we generated the 3D model of DacD using homology modeling and analysed the binding of specific substrates within AGV. Interestingly, the AGV of DacD is about one-third of PBP5 and such a low AGV might reduce the catalytic efficiency of DacD. To validate the result \textit{in silico}, we mutated the secondary amino-acid residues around the DacD active-site by incorporating several transitional and transversional mutations and observed for the changes developed in the AGV. Such mutations brought a 70\% increase in the mutant DacD AGV. PBP5 has higher AGV that can be correlated with DD-CPase activity. In addition, we superimposed PBP5 active-site onto mutated DacD and observed a similar geometric arrangement. Furthermore, the pentapeptide-binding affinity of the mutant DacD was increased as revealed by molecular docking analyses. Higher binding affinity of the mutant DacD towards peptidoglycan mimetic pentapeptide substrate possibly signifies a greater DD-CPase activity.

Conclusions

Active-site groove volume may act as a determinant for DD-CPase activity and the reduced DD-CPase activity of DacD is possibly due to its inadequate active-site groove volume.
Background

The most abundant periplasmic protein of *Vibrio cholerae* grown under inorganic phosphate (Pi) limitation is the high affinity Pi transporter, PstS, of the Pst2 system. It is encoded by *pstS* of the *pst2* operon, *pstSABC*, member of *V. cholerae*’s Pho regulon. Little is known about the regulation of *pst2* expression and the non-stoichiometric production of its genes products.

Objectives

To elucidate the regulatory mechanisms behind *pst2* expression in *V. cholerae*.

Methods

Bioinformatics analysis: Clustal Omega for protein sequences alignment, Mfold for the determination of RNA secondary structures and the corresponding free energy values and MEME/MAST to search for putative Pho boxes. Experimental procedures: SDS-PAGE and MS ESI-Q-Tof Micro for protein analysis and identification and promoter-lacZ fusion, RNA extraction, cDNA synthesis, endpoint-PCR and quantitative Real-Time PCR for the expression analysis.

Conclusions

Based on the bioinformatics analysis we proposed new start codons for *pstS* and *pstC*, a regulatory region upstream *pstS* containing potential Pho boxes and a *pstS-pstC* intergenic region, distinct from those predicted. Moreover, sequences able to form stem–loop structures followed by potential RNase E-cleavage sites were detected in the intergenic regions *pstA-pstB* and *pstS-pstC*. The experimental analyses demonstrated that *pst2* is transcribed under Pi limitation in a PhoB-dependent manner as a full-length mRNA that is processed into minor transcripts of distinct stabilities. The most stable is the pstS-encoding mRNA, followed by pstB, pstA and pstC specific transcripts. The abundance of PstS relative to the other components of Pst2 system in *V. cholerae* seems to correlate to the higher stability of its transcript.
Background
The world’s microbial diversity is vast and the majority of microbes do not readily
grow in culture, complicating the analysis and interpretation of this diversity. In
addition, the lack of a unified and consistent annotation of the world’s microbial
diversity has so far impeded a comprehensive comparative analysis.

Objectives
The “Microbe Atlas”, a global metasource of all publicly available environmental
marker gene sequence data, which will be available as a web resource. A dataset
consisting of 212,000 samples with more than 3 billion identified small subunit
ribosomal RNA sequences. This resource will enable a better understanding of what
are the typical and atypical microbial taxa in samples, by establishing compositional
baselines in terms of microbes that are often found in similar environments.

Methods
An analysis of all publicly available environmental marker gene sequence data.

Conclusions
This resource should become an essential tool for microbial biologists wishing to
compare their samples to previously sequenced microbial communities, and identify
both the typical as well as atypical microbial taxa in their samples.
FEMS-1143
(Human) microbiome

LACCASE FROM PATHOGENIC GUT BACTERIA FACILITATE
BIOTRANSFORMATION OF NON-STEROIDAL ANTI-INFLAMMATORY DRUGS
(NSAIDS)
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Background
Non-steroidal anti-inflammatory drugs (NSAIDs) are used in the treatment of pain, inflammation, cardiovascular diseases, arthritis and cancer by inhibiting cell proliferation. Laccases (EC 1.10.3.2) are multicopper oxidases (MCO), recently reported from Escherichia coli, Salmonella enterica, Campylobacter jejuni, Pseudomonas aeruginosa with role in copper homeostasis, iron acquisition, manganese oxidation and in pathogenesis.

Objectives
To find out novel laccase from different serotypes of Yersinia enterocolitica and clone it into a suitable expression vector for the biotransformation of NSAIDs.

Methods
Amplified laccase gene (yack) from different serotypes of Y. enterocolitica was cloned in pTZ57R/T vector using TA cloning method. The positive clones were sequenced to confirm yack and construct a phylogenetic tree using Mega 5.0. Further, yack was subcloned into the desired restriction sites of pET28a expression vector and transformed into E. coli BL21 (DE3) expression host. The recombinant protein was refolded and purified using His-spin protein miniprep kit, for the biotransformation of aspirin and diclofenac (NSAIDs), which validates our in silico studies.

Conclusions
Laccase from different strains of Y. enterocolitica can be used as a taxonomic tool as it showed serotype specific clades. The HPLC analysis of laccase treated diclofenac and aspirin showed complete biotransformation after 24h of incubation. The FTIR results suggested a change in C-C and C-N bonds. Laccase transform NSAIDs and may have a significant role in the colonization of laccase positive gut microflora.
Background

Environmental characteristics impose some particular constraints to the species living in any given habitat. In many instances, prokaryotic taxa show environmental preferences that shape the diversity found in diverse different environments. It is reasonable to expect that these habitat preferences have a reflection on the metabolism of the prokaryotic organisms. All these metabolic choices require particular sets of genes, and, therefore, gene content is determined to some extent by environmental preferences. But it is unclear to what extent is this relationship determinant, or how extensive is the metabolic rearrangement leading to different environmental adaptations.

On the other hand, it seems obvious that genomic content is determined highly by phylogenetic proximity. The extent to which each of the two processes modulates the genomes has not been quantified.

Objectives

We present a comprehensive analysis of the relationships between phylogeny, metabolism and environmental similarity in the prokaryotic world. We aim to quantify the influences of phylogenetic descent and environmental preferences in genomic content, to answer the question of how much it is shaped by each of the contributions.

Methods

We have calculated measures of phylogenetic, genomic and environmental distances between pairs of prokaryotic taxa using their 16S distances, common gene content and environmental preferences.

Conclusions

The results indicate a strong influence of the phylogeny in the genomic content, although the influence of the environment is notable for some particular metabolic
classes. This allowed to discover sets of genes that can act as environmental markers and predictors of the possible habitats for new species.
DISCOVERY OF PUMILARIN, A NOVEL HEAD-TO-TAIL CYCLIZED PEPTIDE, BY BAGEL3
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Background
In the post-genomic era the amount of (public) genomic data is ever increasing. Bacteriocin mining tools can be used to extract valuable information from this data regarding the presence of post-translationally modified peptides.

Objectives
As a proof of principle we mined all publicly available bacterial genomes using Bagel3. For simplicity we limited the results to head-to-tail cyclized peptides only. We found a large variety of these (predicted) circular peptides in the Bacillus genus. Based on the mining results we screened several Bacillus pumilus strains for the production of novel (head-to-tail) bacteriocins.

Methods
This yielded the production of a novel bacteriocin that we call pumilarin. The overall homology with As-48 is about 50%. We purified pumilarin using protocols that are based on AS-48 purification methods.

Conclusions
Next we analysed its structure using mass-spectrometry and found that its mass was in accordance with its circular structure. After fragmentation masses could be observed that could be explained only by N- to C-terminal linkage of the peptide. The activity spectrum of pumilarin was benchmarked against the prototype head-to-tail cyclized bacteriocin AS-48 and found to show some interesting differences in spectrum.
Background
In nature bacteria are rarely found in isolation, rather they reside in close association and are commonly found within biofilms. Bacteria that develop alternative feeding mechanisms, such as cross-feeding, have a much greater chance of survival in a competitive environment. In the human intestinal tract, the many types of cross-feeding include the use of oligosaccharides and/or monosaccharides resulting from extracellular degradation of polysaccharides by a competing bacterium, the use of such molecules along with metabolites produced by other bacteria and solely using metabolites produced closely associated bacteria.

Objectives
We aimed to develop models to describe the three aforementioned types of microbial cross-feeding and use those models to understand the cross-feeding phenomenon.

Methods
We developed a system of mechanistic differential equations to describe the different types of bacterial cross-feeding. These models were fit to multi-response data found in the literature using nonlinear regression. The multi-response data included bacterial growth (Bacteroides, Bifidobacterium, Eubacterium, Roseburia), substrate degradation (inulin, oligofructose, fructose, and metabolites), and metabolite concentrations (succinate, acetate, lactate and butyrate). To ensure that the estimated model parameters were independent of scale, we minimized the diagonal of the matrix $ZTZ$, where $Z$ is the residual matrix formed from the data and model fit.

Conclusions
Our results suggest that microbial cross-feeding follows second-order chemical kinetics even when the ratios of two substrates are not one-to-one.
Background

The yeasts *Kluyveromyces lactis* and *Kluyveromyces marxianus* are the best-studied species of the *Kluyveromyces* genus. *K. lactis* has been adopted as a model for non–*Saccharomyces* yeasts whereas *K. marxianus* is more widely used for a range of different industrial applications because of relevant phenotypic traits. A *K. lactis* genome sequence has been available for some time but only recently genome sequences for a number of different *K. marxianus* strains have been generated.

Objectives

Our aim to understand the genetic basis of the metabolic and physiological differences between *Kluyveromyces* and *Saccharomyces* and within the *Kluyveromyces* genus.

Methods

We compared the proteomes of *K. marxianus*, *K. lactis* and *S. cerevisiae* by protein clustering using OrthoMCL. We found 4,119 common clusters (in all 3 yeasts) and 596 exclusively shared by the two *Kluyveromyces* species. More detailed bioinformatics as well as phenotypic assays were carried out to verify the findings.

Conclusions

Some key differences arise in sub-telomeric regions, where the same genes can be found duplicated at each end of the chromosome or at the ends of a separate chromosomes. For example, the genes responsible for lactose assimilation are duplicated in the subtelomeric regions of *K. marxianus* but not in *K. lactis*. Indeed, detailed comparison this duplicated region in different *K. marxianus* strains identifies variation that may account for some of the phenotypic differences in lactose assimilation between strains. Other differences that give rise to phenotypic variation
between species have also been verified and new metabolic pathways identified in *K. marxianus*. 
(Human) microbiome

COMPARATIVE GENOMIC ANALYSIS OF NINE SPHINGOBIUM STRAINS: INSIGHTS INTO THEIR EVOLUTION AND HEXACHLOROCYCLOHEXANE (HCH) DEGRADATION PATHWAYS

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Background

Species belonging to the genus Sphingobium are well known for their ability to degrade hexachlorocyclohexane (HCH) isomers due to the presence of lin pathway genes. However, the establishment of the lin pathway in sphingomonads under HCH pressure is still unknown.

Objectives

Here, the nine Sphingobium spp. (strains LL03, DS20, IP26, HDIPO4, P25 and RL3, B90A, UT26S and Sphingobium sp. SYK6) were compared in order to reveal the mode of acquisition of lin genes.

Methods

Genomes of Sphingobium spp. were assembled using ABySS1.3.3 followed by their validation with bwa0.5.9. Phylogeny was constructed with 16S rRNA, single copy genes, ANI and tetranucleotide frequency. Further, draft genomes were annotated using Glimmer-3 at RAST4.0 server. Orthologs were identified using CD-HIT and all versus all BLAST. Functional annotation was performed using KAAS and gene families were predicted using Minpath. dN/dS and dS for each gene pair was calculated using Hyphy2.1.2.

Conclusions

The phylogenetic analysis using genomic data clustered efficient HCH degraders in a closely related group comprising of UT26S, B90A, HDIPO4 and IP26, where HDIPO4 and IP26 were classified as subspecies with ANI value >98%. Total gene content shared among all nine strains was ~10% which increased to ~25% in eight HCH-associated strains. Additionally, an inter-genus plasmid pool between genus Sphingobium and Sphingomonas was observed. Further, the differences in lin gene sequences, copy number and arrangement with respect to IS6100 revealed possible
evolutionary acquisition mechanisms for this pathway. This study also reflected that HDIPO4, IP26 and B90A are better suited organisms for HCH bioremediation.
DIVERSITY OF SURFACE-ASSOCIATED BACTERIA AND ANTIBACTERIAL ACTIVITY OF THE MARINE SPONGES COLLECTED FROM MARINE AREAS OF TURKEY
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Background
Increasing resistance problem of human pathogenic bacteria against commercial antibiotic derivatives has become important problem of all over the world.

Objectives
Anti-bacterial activity of the methanolic extracts of the marine sponges against pathogenic bacteria and diversities of sponge-associated and free-living bacteria were investigated.

Methods
Taxonomic designations of the sponges were carried out using histological sample preparation technique. The samples were extracted using cold methanol extraction technique. The agar disc diffusion method was used to determine the anti-bacterial activity of the sponge extracts. The pure bacterial strains isolated from the sponges were identified using the automated micro identification system VITEK2 Compact30.

Conclusions
The methanolic extracts of all tested sponges displayed positive antibacterial activity at varying levels against pathogenic bacteria. Antibacterial effectiveness rate was found higher in the samples which were collected from the Aegean Sea than the Sea of Marmara. The community profiles of the sponge associated bacteria were found different from free-living bacteria. Though similar bacteria species were found in all sponge samples, the predominant bacterial community profiles were different. Of the 325 unit bacterial strains, isolated from the Aegean Sea sponges, 86% belonged to phylum Proteobacteria. 82% of 102 bacterial strains, isolated from the sponges of the Sea of Marmara, were Proteobacteria. The study resulted in increasing data to understand antibacterial activity of the marine sponges regarding diversities of the sponge/sponge-associated bacteria according to their exposure local environmental conditions in the in the different marine areas.
EFFECT OF TEMPERATURE ON BACTERIOPHAGE-MEDIATED BACTERIAL LYSIS EFFICIENCY

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Background

Bacteriophages (phages) are viruses that specifically target bacteria. This property makes them useful agents for the control of pathogenic bacteria. Despite their great potential there is great uncertainty about the environmental parameters affecting phage-mediated lysis efficiency.

Objectives

In this study, temperature was investigated as a key environmental factor.

Methods

Three distinct phages specific for E.coli were isolated from a single brook water sample. Lysis efficiency was monitored using the double agar overlay method and optical absorbance measurements. Plaque sizes and numbers varied dramatically depending on whether phage-host mixtures were incubated at 20°C, 30°C or 37°C with bigger and more plaques being visible at lower temperatures. Interestingly plaque formation was invisible at the optimum temperature of the host (37°C). Plaque numbers and sizes were found to additionally depend on the temperature history of the bacterial host prior to mixing with the phage. This applied both to the growth temperature of the E. coli (with higher plaque numbers and sizes when cells were grown at lower temperature) and short term temperature exposure of fully grown cells. Exposure of E. coli to sublethal heat stress resulted in a phage resistant phenotype.

Conclusions

These outcomes suggest that many phage-host interactions might be highly temperature-sensitive and phages seem to prefer host cells that were subjected to temperature conditions similar to the ones in the environment where the phages were isolated from. Phages-host interactions should be well characterized for their temperature preference and probably other environmental parameters prior to biocontrol and biosanitization applications to obtain optimal performance.
CHANGES IN MEMBRANE SUBPROTEOME IN VIBRIO HARVEYI DURING THE ADOPTION OF VIABLE BUT NONCULTURABLE STATE BY EFFECT OF STARVATION AND VISIBLE LIGHT
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Background
Vibrio harveyi is a pathogen bacterium for a wide range of marine animals that can be isolated as a planktonic form. The behaviour of Vibrio spp. in natural environments is conditioned, among others, by temperature, light, salinity or nutrient scarcity.

Objectives
The aim of this study was to evaluate the physiological and proteomic changes that take place during V. harveyi survival in seawater under exposure to visible light.

Methods
For this purpose, populations of V. harveyi were maintained in sterile natural seawater at 20°C and under illumination for at least 21 days. Along experiments, total, active and culturable populations were enumerated and the outer membrane subproteome was analyzed.

Conclusions
Experimental conditions had a negative effect upon culturability of V. harveyi, inducing its entry into the viable but nonculturable (VBNQ) state after 6-7 days of exposure, and provoked changes in the membrane subproteome composition.

Along the period of study, some outer membrane proteins remained unchanged (e.g. OmpW), while a set of proteins increased (i.e. lipoproteins and agglutination proteins) or became detectable under tested conditions (in this last group the transport-related proteins are especially relevant). So, all these proteins could be likely essential for sustaining the V. harveyi viability under stress conditions. In contrast, several proteins and especially those related to chemotaxis processes decreased or were undetected along survival.

Therefore, in response to starvation and exposition to visible light V. harveyi experiments an adaptation process which includes the entry into the VBNQ state and the membrane subproteome reorganization.
Background
Marine sponge contains a great number of microorganisms present in their tissues. These ones perform symbiotic associations with their hosts, and are capable of producing secondary metabolites with various biotechnological properties.

Objectives
Aiming to know more about microbial diversity present in aquatic environments and get bacterial species with potential application in biotechnology.

Methods
Three species of marine sponges (Hymeniacidon heliophila, Amphimedon viridis and Aplysina fulva) were collected in two beaches of Rio de Janeiro, Brazil. 38 bacterial isolates from these sponges were tested for the production of amylase, cellulase and caseinase using standard methodology.

Conclusions
Of these, 19 showed enzymatic activity, 5 for amylase, 4 to cellulase and 16 for production of caseinase. Of all isolates tested only one colony was able to degrade the three substrates. The tests of biosurfactants' production, showed that 17 colonies were able to emulsify diesel. Our results show that the species of marine sponges studied are a great substrate for prospecting microorganisms with potential biotechnological applications.
TOXICITY OF TERRESTRIAL CYANOBACTERIAL STRAINS ORIGINATED FROM FORESTS OF THE SERBIAN MOUNTAINS

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Background
As one of the oldest and highly diverse group of photosynthetic prokaryotic microorganisms, cyanobacteria are known as potent producers of various bioactive compounds with toxic effects.

Objectives
Toxicity of five forest terrestrial, filamentous cyanobacterial strains belonging to Oscillatoria and Nostoc genera was investigated.

Methods
The toxicity of intracellular extracts of cyanobacterial strains was tested in Artemia salina bioassay. The LC\textsubscript{50} values were recorded in all tested strains after 24 h (Nostoc genera) and 48 h (Oscillatoria genera). In the case of three Oscillatoria strains, a dose-dependent response in mortality that increased over time was observed after 24 h (1%-8%) and 48 h (72%-98%) of exposure. However, for the intracellular extracts of two strains, Nostoc T7 and Nostoc M2, toxicity was higher after exposure time of 24 h (70% and 97%) and 48 h (93% and 98%). According to the LC\textsubscript{50} values, cyanobacterial strain Nostoc M2 showed to be the most toxic strain with LC\textsubscript{50} value 0.59 mg/ml, followed by strain Nostoc T7 (LC\textsubscript{50}=1.25 mg/ml). Concerning Oscillatoria genera, the recorded LC\textsubscript{50} values were 3.49 mg/ml (Oscillatoria M1), 4.55 mg/ml (Oscillatoria M2) and 5.62 mg/ml (Oscillatoria T18).

Conclusions
The results presented in this paper indicate that toxin production is strain-specific property, whereby intracellular content of three strains belonging to Oscillatoria genera was found to be less toxic to A. salina compared to the strains belonging to Nostoc genera. An additional objective which should be done is analysis and identification of toxic compounds present in the extracts responsible for observed toxicity.
STUDIES ON PRODUCTION AND PURIFICATION OF BIOFLOCULANT BY A BACTERIUM SPECIES ISOLATED FROM ADETI STREAM IN ILESA, OSUN STATE

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Background

Water clarification methods like flocculation, coagulation and sedimentation are often inappropriate because of the high cost and scarcity of chemical coagulants. This study was designed to provide a safe, cheaper and biodegradable flocculating agent.

Objectives

The objectives include isolation of bacterial isolate with flocculating properties from wastewaters and soil samples; selection of a bacterium with the highest bioflocculant-producing potential; determination of the time course of bioflocculant production; and purification of the bioflocculant.

Methods

Wastewater and soil samples were collected from Ilesa and plated on yeast extract, peptone and glucose (YPG) agar. Plates were incubated at 25°C for 24 h. Colonies with mucoid appearance were selected. Pure isolates were stored on sterile YPG agar slants. Bioflocculant production was carried out by growing the organism in YPG medium at 25°C and centrifuged at 5000 rpm for 30 min. Flocculating activity was measured by using a suspension of kaolin clay. Time-course of bioflocculant production was determined by monitoring pH, cell growth and flocculating activity. Bioflocculant was purified by precipitation and gel-filtration chromatography.

Conclusions

The highest bioflocculant –producing isolate was identified as Pseudomonas extremiaustralis ABL19. Maximum bioflocculant production was observed at the 66th h. The optimal bioflocculant activity of 94.4% was achieved with glucose and peptone in the medium. The molecular weight was estimated to be 60.8kDa. The bioflocculant was effective at 80°C and alkaline pH.
CHARACTERIZATION OF BIOACTIVE COMPOUNDS PRODUCED BY SOIL STREPTOMYCES SPP.

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Background

In general special properties of different geographical area could be considered as best sources of bioactive compounds, therefore investigation on these fields might be increased treatment of patients and reduce the rate of occurrence of antibiotic-resistant bacteria.

Objectives

Major purpose of this study was isolation of antimicrobial metabolite producing Streptomyces spp. from soil samples and identification the structure of their antimicrobial metabolites.

Methods

To perform the present study, 70 strains of Streptomyces spp. were isolated from soil samples and evaluated for producing of their antimicrobial products against some pathogenic microorganisms. Then, the antimicrobial producing strains were identified using phenotypic and genotypic methods. The best growth phase of production was determined and finally structures of antimicrobial compounds were assessed by 13CNMR, 1HNMR and FT-IR methods. Regarding to determination of possible structures, all data obtained from 13C NMR, 1H NMR and FT-IR were analysis by NCBI PubChem Structure Search program.

Conclusions

Out of 70 strains of indigenous Streptomyces spp., three strains had potent activity for production of antimicrobial metabolites. The isolates recognized as Streptomyces griseus, Streptomyces phaeochromogenes and Streptomyces olivoviridis. Metabolites
showed antimicrobial activity against *Escherichia coli*, *Salmonella typhi*, *klebsiella pneumonia*, *shigella dysenteriae*, *Bacillus cereus* and *Staphylococcus aureus*. The best growth phase for antimicrobial metabolite production was stationary phase, therefore based on these data all the metabolites recognized as secondary metabolites. Regarding to determination of possible structures, all data obtained from 13C NMR, 1H NMR and FT-IR were analysis by NCBI PubChem Structure Search program and resulted suggestion three possible structures of bioactive compounds.
ISOLATION AND CONSERVATION OF FLUORESCENT PSEUDOMONADS STRAINS FROM RHIZOSPHERE OF WHEAT

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Background

Plant Growth Promoting Rhizobacteria (PGPR) meets those of inhomogeneous rhizobacteria groups which are patented to increase and promote plant growth. Pseudomonas bacteria is one of the most important PGPR bacteria which can increase yield, growth and health of the plant through direct and indirect mechanisms.

Objectives

In the present study, fluorescent pseudomonads isolated from Savadkooh’s soils have been investigated with respect to their biochemical tests and physiological characteristics.

Methods

In the first experiment, 30 soil samples were collected from the rooting zone of wheat at farmer’s field in Savadkooh, Mazandaran province, Iran. The soil samples were serially diluted up to 6–7, plated on King’s B (KB) agar medium and incubated at 28±2°C for 48 h. Distinct colonies showing fluorescence under UV light were picked and streaked on KB agar medium to check the purity. In the second experiment, for the identification of fluorescent pseudomonads, certain biochemical tests and physiological characteristics were conducted according to Bergey’s Manual for determinative bacteriology.

Conclusions

The results showed that 17 pseudomonas strains were detected based on yellow, green and blue pigments by viewing under UV light. Population of bacteria were $2.71 \times 10^5$ – $6.43 \times 10^7$ cell per gram of rhizosphere soil. Based on results of biochemical tests and physiological characteristics we were detected three species, $P. putida > P.$
fluorescens >> P. aeruginosa. Dominate species of fluorescent pseudomonads in rhizosphere of wheat was putida.
SEASONAL DYNAMICS OF SOIL MICROBIAL COMMUNITIES UNDER DOMINANT UNDERSTORY VEGETATION IN SPRUCE SWAMP FOREST

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Background

Spruce swamp forests (SSF) are exceptional ecosystems which are considered to be important sources of greenhouse gases (GHG). Understorey vegetation dominates Eriophorum vaginatum and Vaccinium myrtillus which create patchy environment in SSF may mitigate GHG emissions. Presence of Eriophorum or Vaccinium can affect GHG emissions through the quality and quantity of its litter and root exudates. Their input directly affects microbial community and its decomposition capabilities.

Objectives

Our two main objectives were (i) to describe the seasonal dynamics of bacterial and archaeal communities in spruce swamp forests under the two dominant understorey vegetations (Eriophorum and Vaccinium) and (ii) to evaluate the effect of dominant vegetation on activity of soil enzymes.

Methods

To characterize microbial community we sequenced variable region V4 of 16SrDNA using Illumina MiSeq platform. OTU-picking and taxonomic assignment was performed using the QIIME 1.8.0 bioinformatics pipeline. Hydrolytic enzymes were analyzed by fluorometric method using 4-methylumbeliferone (MUB) labeled substrates.

Conclusions

Methanogenes (Methanomicrobia, Methanobacteria) dominated archaeal community followed by Thermoplasmata, MBGA and MCG (Fig. 1). Relative abundance of methanogens was significantly higher in Spagnum and Eriophorum than in Vaccinium sites. Eriophorum and Vaccinium sites were enriched by Proteobacteria in comparison to Shagnum sites (Fig. 2). On the other hand Sphagnum control site had higher relative amount of anaerobic bacteria (Firmicutes-Clostridia and Chloroflexi). Hydrolytic enzymes were dominated by phosphatase activity showing probably P
limitation which was highest on Sphagnum control site.

Fig. 1. Seasonal dynamics of archaeal community
Fig. 2. Seasonal dynamics of bacterial community
Background

Sulphate reducing anaerobic oxidation of methane (AOM) is a well-established phenomenon occurring in deep marine environments (~ below 100 m). However, relatively shallow anaerobic sediments containing methane and sulphate may harbour anaerobic methanotrophic microorganisms (ANME). Marine Lake Grevelingen is a former estuary with a water depth of ~ 40 m and salinity of 31 g/kg, which is separated from the North Sea by a dam. High rates of degradation and deposition of organic matter have resulted in methane rich anoxic sediments which combined with sulphate from sea water renders the site a potential niche for AOM.

Objectives

The main aim of this research was to explore whether AOM occurs in the surface sediment of Marine Lake Grevelingen.

Methods

Pore water chemical analysis, serum bottle incubation for activity tests, and microbial analysis by fluorescence in situ hybridisation (FISH) were conducted.

Conclusions

AOM was evident at depths of 5 to 15 cm in the sediment with a steep decline in CH$_4$ concentrations from ~5 mM at 20 cm depth to negligible at the sediment surface; concurrently, sulphide concentrations increased to 5 mM. In vitro incubations with CH$_4$ and SO$_4^{2-}$ showed sulphide production coupled to the consumption of sulphate at approximately equimolar ratios at 150 days. In contrast, sulphate and sulphide concentrations remained constant in biotic and abiotic control incubations. ANME and
archaeal cells were observed by FISH, which could be linked to AOM activity. Using three independent approaches, this study, for the first time provides evidence for the occurrence of AOM in sediments of Marine Lake Grevelingen.
EXOPOLYSACCHARIDE PRODUCTION BY RHIZOBIUM ETLI STRAIN ZD13, A HEAVY METAL-RESISTANT SOIL ISOLATE

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Background

Exopolysaccharide (EPS) production functions as a protective strategy which favors the survival of microbes under unfavorable environmental conditions, e.g., heavy metal stress. Rhizobia are Gram-negative, rod-shaped bacteria known for EPS production. Some EPS, due to the presence of negatively charged functional groups, may act as heavy metal-binding agents.

Objectives

Studies on the EPS produced by Rhizobium etli strain ZD13 (RheZD13), an isolate from a postindustrial soil of the Upper Silesia (southern Poland).

Methods

RheZD13 was grown in 4 liters of a culture medium under aerobic conditions (120 h, 30°C, 120 rpm). Bacteria were pelleted by centrifugation (60 min, 4°C, 11806 x g) and the supernatant was sterile-filtered (Ø 0.22 µm) and treated with cold (-20°C) ethanol to precipitate the crude EPS. The latter was dialyzed and analyzed by GC and GC-MS.

Conclusions

4.627 g of RheZD13 EPS were obtained. The EPS contained glucose and galactose (in a molar ratio 3:1) and pyruvic acid that was 4,6-linked to galactose. The acidic nature and the resulting multiplicity of potential binding sites for positively charged particles suggested that RheZD13 EPS may act as a biosorbent for heavy metals.
Water Treatment Plants (WTP) generate waste as residual decanters sludge due to the production of potable water. The objective of this study was to characterize microbiologically the sludge from three samples from the WTP of Londrina, Brazil, through microbiological indicators – as Enterococcus spp, sulphite-reducing Clostridium, and pathogen Salmonella spp. The ‘multiple tubes’ technique was used to isolate bacteria. For physical-chemical analysis parameters, it was used Biochemical Oxygen Demand (BOD), Chemical Oxygen Demand (COD), turbidity, pH and apparent color (according to APHA, AWWA, and WEF-2005). The results showed that in sample 1, the most probable number (MPN) of microorganism per 100mL was 2.1x10^4 Enterococcus and 1.1x10^5 Clostridium; the sample 2 presented absence of Enterococcus and >2.4x10^5 Clostridium; and the sample 3, 1.0x10^5 Enterococcus and 2.4x10^5 Clostridium. Salmonella spp. was not detected in any sample analyzed. For sample 1, the BOD was 113.9 mg of oxygen/L, the COD of 6184.09 mg of oxygen/L, with turbidity of 24000 NTU, the pH 6.0 and apparent color of 100000 uH. For the sample 2, the BOD was 4334.59 mg of oxygen/L, the COD was not determined, with turbidity of 16800 NTU, the pH 6.38 and apparent color of 6000 uH. In sample 3, the BOD was 73.3 mg of oxygen/L, the COD was 2172.20 mg of oxygen/L, with turbidity of 4370 NTU, the pH 6.90 and apparent color of 16700 uH. The microbiological contamination was relevant, indicating high destructive potential for the environmental and healthy human population.
Water treatment plants (WTP) yields waste as decanters sludge and flushing water filter (FWF). The objective of this study was characterizing FWF for presence of Enterococcus spp., Clostridium sulfhyte-reducing and Salmonella spp.. For physical-chemical parameters Biochemical Oxygen Demand (BOD), Chemical Oxygen Demand (COD), turbidity, pH, apparent color, AWWA and WEF 2005 were used. The results showed in WTP–A (collection one), most probable number (MPN) of microorganism per 100mL was 1,2x10^2 Enterococcus and >2,4x10^3 Clostridium; Enterococcus and 1,1x10^4 Clostridium. BOD was 4,8 oxygen/L, COD 218,30mg oxygen/L, turbidity 754 NTU, pH 6,07 and apparent color 300 uH; In WTP-B, for collection 1, absence Enterococcus, 7,0 x10^1 Clostridium; BOD was 3,2 mg oxygen/L, COD 294,5 mg oxygen/L, turbidity 3990 NTU, pH 6,07 and apparent color 3000 uH. For collection 2, absence Enterococcus and 4,3x10^2 Clostridium; BOD 2,43 mg oxygen/L, COD 1,3 mg oxygen/L, turbidity 881 NTU, pH 6,57 and apparent color 5500 uH; In WTP-B, for collection 2, absence Enterococcus and Clostridium; BDO 5,1 mg oxygen/L, COD 106,8 mg oxygen/L, turbidity 889 NTU, pH 6,78 and apparent color 4500 uH. For collection 3, 3,9x10^2; BOD 11 mg oxygen/L, COD 628,83 mg oxygen/L, turbidity 1600 NTU, pH 6,98 and apparent color 54 uH; 4,0x10^1 Enterococcus and 4,0x10^1 Clostridium; BDO 3,7 mg oxygen/L, COD 170,9 mg oxygen/L, turbidity 190 NTU, pH 7,11 and apparent color 1100 uH. Salmonella spp. was not isolated. The microbiological contamination was relevant and high levels of physical-chemical parameters were detected in the flushing water filter, indicating destructive potential for the environment and population.
ENTERIC VIRUSES INACTIVATION BY GAMMA IRRADIATION

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Background
Enteric viruses, like norovirus and adenovirus are frequently detected in environmental systems and have been implicated in many outbreaks. Normally, the viruses exist in the environment and acquire certain capability to endure conventional treatment processes. Alternative disinfection methods must be developed.

Objectives

The main goal of this study was to investigate the inactivation of murine norovirus type 1 (MNV-1), as a Norovirus surrogate, and human adenovirus type 5 (AdV-5) by gamma irradiation.

Methods

Six different substrates: Phosphate buffer saline (PBS), demineralized water, tap water, fetal bovine serum (FBS) and aqueous solutions of 10% and 50% FBS, were inoculated with MNV-1 and AdV-5 and irradiated in a Co-60 source at several doses (1 up to 10 kGy). The inactivation of viral particles was tested by plaque assay using Raw 264.7 and A549 cells. The D₁₀ values were estimated for each virus and substrate.

Conclusions

A reduction on MNV and AdV titers of 4 log₁₀ PFU/ml was achieved after irradiation at 3 kGy on PBS and water suspensions. However, MNV and AdV were approximately 3 times more resistant to gamma irradiation when irradiated in FBS, and at a dose of 10 kGy it was detected the presence of infective viral particles. The observed D₁₀ values ranged between 0.76 kGy (water) and 3.22 kGy (10% FBS) for MNV; and between 0.87 kGy (PBS) and 2.94 kGy (FBS) for AdV. These results show that inactivation of MNV-1 and AdV-5 by gamma radiation strongly depends on the substrate where the viruses are inoculated.
RECOVERY OF AN ACETATE-CONSUMING SULFATE REDUCING CONSORTIUM AT ACIDIC CONDITIONS

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Background
Sulfidogenic reactors have been used to eliminate dissolved metals from industrial wastewaters, due to their possibility to obtain metal sulfides. Sulfate reducing bacteria (SRB) are responsible for the production of sulfide which reacts with dissolved metals, forming precipitates (metal sulfides). However, the major drawback of sulfidogenic reactors is that acetate cannot be degraded completely, reducing the reactor’s efficiency, another problem is that metal containing wastewaters are usually acidic (pH 2-4).

Objectives
The objective of this work was to obtain a consortium from a natural acidic environment (an abandoned sulfur mine) able to work at low pH (<4) and produce sulfide from the complete oxidation of the substrate.

Methods
The enrichments were preformed from sediments cultured in anaerobic bottles (120 mL) and fed with acetate, glycerol, or lactate, and sulfate. The pH was initially 3 or 4, and we evaluated their capability to completely consume the substrate and produce sulfide. When the enrichment consumed the substrate and produced sulfide, we took 10 or 20 % (v/v) as an inoculum to develop a new enrichment of sulfate-reducing consortium.

Conclusions
Supernatant (only the liquid part) used as inoculum (10-20%) was not favorable for the growth of SRB. The slurry (combination of sediment and liquid) used as inoculum (20%) promoted the sulfate-reducing activity at acidic pH. Acetate, used as the sole electron donor, was not suitable for the SRB consortium. Complex electron donor, such as glycerol or lactate, was more suitable to obtain a sulfate-reducing consortium at low pH able to consume acetate and produce sulfide.
BACTERIAL DIVERSITY AND DYNAMICS FROM START-UP TO STEADY CONDITIONS IN A FULL-SCALE MUNICIPAL SOLID WASTE (MSW) ANAEROBIC REACTOR BY 454 PYROSEQUENCING TECHNOLOGY.

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Background

Anaerobic digestion is an environmentally acceptable means of reducing and stabilizing organic fraction from MSW. Functioning and stability of an anaerobic reactor is directly related to the microbial community within it.

Objectives

The bacterial diversity of a full scale anaerobic reactor treating MSW from Madrid (Spain) was investigated using high-throughput 454 pyrosequencing technology.

Methods

The digested MSW samples were taken in two moments of the treatment: start-up phase and steady-state conditions. The bacterial 16S rRNA genes were amplified and sequenced using 454 pyrosequencing FLX machine.

Conclusions

118,392 bacterial 16S rDNA reads were yielded and after trimmed 35,981 (start-up phase) and 18,290 reads (steady conditions) were clustered into OTUs. Fifteen phyla were identified: Bacteroidetes, Firmicutes, Actinobacteria, Proteobacteria, Chloroflexi, SR1, Synergistetes, Planctomycetes, Tenericutes, Deinococcus-Thermus, Acidobacteria, Chlorobi, Cyanobacteria and Gemmatimonadetes. Shifts in the bacterial communities were observed and proteolytic bacteria dominated during all the process, being the major diversity identified in the steady-state condition of the reactor. Fermentative bacteria belonged to Bacteroidetes and Firmicutes phyla predominated, and a great number of reads were identified to genus level. Proteiniphylum, Gallicola and Fastidiosipila genera carried out most biodegradation processes during the MSW treatment. A great number of genera are involved in the
sugars and carbohydrates metabolisms, although its coverage is low. The high concentration of ammonium appears to be the reason for the prevalence of the proteolytic bacteria. The use of the next generation sequencer technology revealed a great diversity of rare organisms and increased our knowledge about the dynamics of bacterial communities in anaerobic MSW reactors.
BACTERIAL DIVERSITY IN RHIZOSPHERE AND SOIL ON MINING WASTE IN THE ARID ENVIRONMENT OF THE SAHARA

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Background

Relatively few studies have been devoted to the microbial diversity of the Sahara. In this study, we have investigated an arid area located in south-west Algeria. In this area, spoil heaps of coal mine waste are colonized by a Saharo-Mediterranean vegetation dominated by Caroxylon sp.

Objectives

As part of an on-going study aiming to understand the role of micro-organisms in the adaptation of this plant to the site, we have studied its associated microflora.

Methods

The bacterial community was investigated by fingerprinting (RISA). The diversity was much higher in the Caroxylon rhizosphere than the surrounding soil, both on the waste heaps and in the desert soil. Libraries of 16S rRNA gene were obtained from rhizospheric and non rhizospheric soil–extracted DNA. Sequences from Firmicutes and Actinobacteria were abundant in all libraries. As expected from the fingerprints, the diversity was higher in the rhizosphere libraries, with 1.6 more phyla detected than in non rhizospheric soil.

Conclusions

Depending on the library, 25 to 65% of the sequences had percentages of identity with known species below the 97% threshold. Potential new species are located in very diverse phyla. The most distant ones (less than 90% identity) belong to Acidobacteria, Chloroflexi, Deltaproteobacteria, Cyanobacteria and Nitrospirae. The results show that we have only begun to explore the diversity of soil bacteria in the arid environment of the Sahara.
Background
Utilization of aromatic compounds by marine microbial communities in nutrition and pollution stress has evolved several catabolic pathways for bioremediation. Of which, biphenyl and cadmium are highly toxic compounds having harmful effects on flora, fauna, and mankind.

Objectives
The main objective of this study was to find out the catabolic potential and pathway of marine bacteria in aerobic utilisation of biphenyl as sole carbon and energy source under toxic metal (cadmium) stress.

Methods
Two bacterial strains *Pseudomonas aeruginosa* (JP-11) and *Achromobacter xylosoxidans* (JP-22) were isolated from the coastal regions of Odisha, India, which could resist up to 1000 ppm and 630 ppm of cadmium respectively. They utilized 97.88 % and 89.6 % of biphenyl respectively. Combination of chromatographic and spectrophotometric techniques identified 2-Hydroxy-6-oxo-6-phenylhexa-2, 4-dienoate and benzopropanol as the intermediate product of biphenyl metabolism in JP-11 and JP-22 respectively. Biosurfactant production was observed which increased the bioavailability of this hydrophobic compound into the cells resulting in their effective removal from the environment. Of both the isolates, the partial structure of the biosurfactant produced by JP-11 was similar to that of rhamnolipid which showed an upregulation of rhamnolipid synthesis gene (*rhlAB*) with increased biphenyl concentration (upto 200 ppm).

Conclusions
Thus, these bacterial isolates can be efficiently utilized for bioremediation of the contaminated sites with toxic metal and organic pollutants.
DIVERSITY OF OIL DEGRADING BACTERIA FROM GEOGRAPHICALLY DISTANT REGIONS
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Background
An investigation of oil degrading bacteria is significant for practical and fundamental reasons. The diversity of metabolic systems allows these microorganisms to utilize a wide spectrum of substrates and produce different biological active substances.

Objectives
The main aim of this research was to characterize oil degrading bacterial strains isolated from different geographical regions (Belarus, Libya, Iraq, and Antarctic).

Methods
Physiological, biochemical and molecular genetic methods were used.

Conclusions
Taxonomy of 22 oil degrading bacterial strains was established. They were determined as Rhodococcus pyridinivorans, R. opacus, R. erythropolis, Bacillus licheniformis, B. beijingensis, B. flexus, Arthrobacter sp., Micrococcus sp., Enterobacter sp., Planococcus maitriensis, Acinetobacter radioresistens, Pseudomonas sp., P. stutzeri, Dietzia sp., Deinococcus sp..

R. pyridinivorans 5Ap was shown to have the widest spectrum of utilizing substrates (it utilized 19 investigated organic substrates). Different strains were able to grow under extreme conditions. For example, B. licheniformis FD9 grew at 54 °C; P. stutzeri, R. erythropolis, Arthrobacter sp. - at 4 °C; Enterobacter sp. FD1 - pH=4, B. beijingensis FD4 - pH=11, P. maitriensis FD3 – with sodium chloride concentration up to 7 %; A. radioresistens and Deinococcus sp. were UV-resistant.

It was shown the presence of naphthalene catabolic plasmids in the strains of R. pyridinivorans and R. opacus.

The ability of oil degrading bacteria to produce the surfactants was shown. The most effective production of surfactants was determined for R. erythropolis A29-k1 when it was cultivated on mineral medium with hexadecane. Lichenisyn biosynthesis genes were found in B. licheniformis FD9.
MICROBIOLOGICAL INVESTIGATION OF MUD VOLCANOES FROM EASTERN ROMANIA FLUIDS

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Background

The largest areas of terrestrial mud volcanoes in Europe are located in the Carpathian Mountains in eastern Romania. Till now the few microbiological investigations were focused on the study of Archean communities implicated in methane- and hydrocarbon cycling.

Objectives

The purpose of this study was to isolate, identify and characterize the pathogenic and resistance features of bacterial strains isolated from the bubbling fluids expelled from the mud volcanoes from Buzau in order to establish the contribution of these protected environments to the natural reservoirs of resistance and pathogenicity.

Methods

All water samples (500 ml) were collected according to current ISO guidelines using sterile glass bottles, at a depth of approximately 30 cm and stored in cold bags at 4\textdegree C until analysis. The samples were diluted and analysed through the standardized membrane filtration method. The identification of aerobic strains obtained in pure culture was based on Gram staining, oxidase reaction and API galleries. Simultaneously, the samples were processed for DNA extraction and molecular assays based on ribosomal DNA amplifications with general bacterial 16S rRNA primers. The isolated strains have been investigated for antibiotic susceptibility and for the production of cell-associated (adherence to inert and cellular substratum, biofilm development) and soluble, enzymatic (hemolysins, lecithinase, lipase, caseinase, gelatinase, amylase, esculin hydrolysis, DN-ase) virulence factors, using phenotypic (disk diffusion method, selective media for enzymatic factors production) and PCR-based methods.
Conclusions

The virulence and resistance profiles of cultivable strains were very poor, suggesting the absence of any selective pressure agents in the investigated areas.
IDENTIFICATION AND CHARACTERIZATION OF GENES INVOLVED IN 
SIDEROPHORE PRODUCTION OF PANTOEA ANANATIS, THE CAUSATIVE 
BACTERIUM OF RICE SHEATH ROT

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Background

Pantoea ananatis is known to cause disease in maize, eucalyptus, onions, and rice. This bacterium is also known as an opportunistic human pathogen. P. ananatis PA13 is a rice grain and sheath rot bacterium, which produces an antibacterial compound.

Objectives

In the present study, we have tried to identify and characterize genes involved in antibacterial activity in P. ananatis PA13.

Methods

We performed a large-scale, mariner transposon-based genetic screening and gene replacement strategy based on homologous recombination. Antibacterial activity was monitored against P. ananatis HY02, onion center rot bacterium, and Yersinia enterocolitica.

Conclusions

A number of proteins that involved in transcription, catalytic enzymes, siderophore transporting systems, and TonB-dependent transporter system were found to affect the antibacterial activity of strain PA13. These data indicate the iron uptake is a major factor of antibacterial activity in P. ananatis PA13. We focused then siderophore biosynthetic genes. P. ananatis has genes homologous to siderophore biosynthesis, aerobactin (iucABCD), alcaligin (alcA) and rhizobactin (rhbF). Single mutant of ΔiucABC exhibited slight reduction of antibacterial activity, whereas ΔalcA and ΔrhbF showed normal antibacterial activity. This result led to generation of double and triple mutants of the genes. Mutants ΔiucABCΔrhbF and ΔiucABCΔalcA exhibited strong reduction in antibacterial activity, whereas mutant ΔalcAΔrhbF showed normal antibacterial activity. These data indicate that siderophores play an important role in antibacterial activity of P. ananatis PA13. This work will give novel insights into iron
uptake regulation and contribute to a better understanding of how these genes function to regulate iron uptakes.
HYDROGEN PRODUCTION AND VFA ACCUMULATION IN A CONTINUOUS STIRRED TANK REACTOR USING MOLASSES WASTEWATER AS FEEDSTOCK

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Background
Molasses wastewater as a sugar-rich waste is a liquid by-product generated in large amount from food industries. Molasses wastewater is one of promising feedstock for bioenergy production because it has high strength of organics and relatively high bioavailability.

Objectives
For bioenergy production and wastewater treatment simultaneously, two-stage fermentation processes employing hydrogenesis and methanogenesis have been developed. Hydrogenesis has advantage of volatile fatty acids (VFAs) as main byproducts as well as hydrogen, and VFA production is necessary to stabilize the waste stream in the secondary stage for methane production. Hydrogen production and VFA production was characterized in a continuous stirred tank reactor (CSTR) fed molasses wastewater as feedstock.

Methods
The CSTR reactor achieved a stable performance at an organic loading rate from 20.3 to 36.1 g-COD·L⁻¹·d⁻¹. Average gas production rate was 1.2 L/d with a hydrogen concentration of 33%. The maximum hydrogen production rate was 1.02 L-H₂·L⁻¹·d⁻¹ at 31.0 g-COD·L⁻¹·d⁻¹. The abundant VFAs were butyric acid (50%) and acetic acid (38%). Total VFA production kept at around 7,135 mg/L level during operation period. Active microbial community was analyzed using rRNA-based massively parallel sequencing technique. Clostridiales, Lactobacillus and Clostridium were relatively abundant in the CSTR reactor. It has been reported that appearance of Clostridium species was in accordance with increase of butyrate and acetate concentration. Lactobacillus species has been known to be involved in anaerobic lactate degradation to acetate.

Conclusions
VFAs produced in the CSTR reactor can be utilized as substrates for methane production in downstream processes.
Background
Biohydrogen and biomethane can be produced in Upflow Anaerobic Sludge Blanket (UASB) reactors treating high strength wastewaters such as molasses wastewater and distillery wastewater.

Objectives
Even though many researches have been reported on the performance of UASB reactors and their microbial properties, there is little information about the effect of starvation on the UASB performance. In this study, the effect of starvation on methane production was characterized in an UASB reactor.

Methods
Anaerobic digested sludge was used as an inoculum source, and the effluent (10,000 mg-COD/L) from an hydrogen-producing UASB reactor treating molasses wastewater was used as influent wastewater for the methane-producing UASB reactor. The UASB reactor was operated for 18 days at hydraulic retention time of 3 d. After confirming the UASB performance reached at steady-state, the UASB was maintained at starvation condition for 14 d or 30 d. After starvation period, the UASB was re-operated by feeding the influent without re-inoculation, and COD removal and methane production were compared with their performance at steady-state (COD removal and methane production rate were 98% and 0.91 L-CH₄ L⁻¹ d⁻¹, respectively).

Conclusions
After 3-days of 14 d-starvation, COD removal and methane production rate were 87% and 1.51 L-CH₄ L⁻¹ d⁻¹, respectively. The UASB performance improved speedy, and the UASB performance could be recovered by 15-days operation after restarting. However just after 30 d of starvation, no COD removal and methane product was observed, and over 27-days operation was necessary to recovery the UASB performance. These findings can be used to plan the operation strategy of methane-producing UASB reactors.
APPLICATION OF BIOLOG PHENOTYPING MICROARRAYS FOR ASSESSING THE FUNCTIONAL DIVERSITY OF ENVIRONMENTAL STRAINS OF BACILLUS

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Background
The ability to link genotypes to corresponding phenotypes is of interest in biotechnological manipulation of metabolic pathways. In order to assess a rapid functional and phenotypic profiling under different metabolic conditions, a high-throughput approach has been developed by BIOLOG Inc. (Hayward, CA): the Phenotype Microarray (PM) technology.

Objectives
The objective of the study was to determine phenotypic changes in environmental strains of *Bacillus* by Phenotyping MicroArrays.

Methods

The four *Bacillus* strains were tested on PM 96-well plates (PM01-02 carbon sources, PM03 nitrogen sources, PM04 phosphorus and sulfur sources, PM09 with osmolytes, PM10 with another pH, PM11-13 with various antibiotics). *Bacillus subtilis* ATCC as reference strain was used to determine the differences between strains. Data were analyzed with Omnilog-PM software. The software suite called 'DuctApe' which analyzes and links together both the genomic and the phenomic data and suggests genetic explanations of metabolic phenotypes was also used.

Conclusions

The environmental isolates as compared to the reference strain were found to be the most metabolically versatile strains, either by looking at the number of 'more active' metabolic features or by the proportion of active compounds for each category. The strains showed higher proportion of active compounds for carbon and nitrogen sources and in the resistance to chemical agents. The DuctApe software is good tool for the visualization of PM data, the exploration of both the genomic information and the phenotypic expression of *Bacillus* spp. tested. Phenotypic profiling is an essential step for understanding genotype differences for environmental microorganisms of interest in contaminant remediation, biofuels production, and climate change.
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THE STRUCTURAL AND FUNCTIONAL BIODIVERSITY OF MICROORGANISMS IN SOIL UNDER EX SITU AIDED PHYTOSTABILIZATION

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Background
The high toxicity of heavy metals causes the need to remove them from the contaminated soil using minimally invasive remediation solutions. One of them seems to be aided phytostabilisation.

Objectives
The aim of this project was to investigate the impact of organic by-products as heavy metals stabilizers in soil on the biodiversity of microorganisms under ex situ aided phytostabilisation.

Methods
Green waste compost and pulp from the processing of grain were introduced into highly contaminated with Zn, Pb and Cd soil. As a phytostabilizer grass Festuca arundinacea was used. After 18 weeks the activity of enzymes: dehydrogenases, phosphatases and urease as well as the structural and functional biodiversity of microorganisms were tested. The biomass of grass after crop was also analysed.

Conclusions
The activity of enzymes in soil supplemented with grain was significantly higher in comparison with their activity in soil amended with compost. Simultaneously, the addition of grain into soil caused the highest increase the metabolic activity and biodiversity of microorganisms in comparison with soil amended with green compost. Additionally, after plant cutting the grass achieved the highest biomass in soil with grain.
Background
Chromium is extensively used in resistant alloys, electroplating, dye productions and leather tanneries. Hexavalent chromium (Cr$^{6+}$) is a toxic, mutagenic and carcinogenic chemical. Physicochemical processes have been applied in Cr$^{6+}$ treatment, however, their setup and operating costs are high for large-scale treatment. Based on this consideration, biotechnology is a potential alternative.

Objectives
In this study, we would like to reveal the characteristics of Ochrobactrum sp. CUST210-1 and its crude chromate reductase in removing Cr(VI).

Methods
We isolated chromium-resistant bacterium from leather industry wastewater by chemostat. Its chromate reductase was purified and further identified by SDS-PAGE. Characteristics of bacterial cell and chromate reductase for Cr(VI) removal were evaluated.

Conclusions
A facultative Ochrobactrum sp. CUST210-1 was isolated and it achieved high removal efficiency at relatively low Cr(VI) concentration (<350 mg/L) under the aerobic condition. Cu$^{2+}$, Zn$^{2+}$, Ni$^{2+}$, SO$_4^{2-}$ and Cl$^{-}$ with 10-150 mg/L did not affect Cr(VI) removal by CUST210-1. Thus, the cell technique could be applied in treating chromium-containing wastewater (e.g. leather industry wastewater). By SDS-PAGE analysis, we confirmed that crude chromate reductase was successfully isolated. NADH rather than carbohydrate was optimal e$^{-}$ donors for the activity of chromate reductase. Thus, immobilized enzyme technique could be applied in treating chromium-containing wastewater with low concentrations of organic compounds (e.g. electroplating wastewater). To our knowledge, this is the first report to demonstrate the application range by bacterial cell or immobilized enzyme in removing Cr(VI).
INVESTIGATION OF METABOLICALLY ACTIVE BACTERIA LEVELS AROUND GÖKÇEADA ISLAND (THE NORTHERN PART OF AEGEAN SEA), TURKEY

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Background

Determination of the metabolically active bacteria level in the studied area is essential in terms of describe of bacteria that contribute to the ecological in aquatic ecosystems. Metabolically active bacteria have a well-developed polysaccharide capsule whereas inactive bacteria rapidly release the capsule (the term "capsulated bacteria was used in reference to "capsule bearing bacteria").

Objectives

The aim of this study was to investigate total and metabolically active bacteria levels at the surface sea water samples taken the coastal and off shore areas which have chosen around Gökçeada Island, northern part of Aegean Sea (Fig.1).
Methods

Sea water samples were taken 19 stations around Gökçeada Island (northern part of Aegean Sea) seasonally for the autumn, winter, spring as monthly for the summer in 12 times in total between March 2012 - November 2013. The frequency of the metabolically active bacteria was determined with modified staining technique comparing the number of the intact cells to the total (live and dead bacterial cells) number of bacteria (Plante and Shriwer, 1998; Stoderegger and Herndl., 2001).
Conclusions

The highest metabolically active bacteria percentage was determined to be 33.12% in the seawater samples which were taken from Aydincik Bay in 2012 summer and the metabolically active bacteria levels in coastal station was found higher than the off-shore stations.

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References


DEGRADATION OF NAPROXEN BY A SELECTED FUNGAL STRAIN

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Background

Pharmaceuticals are widely used for treating human and animal diseases. These active compounds and their metabolites can enter aquatic environments via urine, feces, discharge from production facilities and hospital wastes (Kosjek et al. 2005). Naproxen and its sodium salt are members of the α-arylpipionic acid group of non-steroidal anti-inflammatory drugs. Due to excessive usage of naproxen, this drug has been detected in aquatic environments and even in drinking water (Marco-Urrea et al. 2010).

Objectives

The principal aim of this research was assessment of naproxen degradation capabilities of four fungal strains, include two white-rot fungi (*Phanerochaete chrysosporium*, *Fanalicia trogii*), yeast *Yarrowia lipolytica*, filamentous fungus *Aspergillus niger* and identification of byproducts.

Methods

The LC/MS spectra were taken on a Waters Micromass ZQ connected with Waters Alliance HPLC, using ESI (-) method, with C-18 column. For the structural elucidation of isolated metabolites, low resolution LC-MS and NMR spectroscopy have been used. ¹H (400 MHz) was recorded employing a VARIAN MERCURY 400 MHz FT spectrometer.

Conclusions

*Aspergillus niger* found to the most efficient strain with %98 degradation rate. Two main byproducts of degradation, O-desmethylnaproxen and 7-hydroxynaproxen were identify using LC/MS and ¹H-NMR.

References

THE INFLUENCE OF ENDOPHYTIC BACTERIA IN WHEAT PLANTS FERTILIZED WITH NITROGEN

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Background
The plant growth-promotion bacteria improve nutrient uptake, reduce fertilization cost and also minimize the environmental pollution by decreasing nitrogen leaching. However, there is not enough data about plant growth-promotion bacteria and grasses interaction, especially with endophyte bacteria.

Objectives

The aim of this work was evaluate the possible benefits of three diazotrophic endophytic bacteria about the role in the nitrogen metabolism, ability to synthesize plant hormones and the influence of different N level on wheat plant.

Methods
Wheat plants were fertilized with three nitrogen levels (no-fertilizer, half recommended N-fertilizer and full recommended N-fertilizer) and treated with different nif H+, endophytic bacteria: IAC-AT-8 (Azospirillum brasilense), IAC-HT-11 (Achromobacter insolitus) and IAC-HT-12 (Zoogloea ramigera). The enzymes nitrate reductase, glutamine synthetase and chlorophyll content analyses were analyzed as well as the IAA production by the strains. Before the harvest the nitrogen and the growth promotion were also evaluated.

Conclusions

All the three bacteria were able to modify the nitrate reductase and the glutamine synthetase activities and improve the chlorophyll content, and promote the root and shoot weight. The strains also produced IAA in pure cells culture. The N content and the N-use efficiency index were dependent on the endophytic bacteria strain and on the plant tissue assessed. In general, the performance of the above parameters was strongly affected by the nitrogen rate. The different responses suggest that the successful colonization and the growth promotion are achieved by distinct mechanisms.
ANTIBIOTICS PROMOTE AGGREGATION WITHIN AQUATIC BACTERIAL COMMUNITIES

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Background

The release of antibiotics (AB) into the environment poses several threats for human. Knowledge on the impact of AB on natural bacterial communities is missing both in terms of spread and evolution of resistance mechanisms, and of modifications of natural community composition and productivity.

Objectives

We performed a chemostat-based experiment with 4 coexisting bacterial strains mimicking a freshwater bacterial community to study their response to antibiotics in low and high doses.

Methods

Bacterial abundance rapidly decreased by 75% in the presence of AB, independently of their concentration, and remained constant until the end of the experiment. The bacterial community was mainly dominated by \textit{Aeromonas hydrophila} and \textit{Brevundimonas intermedia} while the other two strains, \textit{Micrococcus luteus} and \textit{Rhodococcus} sp. never exceed 10%. Interestingly, the bacterial strains, which were isolated at the end of the experiment, were not AB-resistant; in addition reassembled communities composed of the 4 strains, isolated from treatments under AB stress, significantly raised their performance (growth rate, abundance) in the presence of AB compared to the communities reassembled with strains isolated from the treatment without AB. By investigating the phenotypic adaptations of the communities subjected to the different treatments, we found that the presence of AB significantly increased co-aggregation by 5-6 fold.

Conclusions

These results represent the first observation of co-aggregation as a successful strategy of AB resistance based on phenotype in aquatic bacterial communities, and can represent a fundamental step in the understanding of the effects of AB in aquatic ecosystems.
ISOLATION AND CHARACTERIZATION OF THERMOTOLERANT PETROLEUM-OXIDIZING BACTERIA FROM GEOGRAPHICALLY REMOTE REGIONS

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Background
Bioremediation of oil-polluted ecosystems in the hot climate regions can be performed by microbial consortia of thermotolerant bacteria.

Objectives
The objective of our work was to isolate thermotolerant bacteria from soil and water samples, physiological and biochemical analysis and taxonomic characterization of the bacteria.

Methods
In the work we used such methods as bacterial cultivation, DNA isolation, PCR, electrophoresis in agarose gel, direct sequencing, phylogenetic analysis, IR-spectroscopy.

Conclusions
Among 85 petroleum-oxidizing bacteria isolated from soil and water samples of Russia, Kazakhstan and Antarctica 15 thermotolerant gram-positive strains have been found which can utilize petroleum and particular hydrocarbons at 45°C and higher. The isolates were identified as the members of genera Gordonia, Paenibacillus and Rhodococcus and were closely related to known species of those genera. The alkB gene (encodes for alkane 1-monooxygenase) has been determined in all Gordonia isolates. Most thermotolerant isolates of genera Gordonia, Paenibacillus and Rhodococcus utilize petroleum in salt-enriched media.

The strains Rhodococcus sp. 5Ap, Rhodococcus sp. Par7 and Gordonia sp. 1D were the most effective thermotolerant petroleum-oxidizing bacteria at 45°C – 27%, 14% and 20% of petroleum, respectively. At the temperature 24°C the most effective petroleum-oxidizing strain was Gordonia sp. 1D – up to 59% of petroleum.
ADAPTATION OF ARTHROSPIRA SP. PCC 8005 TO NITROGEN STARVATION: UNEXPECTED EXPRESSION OF HETEROCYST-LIKE PROTEINS

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Background
Among the most spread microorganisms on Earth, cyanobacteria play a dominant role in nitrogen and carbon cycles. The main limiting factor in cyanobacterial growth results from essential nutrients deprivation (e.g., nitrogen starvation). To deal with that, cyanobacteria develop survival strategies, including morphological and metabolic changes.

Objectives
This study aims to investigate the morphological and metabolic changes within Arthrospira sp. PCC 8005 under N starvation.

Methods
In order to characterize the biological response of this strain, a multidisciplinary investigation was carried out (i.e., proteomic, electronic microscopy, intracellular compounds assay,...).

Conclusions
Arthrospira sp. PCC 8005 underwent to metabolic reprogrammings of C and N metabolisms. Proteomic investigations suggested a degradation of the phycobilisomes, cyanophycin and potential endogenous N sources (e.g., nitrile, urea, cyanates & formamide) to counteract the lack of N in the medium. As N metabolism is regulated thanks to N-to-C ratio, N starvation results in C over-excess, which was stored as glycogen as indicated by proteomic and transmission electronic microscopy investigations. TEM confirmed proteomic results as shown in the decreased of thylakoids, cyanophycin, and in glycogen granules accumulation. Arthrospira sp. PCC 8005 exhibited a survival capacity during long-N starvation, likely by maintaining a basic activity in cells named HFN⁺, probably to sustain colonies viability. Interestingly, proteomic results showed an increased of heterocyst-like proteins (HetR & HglK), while the colonies are non-heterocystous. In order to characterize the role of these heterocyst-like proteins during N starvation in our strain, we carried out immunolocalization of HetR, and studied its potential protein partners by co-immunoprecipitation.
ANALYSIS OF EXTRACYTOPLASMIC FUNCTION SIGMA FACTORS IN THE
PLANT GROWTH-PROMOTING RHIZOBACTERIA BURKHOLDERIA
PHYTOFIRMANS PSJN
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Background
Nutrient availability makes plant internal tissues and rhizosphere attractive spaces for microbial colonization. However, bacteria interacting with plants need to adapt to constant environmental changes, reacting to fluctuations in their immediate surroundings through global gene regulation. Sigma factors are dissociable subunits of RNA polymerase that regulate gene transcription initiation by recognition of specific promoter sequences. Their total number in the genome of a specific bacterial strain is usually determined both by its lifestyle and its genomic size. The extracytoplasmic function sigma factors (ECFs) are the largest and more diverse group of these factors (classified into 43 subgroups), but little is known about their functions, although they are predominant in environmental and plant associated bacteria, which makes them interesting candidates for global gene regulation during plant colonization.

Objectives
The aim of this study was to inactivate and to characterize ECFsf present in the plant growth-promoting rhizobacteria Burkholderia phytofirmans PsJN.

Methods
This strain contains 18 ECFsf, which were inactivated by insertional mutagenesis.

Conclusions
Results suggested that at least one ECFsf could be related with growth and general metabolic processes in strain PsJN, meanwhile others ECFsf may be specifically involved in oxidative stress tolerance and biofilm formation, processes related to plant colonization by bacteria. In general, the results support a possible role of ECFsf in plant bacteria interactions at the level of the rhizosphere. Acknowledgements: FONDECYT grants 3140033, 11121515 and 1110850. Center for Applied Ecology and Sustainability (CAPES FB-0002).
MELANIN SYNTHESIS BY BACILLUS WEIHENSTEPHANENSIS STRAINS ISOLATED FROM SOIL IN NORTHEASTERN POLAND

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Background
Melanin is known for protecting living organisms from harmful physical and chemical factors, however, its synthesis is rarely observed among Bacillus cereus sensu lato. So far, only two melanin-positive wild strains, B. thuringiensis subsp. dendrolimus [1] and B. thuringiensis subsp. kurstaki [2], have been described, but melanin production by B. weihenstephanensis has not been reported yet.

Objectives
B. weihenstephanensis isolates originated from soil in Northeastern Poland were investigated in order to assess physicochemical properties of a blackish-brown pigment they produced and the mechanism of its synthesis in relation to their genotypic and phenotypic characteristics.

Methods

Phenotypic and genotypic investigation of the melanin producers were done using API system, Multi-locus Sequence Typing, 16S rRNA sequencing, and Pulsed-Field Gel Electrophoresis. Fourier transform infrared spectroscopy we used to compare produced pigment with commercial melanin. The mechanism of the pigment production was assessed by biochemical tests and the whole genome sequencing of one B. weihenstephanensis strain.

Conclusions
For the first time, we report that psychrotolerant *B. weihenstephanensis* can produce melanin pigment, which is probably associated with laccase activity. These environmental isolates are closely related to the *B. weihenstephanensis* DSMZ 11821 reference strains. The ability of melanin synthesis by soil *B. weihenstephanensis* strains seems to be a local adaptation to a specific niche.

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References
MICROBIOLOGICAL ANALYSIS OF THE SURFACE OF THE UNDERGROUND REINFORCED CONCRETE STRUCTURES

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Background
In public buildings and facilities, in areas with high humidity and specific climatic conditions, microbiological corrosion is an important factor affecting the reliability and durability of structures made of metal, concrete, and reinforced concrete. Therefore, the problem of potential protection of building materials and structures is very complex and actual.

Objectives
Estimation of the degree of contamination of reinforced concrete structures of Almaty subway by corrosive dangerous microflora.

Methods
Isolation of corrosive dangerous microorganisms was performed by seeding selected water samples and swabs of concrete materials on selective media.

Conclusions
7 stations of Almaty subway were examined in spring 2014. 31 samples were selected, including 5 water samples and 26 swabs from different visually damaged surfaces of reinforced concrete structures. Studies have shown that pH of the water samples and scrapings were neutral or alkaline. Heterotrophic bacteria, filamentous fungi, and denitrifying microorganisms were found in almost all samples. Their number was high enough. This is an alarming fact, because of their intensive development can contribute to a change in environmental conditions that are favorable for the development of thione and sulfate-reducing bacteria, which are corrosive dangerous microorganisms. Further, these group of microorganisms may also participate in the destruction of the concrete. Thione and sulfate-reducing bacteria were detected in small amounts. However, their presence shows that they can grow intensively in creating the favorable conditions and thereby contribute to the development of corrosion processes in reinforced concrete structures.
THE DIVERSITY OF MICROBIAL COMMUNITIES AT ACIDIC MINE WATER OUTLETS IS DRIVEN BY STOCHASTIC PROCESSES

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Background

Explanation how the vast microbial diversity with multiple concurrent organisms may persist at a single site is still elusive for microbial ecology. One of plausible hypotheses is that a large number of spatially separated microcommunities exist within each complex habitat. Thus, each real assembly consists of a small and largely random selection of organisms from the total observed diversity.

Objectives

One of plausible ways to test these hypotheses is to compare microbial communities from homogeneous microhabitats that exist in multiple physically separated copies at a single site. We selected ferruginous stalactites growing in large groups at acidic mine drainage outlets as a model of such microhabitats.

Methods

Two groups of microbial stalactites growing at a single acidic mine drainage outlet and six stalactites from other sites were sampled. Prokaryotic 16S rDNA was amplified using universal primers and microbial diversity was characterized by tRFLP and amplicon pyrosequencing.

Conclusions

Both methods showed that simple and homogeneous microbial communities inhabited all stalactites, but species-level OTU composition substantially differed even between adjacent stalactites. Although the sampling sites influenced composition of microbial communities, all OTUs including the most abundant ones unpredictably alternated in all samples. As a result, a large portion of communities clustered independently of locality or other variables by both phylogenetic beta diversity and OTU abundance metrics. Thus, each community probably contains a random selection from the group of plausible species. The environmental traits influence the probability of the presence of individual species but do not determine composition of simple microbial assemblies.
THE MICROBIOME OF THE ESTERO SALADO MANGROVE: A 16S TAG APPROACH TO DETERMINE DIFFERENCES IN BACTERIAL COMMUNITY, ON SEDIMENTS CONTAMINATED BY HEAVY METALS.
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Background
Mangrove ecosystems are coastal estuarine systems confined to the tropical and subtropical regions. The Estero Salado mangrove located in Guayaquil, Ecuador, has suffered constant disturbances during the past 20 years, due to industrial wastewater release. Previous work determined that this estuary is one of the most disrupted on earth due to its relationship with anthropogenic disturbance.

Objectives
To determine the concentration of heavy metals and nutrients in surface sediments of the Estero Salado in equatorial summer season (February).

To determine the influence of high concentrations of heavy metals in terms of composition of the bacterial microbiome.

Methods
A proper understanding of the spatial variations of microbial communities will provide clues about the underline mechanisms that structure microbial groups. In the present study, the analysis of concentration of heavy metals, nutrients and tag sequencing of 16S rRNA V4 was conducted in surface sediments in two mangroves areas in Guayaquil. The first one under the influence of heavy metals and other pollution free

Conclusions
Using the high throughput sequencing method, revealed a detailed picture of the spatial variations of the bacterial community structure in the superficial sediment on Estero Salado mangrove. heavy metals directly influence on bacterial community due to in the contaminated area had a relatively lower alpha-diversity than the area without its presence. We were able to identify bacterial groups significantly enriched in specific locations. This is the first approach in a mangrove area using tag sequencing and correlating with heavy metals levels.
Background
The cape Bon area (Tunisia) is the world leading producer and exporter of Maltese half blood oranges. However the sour orange (Citrus aurantium) rootstock is naturally infested by a phytopathogen nematode, Tylenchulus semipenetrans, a major nematode pest. Some bacteria and nematophagous fungi demonstrated a nematicidal effect.

Objectives
Only few studies have been conducted on the interaction between bacteria and nematodes in the rhizosphere of citrus trees. The aim of this study is to better understand the role of some bacteria in the complex microbial ecosystem of the Tunisian Citrus rhizosphere.

Methods
Sixty bacterial strains were isolated from Citrus root trees and egg masses of nematodes. The isolates were screened for i) their nematicidal activity on eggs and on second stage juvenile of Tylenchulus semipenetrans, ii) their antifungal activity and iii) their effect on the growth of sour orange roots. To identify the isolates rDNA and/or rpoB gene sequencing was carried out.

Conclusions
Among the 60 bacterial isolates, the majority belonged to either Bacillus subtilis or Bacillus cereus. However, the strains showed a great diversity in their effects i) on the plant host Citrus, ii) on the nematode Tylenchulus semipenetrans, iii) on the rhizosphere-associated fungi (i.e. the phytopathogen Fusarium solani and predatory nematodes Monacrosporium cianopagum and Arthrobotrys conoides). Almost 30%
showed antifungal activities against F. solani. These bacterial strains could be used to improve the quality of soil when used in the field by mixing various bacteria, thereby combining antifungal activity and plant growth-promoting bacteria. Large-scale trials should be performed to confirm these promising results.
ICE NUCLEATION ACTIVITY IN THE WIDESPREAD SOIL FUNGUS MORTIERELLA ALPINA

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Background
Biological residues in soil dust are a potentially strong source of atmospheric ice nucleators (IN). So far, however, the sources and characteristics of biological - in particular, fungal - IN in soil dust have not been characterized.

Objectives
Thus, the objective of this study is a regional investigation of the identity and relative abundances of culturable INA fungi in topsoils, an essential base for improving our understanding of the effects of microorganisms on climate and the hydrological cycle.

Methods
Soil fungi, from a range of different land use and ecosystem types in south-east Wyoming, were analyzed for ice nucleation activity. Ice nucleation active (INA, i.e., inducing ice formation in the probed range of temperature and concentration) fungi were found to be both widespread and abundant, particularly in soils with recent inputs of decomposable organic matter. Across all investigated soils, 8% of fungal isolates were INA. All INA isolates initiated freezing at -5°C to -6°C, and belonged to a single zygomycotic species, Mortierella alpina (Mortierellales, Mortierellomycotina). The IN produced by M. alpina seem to be proteinaceous, <300 kDa in size, and can be easily washed off the mycelium.

Conclusions
Ice nucleating fungal mycelium will ramify topsoils and probably also release cell-free IN into it. If these IN survive decomposition or are adsorbed onto mineral surfaces, these small cell-free IN might contribute to the as yet uncharacterized pool of atmospheric IN released by soils as dusts.
DEVELOPMENT OF GREEN ALTERNATIVE COATINGS TO PROTECT THE METALLIC STRUCTURES AGAINST THE MICROBIAL INDUCED CORROSION PROCESS

Background

Microbial Influenced Corrosion (MIC) is a very aggressive form of corrosion with many proposed mechanisms for its prevention, but existing antifouling solutions include not environmentally friendly biocides. This fact has caused an urgent demand for greener, non-toxic or low-toxicity and longer lasting antifouling compounds and technologies.

Objectives

BIOCORIN project, within FP7-ENVIRONMENT program, aims to develop a green alternative to the coatings and solutions used up to date for MIC corrosion protection and prevention in infrastructures.

Methods

The project is based on a biological phenomenon commonly occurring in nature for microbial population regulation. Microorganisms secrete compounds that inhibit the growth of other microorganisms due to existing antagonism between microbial populations. First step was the isolation and identification of those microorganisms that play a major role in fouling and MIC for different environmental conditions. Several samples were collected from different climate locations for the isolation and identification of microbial populations from metal surfaces. As a result, several microorganisms belonging to bacteria, fungi and yeasts were isolated from the four
different samples and were identified by means of 16S and 18S rRNA gene sequencing.

Conclusions
The search of microorganisms with anti-MIC properties resulted in the identification of two candidates with the ability to synthesize compounds with antifouling properties. These candidates were integrated in a coating based on sol–gel technology to prevent the MIC corrosion phenomena in a green way. Currently, this technology is under evaluation by means of three case studies with different environmental conditions.
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Environmental microbiology - 2

METABOLOME ANALYSIS OF THE ANTIMICROBIAL COMPOUNDS SECRETED BY ENVIRONMENTALLY ISOLATED ANTI-MIC MICROORGANISMS
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Background
Corrosion is a worldwide problem and the direct economic loss due to metal corrosion of infrastructures and equipments is estimated to exceed €1.32 trillion per year, which accounts for 3 to 4% of the Gross Domestic Product of industrialized countries. Among corrosion types, Microbial Induced Corrosion (MIC) is involved in at least 10% of the corrosion of structures, and up to 50% in the case of subterranean pipes.

Objectives
BIOCORIN (New Biocoating for Corrosion Inhibition in Metal Surfaces) is a project funded by the European Union under the activities of the Seventh Framework Programme (FP 7-ENVIRONMENT) that aims to develop a green alternative to the coatings and solutions used up to date for MIC corrosion control. Some of the results of this project are presented here, with the identification of several relevant antifouling compounds secreted by environmentally isolated anti-MIC strains via a metabolomic approach.

Methods
Metabolites were extracted from three anti-MIC strains. 100 ml cultures incubated in Terrific Broth (TB) at 30°C for 24 h in the presence and absence of a pool of MIC extracts (inducers) originated from several climatic locations around Europe. Each culture broth was recovered and analyzed by means of LC-MS and GC-MS (Acquity UPLC - SYNAPT G2 HDMS system QToF with ESI injection and a Thermo Scientific Focus GC - DSQ II respectively).

Conclusions
The metabolomics analysis pointed out the differential expression of 2 compounds by GC-MS and more than 10 compounds for LC-MS (positive mode) between the analyzed anti-MIC strains.
Background

Air quality is an issue that has come to be of great concern in recent years due to increased production of particulate material. The presence of fungi in the air is partly responsible for some allergies, infections and diseases. Because of this there is a need to study fungal species associated with inhalable particulate matter and its effects on health.

Objectives

The aim of the research is to isolate the fungi present in PM10 filters in sampling two Network Monitoring Air Quality in Aburrá Valley, which presented the highest levels of pollution in the city. After isolation fungi are identified and characterized by the types of pathogens.

Methods

The microorganisms are absorbed into quartz filters that capture particles diameters smaller than 10 microns (PM10). The filters are exposed to atmospheric air for 24 hours in the HI-VOL PM10 equipment. Subsequently, the filter was incubated in enrichment medium, serial dilutions were made and plated on selective media for fungi (PDA and Sabouraud in this case). After growth, morphological identification is performed.

Conclusions

The results of the tests reveal the presence of Cladosprium sp., Fusarium sp., Alternaria sp. y Rhodotorula sp. These microorganisms are considered allergens and are associated with the generation of respiratory diseases like asthma, rhinitis, tracheitis, bronchitis, pneumonia, bronchopulmonary mycosis, among others. These findings demonstrate the importance of knowing the microorganisms associated with particulate material breathe as increased air pollutants in the atmosphere increases morbidity and mortality.
DIFFUSION SANDWICH SYSTEM, A TOOL TO ACCESS THE MICROBIAL DARK MATTER

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Background

Prokaryotes are the most abundant and diverse life form on Earth. However, to date, we know that only between 0.001% and 1% of total environmental bacteria have been cultivated through standard culture techniques and media, remaining then a rich diversity of bacterial taxa to be isolated and cultured, the so-called microbial dark matter.

Objectives

The aim of this study was to carry out a proof of concept of a system of miniature diffusion chambers which we have named Diffusion Sandwich System (DSS), as a cheap and user-friendly tool to gain access to previously uncultured bacterial taxa.

Methods

We did a comparative study of the cultivability and bacterial diversity which could be isolated by using the DSS and with the classical approach based on picking up bacterial colonies from Petri dishes. Further both culture-dependent approaches were compared with the bacterial diversity present in the same ecological niche but analyzed in-depth by pyrosequencing the metagenomic 16S rRNA gene.

Conclusions

The culturability obtained by using the miniaturized chambers was 2-log10 units higher than with the classical approach. Unlike the classical approach, the relative abundances of taxa isolated with the DSS was in line with those actually present in the ecological niche, allowing in fact to isolate representatives of several underrepresented phyla such as Acidobacteria, Verrucomicrobia and Gemmatimonadetes, which constitute new taxa into these phyla.
MARINE PHAGES AS MARKERS OF REACTIVE TRANSPORT OF THE COLLOIDAL PARTICLES IN THE CRITICAL ZONE

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Background
Little is known about the factors determining the eligibility of marine phages as specific markers of hydrological flow and reactive transport of colloidal particles in the Earth’s Critical Zone (CZ). Marine phages and their bacterial hosts are naturally absent in soil, can be applied as tracers at high concentration and be detected as little as one phage/mL in water.

Objectives
Within the DFG Collaborative Research Center – AquaDiva we evaluated the effects of phage characteristics (morphology, surface property), water flow regimes as well as the role of mycelial networks on transport of marine phages in laboratory experiments.

Methods
Phages were characterized by electron microscopy, dynamic light scattering and water contact angle analysis (CA) for their size/morphology, surface charge ($\zeta$) and hydrophobicity. Sand-filled percolation columns and a modified high-throughput plaque assay combined with fluorescence microscopy counting were used to quantify phage deposition during percolation.

Conclusions
Our data show that marine phages exhibit differences in their deposition efficiency depending on their size, surface charge and hydrophobicity. For instance, the bigger (60 nm), more hydrophobic (CA = 78°) and poorly charged ($\zeta_{M, pH=7.8} = -13$ mV) PSA-HM1 phage exhibited a higher deposition efficiency than smaller (30 nm) and less hydrophobic (CA = 52°) H6/1 phages. Interestingly however, both marine phages showed lower retention than terrestrial T4 phages exhibiting similar characteristics than HM1. We conclude that marine phages have a high potential for the use as sensitive tracers in terrestrial habitats with their surface properties playing a crucial role for their transport.
Background
Pentachlorophenol (PCP) is a hazardous agent in water and soil that poses a significant risk to human health. The toxicity associated with PCP occurs in low doses and mainly affect liver, kidney and central nervous system.

Objectives
The purpose of this study was to investigate the biosorption of PCP on Aspergillus niger biomass as a method for removal of PCP from aqueous solutions.

Methods
The modified A. niger biomass with NaOH was used to adsorb the PCP. The impacts of various experimental parameters such as initial PCP concentration, pH of solution, contact time, and biomass dosage were investigated and optimal conditions were identified. The experimental data were evaluated by various isotherm and kinetic models.

Conclusions
The correlation of contact time, pH and initial concentration with the biosorption of PCP by A. niger biomass was statistically significant ($P<0.001$). The PCP removal increased with decreasing of solution pH and maximum adsorption was obtained at the pH 3. The equilibrium sorption capacity was increased by increasing initial PCP concentration from 10 to 40 mg/l of 4.23 to 11.65 mg/g, while the PCP removal efficiency decreased from 87 to 55%. Both Langmuir and Freundlich isotherms well described adsorption equilibrium of PCP on A. niger biomass. For all cases, the correlation coefficients for the second order kinetic model were close to one.

A. niger biomass can be used to reduce the toxicity of aqueous solutions containing PCP in acidic pH conditions. PCP concentration in water and soil poses a great hazard to human health, so its removal by fungi is applicable and commercial.
Background
The microbiological purity of haemodialysis water and dialysis fluid is crucial in the treatment of patients with chronic renal insufficiency. The presence of bacteria and bacterial-derived products (i.e., endotoxins, sphingolipids, oligonucleotides) can contribute to silent chronic inflammation, secondary amyloidosis, pyrogenic reactions and anemia.

Objectives
The main objective was to characterize the microbial community present in the haemodialysis water used to reconstitute the dialysis fluid at the Hospital Universitario Son Espases (Majorca, Spain). Bacterial community was assessed in samples obtained in November 2012 and 2013 to evaluate the evolution of the species colonizing these pure waters.

Methods
Dialysis water was filtered through 0.22 µm pore size filters. DNA was extracted from the filters, purified and was amplified with two different set of primers: i) 16S rDNA universal primers and ii) selective \textit{Pseudomonas} primers designed for the $\beta$-subunit of the RNA polymerase sequence ($\text{rpoD}$ gene). Amplicons were pyrosequenced with a 454 System+ GS FLX platform from Roche. Reads obtained were analyzed in order to assess the phylogenetic identification of bacteria present in the community.

Conclusions
The bacterial community present in the dialysis water is very complex, with microorganisms highly diverse and adapted to the oligotrophic habitats, not usually detected routinely in clinical microbiology laboratories. This community is maintained in the two years period of this study. Based on the 16S rRNA gene pyrosequencing analysis the \textit{\alpha-Proteobacteria} class is the most abundant. The pyrosequencing of the $\text{rpoD}$ amplicon showed a high number of putative novel species of \textit{Pseudomonas} showing a high diversity still not described.
Background

Because of their massive utilization, hydrocarbons are major pollutants of soils and aquifers. Biodegradation is a key aspect of the fate of pollutants in the environment.

Objectives

It is based on the Gas Chromatographic and Thin Layer Chromatography (TLC) coupled with a Flame Ionization Detector (FID) analysis of hydrocarbons, after incubation in optimal conditions of major compounds in crude oil (saturates and aromatics fractions).

Methods

Using a method of gene sequencing 16S rDNA, the identification of bacteria adapted to soil was determined. Based on nearly full length 16S rRNA gene sequencing analysis, a phylogenetic trees was constructed. Capacity of degradation of selected bacteria and the presence or absence of functional genes coding for the initial oxidation of hydrocarbons (alkB, nahAc, nidA) were studied.

Conclusions

The results indicate that the **Gamma-Proteobacteria** group was the main actor of this degradation. *Pseudomonas* sp, specific bacterial group suitable for petroleum hydrocarbon pollution has the ability to metabolize (single culture) a high mass residues, Pristane (n-C19) at 35.11% and Benzo[a]Pyrene (n-C20) at 33.93% and co-metabolize (in consortium) fractions in petroleum-hydrocarbon, 50.44% of saturates and 30.42% of aromatics compounds. Biodegradation efficiency of soil microflora depended on the specific metabolic pathways of some microorganisms and on the cooperation within microbial population. This study provides a better understanding of the adaptation of bacteria inhabiting polluted environments and for developing and implanting adequate bio-strategies in the future to enhance oil in contaminated soil and the biotreatment of oily waste water in refineries.
EXTRACTIVE MEMBRANE BIOFILM REACTORS (EMBFR) FOR BIOREMEDICATION OF METHYL TERT-BUTYL ETHER CONTAMINATED GROUNDWATER: A LAB-SCALE STUDY.

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Background
Among the strategies developed for contaminated groundwater bioremediation, those based on the use of bacteria adhering to inert supports and established as biofilms, have gained great importance in this field. The EMBFR technology offers productive solutions for the removal of volatile compounds like methyl tert-butyl ether (MTBE) and other contaminants from water bodies. The EMBFR is based on extractive semipermeable (silicon) membranes through which the contaminants migrate to the biological compartment in which microorganisms with MTBE biotransformation and/or mineralization capacities grow in mineral salts medium, forming a catalytic biofilm on the membrane surface.

Objectives
The objective of this study was to assess the use of three bacterial strains (\textit{Paenibacillus} sp. SH7 CECT 8558, \textit{Agrobacterium} sp. MS2 CECT 8557, and \textit{Rhodococcus ruber} EE6 CECT 8612) previously isolated from gasoline contaminated environments, and used as inoculum in a lab-scale EMBFR running for 28 days under aerobic conditions.

Methods
Three different retention times (1h, 6 h, and 12 h) were employed. Degradation rates were determined daily by gas chromatography - mass spectrometry, and the biofilm established by the bacterial strains on the semipermeable membrane was observed by FESEM at the end of each experiment. Acute toxicity of the effluents was determinate by Microtox\textsuperscript{©} assay (EC\textsubscript{50}).

Conclusions
The results achieved from MTBE degradation, biofilm formation and toxicity analysis showed that these bacterial strains could be excellent candidates for use as selective inocula in EMBFR technology for MTBE bioremediation.
DIARRHEAL BACTERIAL PATHOGENS ARE FREQUENT IN THE CHOQUEYAPU RIVER IN BOLIVIA, AND POSE A RISK OF TRANSMISSION TO FRESH PRODUCE BY CONTAMINATED IRRIGATION WATER

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Background
Water borne diarrheal pathogens might accumulate in river water and present a potential contamination risk of drinking and irrigation water.

Objectives
In this study, we show how pathogens can accumulate and be transported in the river Choqueyapu in La Paz and contaminate fresh produce irrigated by river water.

Methods
Using qPCR absolute quantification methods, we found a seasonal and spatial variation in the number of pathogen gene copies per ml of river water with a high peak of Campylobacter ssp. that contaminated otherwise clean water in a watershed at the Altiplano plateau, after the peak of the rainy season in February 2014. High levels of Campylobacter, Salmonella and enterotoxigenic E. coli (ETEC) were subsequently isolated in sampling points downstream along the river and finally identified in vegetables rinse water.

Conclusions
These results indicate that distant water contaminations may be transported all the way to the agricultural area. In addition, we found that ETEC carrying the heat labile toxin (LT) alone was much more prevalent in water than other ETEC carrying the heat stable toxin (ST). Hence ETEC with different toxin profiles might have different reproduction and survival ability in water. The results show that the absolute levels of several diarrheal pathogens are high in contaminated river water in the central part of La Paz and although this water is not accessible by humans at this spot several of the pathogens end up in the downstream river water used for irrigation of crops where they might contaminate vegetables.
STUDY ON O, M, P-XYLENE DEGRADATION AND ITS RELATED GENES IN RHODOCOCCUS SPP. J7
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Background

Xylene, an aromatic hydrocarbon, can be easily found in gasoline contaminated soil and composed of three isomers [ortho (o-), meta (m-) and para (p-) xylene]. Many bacterial strains have been reported to grow on omp-xylene isomers, but few studies have been performed to degrade them simultaneously. Xylene is known for its wide usage in tissue processing, staining and cover slipping and can find easily in ground water. Due to volatile property of xylene isomers, they can spread easily from groundwater to the air.

Objectives

Once human is exposures to xylene, central nervous system could be damaged. So we tried to decrease amount of xylene emitted from factory using soil bacteria.

Methods

Strain J7, belong to the genus Rhodococcus was isolated from total petroleum hydrocarbon contaminated site in Korea. Xylene degradation ability was evaluated using 90ppm of xylene mixture (30 mg/L individually) and analyzed by Agilent GC 6890N with Flame ionization detector (FID). The metabolites are analyzed using GC-MS and the pathway of degradation was construed. Target was amplified by PCR using genomic DNA and PCR products were sequenced at Marcrogen (Korea) and compared with other reference genes.

Conclusions

From this study, we confirmed that strain J7 can degrade o, m, and p-xylene at the same time and based on the data, we have constructed that it follows TOL pathway with high efficiency. The catechol-1,2-dioxygenase gene was amplified and conforming that it followed ortho-cleavage pathway.
KERATIN AZURE AND FEATHER DEGRADATION IN SOIL BY SOME FUNGI USING SINGLY AND IN COMBINATION

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Keratin azure and feather degradation in soil by some fungi using singly and in combination

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Abstract

The large amount of keratinous waste produced and their localized accumulation create a serious disposal problem leading to environmental pollution. Keratin in forms of animal waste such as hairs, nails, horns, hooves and feathers ultimately comes to soil and if it remains there make pathogenic reservoirs because it is degraded by fungi which have pathogenic potential. On the other hand keratin when degraded by fungi nitrogen is slowly released in the soil and may be utilized by plants as manure. Keratinophilic fungi frequently occurring in soil were selected for hen feather and keratin azure degradation individually and in combination in different sets in soil. Five selected fungi Acremonium implicatum, Chrysosporium queenslandicum, Chrysosporium pannicola, Malbranchea pulchella and Verticillium lecanii, when used in combination with Chrysosporium keratinophilum showed feather and keratin azure degradation. Further feather and keratin azure degradation was monitored in soil when these were used individually and in combination with C. keratinophilum and almost above 90% degradation was recorded. The determination of amount of keratin degradation in soil by using individual fungus and fungi in combination is of immense importance in order to find out how much keratin is degraded in soil. The measurement of feather and keratin azure degradation in soil by using five fungi singly and in combination with C. keratinophilum presented here for the first time.
LOW BIOMASS DNA AND RNA EXTRACTION IMPROVES BY USING G2 BLOCKINGREAGENT WITHOUT ANY BIAS IN METAGENOMIC ANALYSIS
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Background
Low biomass samples can be extremely challenging when extracting DNA and RNA. Many scientists have been adding various sources of RNA or DNA to the samples to aid a high recovery, however this makes the samples only useable for specifically directed analysis, while metagenomic analysis is hampered.

Objectives
A new product G2 that are shown to improve DNA and RNA yield from low biomass samples are tested for use in metagenomic analysis as well as if several labs can repeat the results in a ring test.

Methods
We have included analysis of influence of G2 on both full genome sequencing and various amplicon sequencing techniques. Further a Q-PCR analysis was performed after 12 laboratories in a ring test were applying G2 to one sampltype.

Conclusions
G2 was shown to be free of contaminating DNA both using 16SrDNA based Q-PCR and amplicon sequencing, further a metagenomic analysis clearly indicated that no traces of the original DNA could be found in a metagenome analysis. The ringtest showed that the participating laboratories all had a higher yield using G2 but also that the participating laboratories did perform very different.
Background
Terrestrial microorganisms, especially those from the order Actinomycetales, formed the backbone for the golden age of antibiotic discovery, and are the source of the vast majority of FDA-approved antimicrobial agents.

Objectives
In this study, the antimicrobial activity and diversity of genes associated with secondary-metabolite biosynthesis of Actinobacteria isolated from fresh fish gut microbiota namely *Schizothorax zarudnyi* and *Schizocypris altidorsalis* were investigated.

Methods
Strains of six groups of fish and human pathogenic bacteria were selected and all the isolated actinomycetes were tested for their antibacterial activity against the pathogenic bacteria The antagonistic activity of actinomycetes was tested by the perpendicular cross streak method. The PCR-based approach was applied to detect the presence of three important biosynthetic genes including polyketide synthases (PKS-I and PKS-II), non-ribosomal peptide synthetase (NRPS). The PCR amplicons and their origins were further confirmed by sequencing.

Conclusions
According to results all isolates possess at least two types of the investigated biosynthetic genes. This study demonstrates the significant diversity of genes associated with secondary-metabolite biosynthesis of Actinobacteria in the fish gut microbiota and it’s potential to produce biologically active compounds.
EFFECTS OF NITROGEN FERTILIZER ON NITRITE-DEPENDENT ANAEROBIC METHANE OXIDATION BACTERIA IN A FLOODED RICE PADDY

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Background
Nitrite-dependent anaerobic methane oxidation (n-damo) may be an important process in the biogeochemical cycles of carbon and nitrogen. However, the inquiry of n-damo bacteria in rice paddy is rarely explored.

Objectives
The aim of this work was to investigate the effects of nitrogen fertilizer on the communities of n-damo bacteria in a flooded rice paddy.

Methods
Different amounts of urea (0, 45, 90, and 180 kg ha\textsuperscript{-1}) were applied to a flooded rice paddy transplanted with a rice cultivar (\textit{Oryza sativa}) and soil cores were sampled after 15 days of third urea application. The soil cores were divided into top (0–5 cm), middle (10–15 cm), and bottom (20–25 cm) layers and the diversity and abundance of n-damo were investigated using T-RFLP (Terminal-Restriction Fragment Length Polymorphism) and quantitative PCR approaches, respectively. A clone library was constructed for the T-RFLP analyses of 16S rRNA gene fragments of n-damo. DNA samples were retrieved from the soil core samples and the abundances and diversities of n-damo were investigated using T-RFLP.

Conclusions
Abundance of n-damo in a flooded rice paddy was not significantly different although different amounts of urea were applied. However, the phylogenetic analysis of \textit{Methylomirabilis oxyfera}-like 16S rRNA gene sequences revealed that n-damo was a little diverse depending on urea application and soil depths. Sequences of Top 90 soil sample fell into just one group, while sequences of Bottom 180 were classified into three groups. In addition, the diversity analysis of n-damo in response to different amounts of urea will be performed and discussed.
MICROFLUIDIC CHIP-ASSISTED DNA PREPARATION FROM ENVIRONMENTAL SAMPLES SPIKED WITH FRANCISELLA TULARENSIS AND BACILLUS THURINGIENSIS
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Background

The detection of bacteria from environmental samples using nucleic acid amplification depends on efficient DNA extraction and purification to remove PCR Inhibitors.

Objectives

DNA extraction and purification from spiked air, natural water, swab and soil samples was performed using a microfluidic chip device. A protocol was established that was suitable for the detection of two representative bacteria, Francisella (F.) tularensis (Gram-negative) and Bacillus (B.) thuringiensis (Gram-positive).

Methods

The procedure is composed of thermal, chemical and enzymatic lysis methods combined with on-chip DNA purification using paramagnetic particles for reversible DNA attachment. Polymeric microfluidic chips with rhombic cavities were mounted on a device with a regulated heating unit and a movable magnet that enables particle mixing for careful washing and elution. Serial 10-fold dilutions of the bacteria suspensions were tested. DNA preparations were analysed by real-time PCR assays to determine the detection limits that can be achieved.

Conclusions

Detection of both species of bacteria was possible from all tested matrices. The detection limits in air samples corresponded to 7 GE/10 l for F. tularensis and 0.7 GE/10 l for B. thuringiensis. In river water the limits were 10² GE/ml B. thuringiensis and 10³ GE/ml F. tularensis. The achieved detection limits for soil samples corresponded to 2.7·10³ GE/mg F. tularensis and 2.7·10² GE/mg B. thuringiensis and
with swab samples $4 \cdot 10^3$ GE/cm$^2$ *F. tularensis* and $4 \cdot 10^2$ GE/cm$^2$ *B. thuringiensis* were detected. Future developments will focus on the development of integrated lab-on-a-chip system combining sample preparation with on-chip PCR technologies.
PLANT GROWTH PROMOTING RHIZOBACTERIAL EFFICACY IN COWPEA (VIGNA UNGUICULATA (L.) WALP.)

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Background

Plant growth promoting rhizobacteria (PGPR) are known to influence the growth of the plant by various plant growth promoting activities such as Indole Acetic Acid (IAA) production, Phosphate solubilization activity, Ammonia production (NH3), Nitrogen fixation, Siderophore production and Hydrogen cyanide production (HCN). In search for the potential PGPR from the Rhizosphere soil of cowpea, a total of 16 rhizobacteria (B1-B6 & D1-D9) were isolated. By comparing the results two potential rhizobacteria (B6 & D4) have been selected for the further evaluation of Plant Growth Promoting (PGP) activity. These isolates biochemically characterized, B6 and D4 confirmed as **Pseudomonas** and **Bacillus** by 16SrRNA gene sequencing. *Invitro* and *Invivo* Nursery field studies revealed that 100% seed germination rate using **Bacillus safensis** and 92% for **Pseudomonas aeruginosa** showing promising results. The study showed the characters of benefits to use these organisms as biofertilizers for sustainable agriculture to improve crop yield.

Objectives

This study was aimed to focus on the PGP activities of the two potential rhizobacteria and growth promotion

Methods

**IAA production** (Lwin et al. 2012)

**Siderophore production** (Arnow 1987)

**Phosphate Solubilization Activity** (Qureshi et al. 2012)

**Hydrogen Cyanide Production** (Ahmad et al., 2008)

**Ammonia Production** (Cappucino and Sherman, 1992)

**Nitrogen fixation activity** (Lwin et al. 2012)

**16S rRNA Sequencing**

**Seed germination rate and seedling vigor index** (Ng LC et al. 2012)
Conclusions

Rhizobacteria isolated from the cowpea rhizosphere *Pseudomonas aeruginosa VRKK1* and *Bacillus safensis VRKK2* acts as the potential PGPR. *Pseudomonas aeruginosa* showed 100% germination rate, *Bacillus safensis* showed 92% germination.
Background

Baltic Sea constitutes a unique environment of brackish water conditions, with dynamic, regional shifts in salinity, mainly due to varying freshwater inflows.

Objectives

The goal of the study was to identify physiological and molecular strategies that allow Baltic Sea bacteria to confront this challenging habitat.

Methods

Three bacterial Baltic Sea representatives, identified as *Shewanella baltica*, *Flavobacterium* sp. and *Paracoccus* sp. on the basis of 16S rRNA sequencing and MALDI-TOF analyses, were cultured under controlled growth conditions in a chemostat system and analyzed with a combined approach of 2-DE, multiplex fluorescent staining of proteins in gels and LC-ESI-MS/MS and/or MALDI-TOF/TOF-driven protein identifications.

Conclusions

Tracking down of proteome dynamics in *Shewanella baltica*, *Flavobacterium* sp. and *Paracoccus* sp. in response to the salinity shifts characteristic of the Baltic Sea (2, 7 and 20‰) allowed the identification of a subset of proteins, both of the general stress response group and of other functional categories, differentially expressed under osmotic challenges tested. Wide variations in adaptations to osmotic stress were revealed in the three strains studied, with *Paracoccus* sp. requiring a significantly less
number of proteins to survive in the osmotic stress conditions in comparison to *Shewanella baltica* and *Flavobacterium sp*.

Taken together, our study uncovered well-coordinated and highly regulated protein inventories in the marine bacteria studied, with a large number of novel candidates for stress response players, thus laying a ground work for understanding of the complexity of microbial adaptive processes in the intriguing ecosystem of the Baltic Sea.
BIOSORPTION OF CHROMIUM BY PSEUDOMONAS SP ISOLATED FROM SOIL CONTAMINATED WITH PETROLEUM OF KHOUZESTAN, IRAN

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Background
In recent decades, the biological methods have been used for remove environmental contaminants such as heavy metals. Resistance to metals is important for this aim.

Objectives
The objective of this research is the isolation of chromium resistant strains.

Methods
Five samples of soil were collected under sterile conditions and transferred to laboratory immediately. Soil samples were homogenized and diluted up to 10-10 with sterile normal saline. These samples were cultured in LB agar containing 5ppm of chromium in order to isolation of resistant strains. These strains were isolated and cultured in Macconkey agar for isolation of appropriate strains. Isolated bacteria were identified by biochemical tests. Then, the MIC test was used for screening of resistant strains. The best conditions of bacterial growth were found in the presence of chromium in various temperatures, rate of shaking and values of pH by spectrophotometry at 600nm in the overnight of cultivation. Metal adsorption test measured under optimal conditions by atomic absorption spectroscopy.

Conclusions: From total of 24 strains of isolated Pseudomonads, 14 strains were resistant to chromium. The top strain (Mac2) has eliminated 35.60% of chromium from aqueous culture mediums in optimal conditions (pH: 8, Temp: 40°C and Shaking rate: 200rpm). The results were verified using statistical method named the general linear model. The contaminated zones should be clean, because heavy metals in various physicochemical forms have been considered as environmental pollutants. This bacterium can be used for studies of bioremediation of contaminated sites to chromium.
Microorganisms in caves: importance, diversity and conservation

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Background

Harsh conditions and isolation lead to formation of unique microbial communities, making caves a promising yet poorly understood field for study of biodiversity. Studies of microbial communities in caves may provide insights of microbial relationships, biogeochemical processes, evolution and even functioning of extraterrestrial life. Furthermore, they can lead to the discovery of novel bioactive compounds and draw attention to the importance of cave conservation.

Objectives

The goal of the project was to analyze the microbial communities of different caves and evaluate the impact of human activity within pristine and disturbed cave areas.

Methods

Barcode pyrosequencing of 16S rRNA genes was used to investigate the microbial diversity of two oligotrophic Karabi Jaila (Crimea) caves and the world’s deepest cave Krubera-Voronja (Caucasus).

Conclusions

It was found that 12 % of 16S rRNA gene sequences from the more frequently visited areas of Crimean caves belonged to previously unclassified bacteria. In the samples of rarely visited branches the sequences belonging to unclassified bacteria comprised 46 %. On the other hand, only 5 % of sequences found in more pristine areas of Krubera-Voronja cave belonged to unclassified bacteria. 6 % of unclassified sequences were found in the main branch of the cave where human activity is particularly intense. Disturbed areas of all caves demonstrated broader diversity of known phyla as well as significantly higher abundance of sequences belonging to Bacteroidetes, which are known to be indicators of fecal pollution.

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IDENTIFICATIONS OF PROPER METHODS TO DETERMINE LEGIONELLA PNEUMOPHILA BACTERIA EXPOSED TO DIFFERENT ENVIRONMENTAL CONDITIONS

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Background

Legionella which is the agent of Legionnaires’ disease in natural and man-made water systems. Legionella bacteria pass to the viable but non-culturable (VBNC) phase due to the environmental conditions such as temperature, pH and disinfectants. After examining under laboratory conditions the water samples containing Legionella bacteria, have been exposed to environmental factors, it was shown that those bacteria couldn’t colony form medium, but this result does not mean that the bacteria are not present in a sample or an environment.

Objectives

The aim of the present study is to detect the most suitable methods for the recovery of L. pneumophila bacteria exposed to different environmental conditions.

Methods

In the current study, first of all, the detection limits of culture, fluorescent in situ hybridization (FISH) and semi-nested polymerase chain reaction (PCR) methods for different L. pneumophila concentrations (10²-10¹⁰ cell/L) was determined. Later, the recovery of L. pneumophila bacteria from the water samples including L. pneumophila bacteria that expose to different environmental conditions (temperature, pH and biocide) has been assessed by culture, FISH and semi-nested PCR methods.

Conclusions

In the present study, the detection limit of L. pneumophila bacteria was determined as 10³ cell /L for the culture method, 10⁵ cell/L for FISH method and 10⁶ cell /L for semi-nested PCR method. It was determined that FISH and semi-nested PCR methods are suitable methods for the recovery of L. pneumophila bacteria that are subject to different environmental conditions from the water samples.
ANTIBIOTIC RESISTANCE PATTERN OF PSEUDOMONAS SPP. ISOLATED FROM THE TOTAL COURSE OF THE RIVER DANUBE

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Background
This study was part of the Joint Danube Survey 3 (JDS3), the world's biggest river research expedition of its kind.

Objectives
The aim of the study was to determine the presence of acquired antibiotic resistance of different bacterial species with clinical impact.

Methods
During JDS3 180 water samples were taken over the total course of the river Danube. Samples were mixed with glycerol and stored at -70°C. For isolation five ml of the thawed samples were plated in 0.5 ml portions on Pseudomonas selective agar and incubated at 37°. The isolates were tested for species identification with mass spectrometry MALDI-TOF MS (Shimadzu, Japan). Resistance testing was performed as recommended by the European Committee on Antimicrobial Susceptibility Testing (EUCAST). To estimate the presence of carbapenemase modified hodge tests were performed with all isolates resistant to at least one carbapenem.

Conclusions
Up to now 496 Pseudomonas were isolated, 343 (67%) Pseudomonas putida, 120 (25%) Pseudomonas fluorescens, all other species were represented by less than 10 isolates but no Pseudomonas aeruginosa was observed. Most common resistance was against meropenem (20.4%/76 isolates) piperacillin/tazobactam (4.3%/16 isolates) and ciprofloxacin (4.3%/16 isolates). None of the antibiotics were susceptible in all tested strains. Resistances were present all over the tested sampling points with no significant chances downstream. One isolate (Pseudomonas fluorescens) was tested positive for the presence of a carbapenemase in modified hodge test. Although the isolated Pseudomonas species are clinically less frequent, they are a
possible reservoir for resistance acquisition for other species (especially *Pseudomonas aeruginosa*).
Background

Soil bacteria play a central role in many ecosystem services such as formation of rhizosphere communities, nutrient transformation and global biogeochemical cycles. Although molecular techniques have uncovered the tremendous bacterial diversity in soil, the mechanisms that control the assembly, functioning and maintenance of complex microbial communities remain largely unknown.

Objectives

The study aims at linking dynamics of soil hydration conditions and related biophysical factors with variations in microbial composition and ecological functioning. Understanding factors that shape the dynamics of species abundance is essential to get an insight into the assembly of a microbial community, its functioning and response to induced changes in water availability, a key parameter of soil environments.

Methods

To assist with definitive community level observations we designed a synthetic microbial community comprised of 10 well-characterized bacterial species spanning a wide range of soil phyla to be inoculated onto model porous surfaces mimicking soil habitats. The experimental system consists of sand layer placed on porous ceramic surface connected to nutrient reservoir. Fluctuations in hydration conditions are induced by changing the reference nutrient reservoir level to simulate wetting-drying cycles. The activity and composition of the bacterial community is studied using 16S fingerprinting and quantitative PCR.

Conclusions

Spatial self-organization of microbial community is highly dependent on the hydration level of their habitat. Subjecting the synthetic community to a range of controlled physico-chemical conditions allows us systematic evaluation of the role of environmental fluctuations on changes in community composition.
Background

The Baltic Sea constitutes very specific environment. One of its characteristics are brackish water conditions that are caused by high water input from the surrounding lands and rivers. However, because of its characteristic geographical location, salinity levels change significantly in different parts of the Sea and can extend from 2, through 7 and even up to 20‰.

Objectives

The aim of our work is to assess which genes are essential for marine bacteria to adapt to the changes in salinity of their environment.

Methods

Three bacteria isolated from Baltic Sea were sequenced using Illumina Hiseq 2,000 platform and characterized as *Shewanella baltica*, *Paracoccus sp.* and *Flavobacterium sp.*. Then, they were cultured in a chemostat system in the minimal marine medium at various salinity conditions. During steady-state growth cells were harvested for RNA extraction and Illumina-based RNA-Seq reads were analyzed with DESeq2 software.

Conclusions

The transcriptome analysis of the three strains *Shewanella baltica*, *Paracoccus sp.* and *Flavobacterium sp.* at various salinity levels (bacteria cultured in 2, 7 and 20 ‰) resulted in identification of groups of genes responsible for bacterial survival in the
challenging environment of the Baltic Sea. Each strain is characterized by different response to osmolarity stress, underlying the importance of diverse categories of functional genes in this marine habitat.
FUNCTIONAL RESILIENCE OF MICROBIAL ECOSYSTEMS IN SOIL: HOW IMPORTANT IS A SPATIAL ANALYSIS?

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Background
Microbial life in soil is exposed to fluctuating environmental conditions influencing the performance of ecosystem services such as biodegradation.

Objectives
However, as this environment is typically very heterogeneous, spatial aspects can be expected to play a major role for the ability to recover from a stress event.

Methods
To determine key processes for functional resilience, scenarios with varying stress intensities were simulated within a microbial simulation model and the biodegradation rate in the recovery phase measured. Besides temporal monitoring, results were analyzed regarding spatial and mechanistic aspects. Parameters including microbial growth and dispersal rates were varied over a typical range to consider microorganisms with varying properties.

Conclusions
Results of the mechanistic and spatial view show that key factors for functional recovery depend on stress intensity and the location of the observed habitats. The limiting factors near unstressed areas are spatial processes (motility and substrate diffusion), with increasing distance microbial growth becomes more important. To confirm this, we repeated the simulations including a dispersal network representing fungi in soil. The system benefits from an increased spatial performance due to higher bacterial mobility.

With these simulations we show the importance of spatial aspects even at the mm-scale for recovering after a severe stress event in a highly heterogeneous environment such as soil. In consequence a spatial-mechanistic view is necessary for examining the functional resilience as the temporal view alone could not have led to these conclusions.

Further research should explore the importance of a spatial view for quantifying functional resilience also after complex stress regimes.
UNCOVERING STABILITY MECHANISMS IN MICROBIAL ECOSYSTEMS - COMBINING MICROCOSM EXPERIMENTS, COMPUTATIONAL MODELING AND ECOLOGICAL THEORY IN A MULTIDISCIPLINARY APPROACH

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Background
Although bacterial degraders in soil are commonly exposed to fluctuating environmental conditions, the functional performance of the biodegradation processes can often be maintained by resistance and resilience mechanisms.

Objectives
However, there is still a gap in the mechanistic understanding of key factors contributing to the stability of such an ecosystem service.

Methods
Therefore we developed an integrated approach combining microcosm experiments, simulation models and ecological theory to directly make use of the strengths of these disciplines. In a continuous interplay process, data, hypotheses, and central questions are exchanged between disciplines to initiate new experiments and models to ultimately identify buffer mechanisms and factors providing functional stability. We focus on drying and rewetting-cycles in soil ecosystems, which are a major abiotic driver for bacterial activity.

Conclusions
Functional recovery of the system was found to depend on different spatial processes in the computational model. In particular, bacterial motility is a prerequisite for biodegradation if either bacteria or substrate are heterogeneously distributed. Hence, laboratory experiments focussing on bacterial dispersal processes were conducted and confirmed this finding also for functional resistance. Obtained results will be incorporated into the model in the next step. Overall, the combination of computational modelling and laboratory experiments identified spatial processes as the main driving force for functional stability in the considered system, and has proved a powerful methodological approach.
Background

There is an emerging need for environment-friendly soil inoculants with favorable effects on crop plants.

Objectives

We aimed to develop BioeGO, a fungal-bacterial consortium-based soil inoculant ensuring increased nitrogen fixation, phosphorous mobilization, stem degradation and humus production on the treated fields and providing protective effects against phytopathogenic fungi.

Methods

Nitrogen-fixing component was selected from bacteria based on growth capabilities in nitrogen-free medium. Phosphorous-mobilizing and stem-degrading component was selected from \textit{Trichoderma} strains based on cellulase- and phosphatase-producing abilities. Humus-producing component was selected from bacteria based on peroxidase-producing abilities, while biocontrol component was selected from \textit{Trichoderma} strains based on \textit{in vitro} antagonism towards phytopathogenic fungi.

Conclusions

An \textit{Azotobacter vinelandii} strain is the nitrogen-fixing component of the soil inoculant with the potential to provide excess nitrogen for crops. Phosphorous mobilization and stem degradation are ensured by a \textit{Trichoderma harzianum} strain producing cellulose-degrading enzymes in the absence of stem residues, while this ability is increased 10-15 fold in the presence of grinded maize stem. It also produces large amounts of enzymes liberating organically bound phosphorous. A \textit{Streptomyces albus} strain with excellent peroxidase-producing abilities was selected as the humus-producing component, while a \textit{Trichoderma asperellum} isolate with outstanding antagonistic abilities towards \textit{Fusarium}, \textit{Phoma}, \textit{Alternaria}, \textit{Botrytis} and \textit{Rhizoctonia}
strains is the biocontrol component of BioeGO. Microbial components of the consortium do not have negative effects on each other, thereby all beneficial effects can occur simultaneously, complementing each other. This research was supported by grant TÁMOP-4.1.1.C-12/1/KONV-2012-0012. Csaba Vágvölgyi thanks the visiting professor program, Deanship of Scientific Research, King Saud University, Riyadh.
CHARACTERIZATION OF POTENTIAL AEROSOLIC IN ACTIVITY OF FUNGAL SPECIES

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Background

Biological aerosol particles are ubiquitous in the atmosphere. Recent findings identified a pool of these particles as potential ice nucleators (IN), which are capable of catalyzing ice formation at relatively warm subfreezing temperatures and thus impact cloud formation and precipitation. Some fungal species have been identified as ice nucleation active (INA) e.g., the ice nucleation capacity of \textit{Fusarium acuminatum} was described already some decades ago but recently more species were discovered to possess IN activity (e.g., \textit{Mortierella alpina}). For fungal IN activity, a protein or at least a proteinaceous compound is hypothesized to be responsible.

Objectives

The aim of the study is to analyze the ability to release cell-free IN into the atmosphere. Therefore, the IN machinery of fungal species is characterized by analytical and molecular biological methods as well as chamber experiments with the ultimate goal to clarify their potential impact on atmospheric cloud formation and water precipitation.

Methods

In this study, liquid chromatography and mass spectrometry is performed to identify proteinaceous compounds of fungal IN. A specific IN trap chamber (IN-TC) is designed to analyze the aerosolic transport capacity of fungal IN. For testing functional IN activity a customized droplet freezing assay is used.

Conclusions

Functional IN active fractions of fungal protein surface extractions can be identified by liquid chromatography and SDS-PAGE. Preliminary IN-TC results show a successful proof of principle suggesting the chamber is capable of collecting aerosolic IN generated of fungal washing water.
DEVELOPMENT OF HEAVY METAL ION BIOSENSORS USING SYNTHETIC GENETIC CIRCUITS

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Background

We previously determined the genome of *Bacillus oceanisediminis* 2691 that was isolated from marine sediment of the South Korean coast and found that many genes encoded heavy metal resistance and efflux systems in the genome. Genes encoding putative cadmium efflux pumps, arsenic efflux pumps, a chromate transporter, and lead-, cadmium-, zinc-, and mercury-transporting ATPases were found in the genome, which are apparently regulated by CadC homologous repressor-type transcription factors.

Objectives

We attempted to develop heavy metal ion biosensors using synthetic genetic circuits with *cadC* genes.

Methods

It was confirmed the transcriptional modulations of CadC-controlled genes in response to various heavy metals in *B. oceanisediminis* 2691 using real-time PCR. Six *cadC* promoter-operator-structural genes were transcriptionally fused with *egfp* gene to make recombinant *E. coli* cells. A variety of heavy metals were treated and specific fluorescence intensities were measured.

Conclusions

Taken together, the results showed that CadC proteins specifically respond to heavy metals and may play separate roles in heavy metal resistances, which have been evolved in the heavy metal abundant marine sediment milieu. In biotechnological aspects, CadC-controlled transcriptional modules could be used in the development of harmful heavy metal bio-sensor.
CHARACTERIZATION OF COD REMOVAL, ELECTRICITY GENERATION AND MICROBIAL COMMUNITY IN MICROBIAL FUEL CELLS TREATING MOLASSES WASTEWATER

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Background

Wastewater microbial fuel cells (MFCs) have advantages in treatment wastewater and electricity production simultaneously. Molasses wastewater (MW) is one of suitable feedstock for wastewater MFCs.

Objectives

In this study, electricity generation, COD removal, and microbial communities were investigated using 3-types of MFCs which were single-chamber MFCs without proton exchange membrane (PEM) and with PEM (AC-MFCs and ACM-MFCs, respectively) and two-chamber MFCs (H-MFCs).

Methods

Diluted MW (10,000 mg·L⁻¹ COD) was continuously fed into the MFCs. Reactor performances in the single-chamber MFCs were similar, indicating that PEM couldn’t enhance the reactor performances. COD removal in the single-chamber MFCs (90%) was higher than in the H-MFCs (50%). However, current density in the H-MFCs (80 mA·m⁻²) was 1.4 times of that in the single-chamber MFCs (57 mA·m⁻²). Power density in the H-MFCs (17 mW·m⁻²) was 2.2 times that in the single-chamber MFCs (7.7 mW·m⁻²). Microbial communities of the MFCs were examined using 16S rRNA-iron torrent sequencing. For bacterial community, Clostridium (7.6%) and Geobacter (4.2) were abundant in the AC-MFCs, and Clostridium (7.6%) and Desulfovibrio (5.1%) in the ACM-MFCs. Bacterial community in the H-MFCs was different with the single-chamber MFCs. Pseudomonas (12.9%) was dominated in the H-MFCs, followed by Clostridium (7.0%) and Geobacter (2.9%). For archaeal community, Methanothrix (96.4%) was remarkably dominated in the single-chamber MFCs. However, Methanobacterium (35.1%) and Methanosarcina (28.3%) were abundant in the H-MFCs.

Conclusions

These results suggest that chamber design is a key factor to influence on COD removal and electricity generation in the MFCs treating MW.
GENOMIC INSIGHTS INTO THE CATABOLIC VERSATILITY OF RHODOCOCCUS SP. STRAIN P52 TOWARDS ENVIRONMENTAL POLLUTANTS
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Background
Rhodococcus sp. strain p52, an isolated dioxin-degrader, can aerobically utilize a variety of aromatic compounds, such as dibenzofuran, dibenzo-p-dioxin, 2-chlorodibenzo furan, 2,8-dichlorodibenzofuran, dibenzothiophene, biphenyl, naphthalene, fluorene, phenanthrene, and anthracene. Degradation of dioxin by strain p52 involves two dioxygenases, DbfA and DfdA, encoded by genes on two plasmids. Additionally, strain p52 can use linear alkanes (tetradecane, tetracosane, and dotriacontane) and branched alkane (pristane) as sole carbon and energy sources.

Objectives
The objective of the present study is to gain insight into the underlying genetic information for the catabolic versatility, and biodegradation potential of strain p52.

Methods
Complete nucleotide sequence of the strain p52 genome was sequenced with the PacBio RS II system. The genome was annotated by BLAST against database, including KEGG, COG, SwissProt, TrEMBL and NR. The predicted genes involved in degradation of aromatic compounds and alkanes were tested for their transcriptional activity. And the function of the genes was confirmed by heterologous expression in recombinant hosts.

Conclusions
Co-presence of catabolic plasmids and multiple catabolic genes, as well as genes involved in chemotaxis, solvent tolerance, and transportation in the strain favors its catabolic ability and environmental adaptation. The present study provides insights into the bioremediation potential of strain p52.
BIODEGRADATION OF P-NITROPHENOL BY DIFFERENT MIXED CULTURES

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Background

One of the most important environmental pollutant among nitroaromatic compounds is p-nitrophenol (4-nitrophenol, PNP). It is used in the manufacture of a wide range of products and is the intermediate of some organophosphorus pesticide degradation (1). PNP has been classified as a priority pollutant by United States Environmental Protection Agency (EPA), and its concentration in natural waters is restricted to less than 10 ng/L (2).

Objectives

The aim of this study was to investigate the biodegradation of PNP by three different mixed cultures (TUS, AM and KOV) isolated from contaminated soil and sediment.

Methods

The samples were inoculated into minimal medium with increasing PNP concentrations. The biodegradation process was monitored by UV spectroscopy. The toxicity of PNP solution and PNP degradation intermediates to luminescent bacteria Aliivibrio fischeri was evaluated according the ISO 11348-3.

Conclusions

The mixed culture TUS showed the greatest biodegradation potential. Mixed microbial population was capable of degrading up to 100 mg/L of PNP as a sole carbon and nitrogen source. Toxicity was significantly reduced after the degradation process.

References:
CHARACTERIZATION OF A THERMO-STABLE BIOFLOCCULANT PRODUCED BY METHYLOBACTERIUM SP

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Background

Chemical and organic synthetic flocculants are, currently, predominantly employed in different industrial processes such as wastewater treatment and drinking water purification. The use of bioflocculants is desirable as most chemical and organic synthetic flocculants have been shown to possess deleterious effects to human health and, also, are recalcitrant to biodegradation thus, polluting the environment.

Objectives

This study assessed the bioflocculant production potential of Methylobacterium sp. Obi isolated from Tyume River in the Eastern Cape Province, South Africa.

Methods

*Methylobacterium* sp. Obi (accession number HQ537130) identified through 16S rDNA and sequence BLAST analysis was evaluated for bioflocculant production and the physicochemical properties optimal for the production of bioflocculant was determined using standard technique. The bioflocculant was characterized for novelty using FTIR and thermo-gravimetric analysis.

Conclusions

Culture conditions for optimal production of the bioflocculant included the following: glucose as carbon source; complex nitrogen source of urea, yeast extract and (NH₄)₂SO₄; inoculum size of 1% (v/v); initial pH 10; and 1% Ca²⁺. Maximum flocculating activity of 90% was attained after 36 hr of cultivation with the bioflocculant exhibiting thermal stability up to 100°C. Optimal culture conditions resulted in purified bioflocculant yield of 4.61 g/l. Optimal dose for kaolin suspension clarification 0.3 mg/ml in the presence of 1% Ca²⁺. Fourier transform infrared (FTIR) spectrum of the purified bioflocculant showed the presence of carboxyl, amino and hydroxyl groups known to be critical in the flocculation process. Thermo gravimetric analysis revealed that the produced bioflocculant was thermo stable.
COMMUNITY STRUCTURE OF BENEFICIAL RHIZOBACTERIA IN APPLE ORCHARDS OF TRANS HIMALAYAN REGION AS INFLUENCED BY PHYSICAL GEOGRAPHY OF AN APPLE HILL FARM

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Background

Over the years, diversity of beneficial rhizobacteria associated with agronomical/horticultural crops, especially the influence of geographical distribution (altitudinal variation) has been well documented. However, the influence of physical geography (eastern and western aspect of a hill farm), on the community structure of beneficial rhizobacterial population, irrespective of the altitudinal variation in unclear.

Objectives

The nutrient and health status of crops growing on two opposite geographical aspects of a hill farm could be influenced by presence or absence of beneficial rhizobacterial communities, with exceptional abilities of nutrient mobilization and pathogen antagonism.

Methods

In the present study we investigated rhizobacterial population associated with apple trees (four different apple cultivars) grown on hill farms at similar altitudes on two opposite geographical aspects. In the present study, a total of 259 rhizobacteria were isolated from the rhizosphere of apple trees, of which 50 were selected and further examined on the basis of their abilities of nutrient mobilization and pathogen antagonism. Beneficial rhizobacterial diversity analyzed using molecular analysis (16S rDNA-ARDRA) and supported by statistical tools revealed the presence of dominant siderophore producing bacterial populations (nutrient mobilizers and plant pathogen antagonists) on the eastern aspect of hill farm.

Conclusions

Results from microbial diversity study correlated with the survey analysis, for the presence of 40-60% of diseased apple trees and high (80-90%) non-survivability of re-planted apple plants on the western aspect of hill farm, as compared to 10-15%
diseased apple trees and low problem (15-20%) of survivability of re-planted apple plants on eastern aspect.
ENTEROCOCCUS FAECIUM REDUCED MALODOROUS COMPOUNDS IN SWINE MANURE SLURRY IN VITRO AND IN CONFINEMENT HOUSES

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Background
Reducing malodorous compounds are still swine producers’ problem.

Objectives
This study evaluated different bacteria for their ability to reduce malodorous compounds.

Methods
Previously isolated and identified bacteria with 99% similarities were used in this study. These were Enterococcus faecium(M1), E. faecalis(M2), Acetobacter tropicalis(M3) and Bacillus subtilis subsp. subtilis(M4). The bacteria were tested using in vitro technique (serum bottles and plastic boxes) and the best bacterium was selected and used as spray in the confinement pig houses.

Conclusions
In serum bottles technique, pH values were reduced (P<0.05) and hydrogen sulfide (H₂S) gas decreased (P<0.05 and M4 from 12 to 24h while opposite was observed in NH₄⁺-N concentration in the liquid phase. Total volatile fatty acid, histamine, methyamine, ethylamine, putrescine and cadaverine as well as total biogenic amine concentration was lowest (P<0.05, H₂S, total mercaptans) and liquid NH₄⁺-N were comparatively lower (P<0.05 and M4. M1 was selected and used for spray to confirm the odor reduction at different periods in the confinement pig houses. Odor compounds were lessened by 28.21%, 42.15% and 15.02% for NH₃, H₂S, and total amines, respectively up to 3 days after spraying. E. faecium can reduce malodorous compounds in swine manure slurry in vitro and in confinement houses.
Background
Bacteria play a significant role in odor production.

Objectives
This experiment was conducted to determine the appropriate bacteria for reducing malodorous compounds during fermentation in pig.

Methods
The bacteria were isolated and identified from rectum of pigs and were used in this study. They have 99-100% similarities to: Enterococcus faecium CWBI B1430(M1), E. faecium DSM 20477(M2), E. faecalis(M3), Lactobacillus acidophilus(M4), L. plantarum(M5), Bacillus subtilis ZHA10(M6), Bacillus subtilis XJASZB15(M7), Acetobacter malorum(M8), and E. faecium NRIC0114(M9). One hundred ml of fecal slurry was transferred to serum bottles containing 1g of soluble starch, inoculated with or without 1ml of different microbial cultures (1.0×10⁷CFU/ml) and incubated anaerobically for 12 and 24h.

Conclusions
Ammonia-nitrogen (NH₃-N) contents (gas phase) were decreased rapidly from 12 to 24h but was not found in M7 and M9 at 24h while NH₃-N contents (liquid phase) of M6 was significantly lowest after 24h. Hydrogen sulfide (H₂S) contents (gas phase) were detected in M5, M6, M7, M8 and M9. Total biogenic amine was found lowest (PEnterococcus faecium NRIC 0114(M9) is the best among the all of the treatments on the basis of toxic compound production.
GRAZING BY NEMATODES AFFECTS MAIZE RHIZOSPHERIC BACTERIA

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Background

Both mutualistic and pathogenic bacteria colonize the plant rhizosphere. Mutualists promote plant growth by providing nutrients or defense against pathogens. Nematodes and protozoa are also enriched in the rhizosphere, but little is known about the interactions of the grazing microorganisms with the bacterial community. The diversity of protozoans and nematodes in the corn rhizosphere has’t been studied and will provide a basis for the further analysis of their grazing preferences and their impact on bacteria.

Objectives

Identify and isolate protozoans and nematodes from the corn rhizosphere with metagenomic and culture-dependent approaches.

Methods

The diversity of the eukaryotic microorganisms was assessed analyzing 18S rRNA gene sequences from DNA extracted from corn rhizosphere samples. Nematodes were cultured in Nematode Growth Medium (NGM) and identified as described above. Feeding preferences were assayed in Petri dishes with NGM medium in the presence of different bacteria. The effect of nematodes was tested by adding the nematode to roots of maize plants growing in flasks with vermiculite.

Conclusions

The metagenomic analysis was performed with DNA samples from Huitzilac in the state of Morelos and in Amecameca in the state of Puebla. In cultures, the single nematode present was identified as *Oscheius tipulae*. This nematode has a preference for the bacterium *Rhizobium phaseoli* Ch24-10, and decreases the growth of corn plants in laboratory assays. However, in the presence of a group of selected root bacteria such as *Rhizobium phaseoli*, *Methylobacterium extorquens* and *Chromobacterium violaceum*, the growth of the corn plants was enhanced compared to control plants without the nematode.
VIBRIO VULNIFICUS EMPLOYS DIFFERENT DEFENCE MECHANISMS AGAINST PROTOZOAN GRAZING

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Background

*Vibrio vulnificus* is an opportunistic pathogen responsible for wound infections and sepsicaemia following ingestion of contaminated seafood, and has the highest reported mortality rate for seafood-related diseases. It is an autochthonous inhabitant of coastal marine environments where it is exposed to predation by heterotrophic protozoa.

Objectives

Protozoan predation on bacteria acts as a selective force, leading to evolution of antiprotozoal mechanisms that may also function as virulence factors. This study investigated the correlation of the place of isolation, genotype and virulence potential with grazing resistance of *V. vulnificus*.

Methods

Twenty two strains of *V. vulnificus* were exposed to grazing by *Tetrahymena pyriformis*, a filter feeding ciliate, and *Acanthamoeba castellanii*, a surface browsing amoeba in microtitre plates. *V. vulnificus* numbers in planktonic phase were determined by plate counts and photometry while biofilm biomass was assessed by crystal violet staining. The health and number of protozoa were determined by microscopy.

Conclusions

Although there was no significant difference in grazing resistance between C-genotype (clinical origin) and E-genotype (environmental origin), an environmental and a clinical isolates exhibited unique grazing defence mechanisms against the protozoa: the oyster isolate showed toxicity against *T. pyriformis* while the blood
isolate employed biofilm and pellicle formation to escape predation. Furthermore, the role of capsular polysaccharide (CPS) in grazing resistance of V. vulnificus was examined; no statistically significant differences were found between the encapsulated, "opaque" strains and the non-pathogenic non-encapsulated, "translucent" strains. These results show that the defence mechanisms of different strains of V. vulnificus have evolved along different evolutionary pathways.
Background

The start-up of anaerobic digester has not been studied in detail from the microbiological point of view. Knowledge of microbiology process could help to determine whether an anaerobic digester has reached steady state, in the event that physicochemical data are not successful.

Objectives

The objective is to determine the dynamism of the microbial community during the start-up of an anaerobic digester.

Methods

The start-up of two-mesophilic anaerobic digester (fed with mix sludge) was studied through methane productivity, volatile suspended solids (biomass), along with other chemical variables. The inoculum was obtained from "La Farfana", wastewater treatment plant located in Santiago, Chile.

DNA was extracted from samples of sludge operation days 0, 13, 25 and 50. The genes studied were: 16S (Bacteria and Archaea) through DGGE and 4 functional genes (Hydrolytic, Homoacetogenic, Methanogenic and Acidogenic) through real time PCR.

Conclusions

The digester reached steady state after 30 days of operation, with productivity of 140 mL methane/ (Liter day) and 40% increase in biomass.
When the digesters reached the steady state the number of haplotypes (DGGE) increased about 40%, while the copies number of functional genes increased twice in
relation to the beginning of the process.

Figure 2 DGGE 16S of digester 1: (A) Archaea and (B) Bacteria
Fig 3. Copy number of hydrolytic gene during start up of two anaerobic digester
The digester 1 is represented for • and digester 2 is represented for ○
MICROBIOLOGICAL SOIL QUALITY IN CITRUS ORCHARD UNDER DIFFERENT MANagements

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Background
The inter rows of citrus orchards in Brazil are generally managed with mowing, while in the lines mainly use the herbicide glyphosate. There is a great predominance of \textit{Urochloa decumbens} in the inter rows of orchards. As there are reports of allelopathy of \textit{U. decumbens} to citrus and damages that result from the continued use of glyphosate in other cultures.

Objectives
This project aims to study through microbiological parameters influences on soil quality using two vegetation in inter rows, managed with different mowing, with and without herbicide in the lines of Tahiti acid lime orchard.

Methods
Since 2010, trial was conducted in Mogi Mirim, Sao Paulo State, Brazil, in split plot design, where in the plots were composed of two types of vegetation: \textit{U. decumbens} and \textit{U. ruziziensis}; the sub plots for two types of mowing: conventional and ecological; and the sub sub plots: by applying and absence of the herbicide glyphosate on planting line. Samples of soil from the rhizosphere of plants were collected 0-10 cm depth, in three seasons and the following parameters evaluated: microbial biomass carbon (BMC), basal respiration (RB) soil and metabolic quotient.

Conclusions
The results showed that there were significant differences for the microbiological parameters and soil treatment with \textit{U. decumbens}, ecological mowing and herbicide use were better when compared with others. There was no damage to the vegetative and productivity development of Tahiti acid lime, so the changes in management can be recommended.
Background

In the framework of the BMBF-funded consortium project BIOACID (Biological Impacts of Ocean Acidification) we are interested in the response of marine epibacterial communities attached to biological surfaces to increased $pCO_2$ and temperature. In spring 2014, we performed a three-month experiment in the benthic mesocosm facility at the Alfred Wegener Institute in List (Germany) which allows low and high tide simulation to mimic Wadden Sea conditions. We manipulated the seawater $pCO_2$ and temperature as predicted by the end of this century to simulate ocean acidification and warming events of the future North Sea.

Objectives

Both stress factors were tested for single and combined effects on epibacterial communities on the surface of the brown macroalgae *Fucus vesiculosus* forma *mytili* and in comparison on the planktonic microbiota of the surrounding seawater.

Methods

Changes in the bacterial phylogenetic diversity were analyzed by bacterial 16S rDNA (V1-V2) amplicon sequencing using the Illumina MiSeq technology.

Conclusions

The generated data indicated significant shifts in the bacterial community structure over a period of three months (April to June), driven by rising temperature and lowered seawater pH levels. Overall, our findings suggest significant changes in the microbial diversity under anthropogenic pressures such as global warming and ocean acidification with potentially severe consequences for the future Wadden Sea ecosystem.
Background
Biosorption of heavy metals is effective technology for removal of toxic metals. Algae, bacteria and fungi are useful biosorbents for metal elimination from polluted zones.

Objectives
The objective of this study was isolation of lead resistant *Pseudomonas* strains.

Methods
Soil samples were homogenized and diluted with sterile normal saline. These samples were cultured in LB agar containing 5ppm of lead in order to isolation of resistant strains. These strains were isolated and cultured in Macconkey agar for isolation of appropriate strains and identified by metabolically tests. Maximum lead resistance of strains was evaluated by MIC test. The bacterial growth were optimized in the presence of lead in various temperatures, rate of shaking and values of pH by spectrophotometry in 600nm at 24h of cultivation. The bacterial strains were tested for ability to grow in the presence of different antibiotic by disc diffusion method. Metal adsorption test measured under optimal conditions by atomic absorption spectroscopy.

Conclusions
In this study 24 colonies were resistant to lead and the bacterium, *Msap*, had more resistant than others. The best conditions of growth in temperature, pH and rate of shaking were 37, 9 and 200. The results were verified using the general linear model. The highest of biosorption capacity was 91.79%. The strain, *Msap*, showed resistance to ampicillin, tetracycline, chloramphenicol, erythromycin, and kanamycin. *Msap*, have a great potential for bioremoval of lead from aquatic polluted environments. In conclusion, this study reveals the significance of using the *Pseudomonas* sp in bioremediation of lead contaminated zones.
CHARACTERIZATION OF BIOAEROSOLS - VALIDATION OF A REDUCED SAMPLING METHOD -
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Background

Bioaerosol monitoring is a rapidly increasing field of environmental risk assessment, which includes measurement of different microbiological and chemical parameters in both indoor and ambient air. Generally, bioaerosols are particles of biological origin with an aerodynamic diameter of up to 100 nm, containing bacteria, fungi, viruses, pollen, plant debris as well as their metabolites like endo-/mycotoxins, allergens and (M)VOC’s. In the last years, numerous studies showed associations between biological particles and a wide range of adverse health effects such as infections, intoxication and sensitization.

Objective

The aim of the study was the validation of a reduced and optimized sampling procedure necessary for a comprehensive bioaerosol risk assessment.

Methods

We investigated bioaerosols from laying hen-, swine- and cattle livestocks on different measuring sites (within the barn, 50 m downwind and background) using impaction and impingment. For the microbiological testing the total viable count of bacteria and fungi as well as a selective enrichment for MRSA, ESBL and VRE was carried out. Additional species identification (bacteria/ fungi) was performed by MALDI-TOF-MS. The chemical characterization included the detection of particulate matter (PM_{10}, PM_{2.5}, PM_{1}, UFP), endotoxin, metal and VOC content.

Conclusion

The interspecies comparison show clear variation in the microbiological (bacterial/ fungal) and chemical components (PM, endotoxin and (M)VOC level) profile between the livestocks (indoor). Up to 50 m downwind no differences compared to the background samples of the corresponding livestock were detected. Due to the harmonized combination of selected chemical and microbiological indicator parameter, the reduced sampling program constitutes a constructive alternative for a comprehensive risk assessment.
ISOLATION AND IDENTIFICATION OF CYANIDE TOLERANT BACTERIA FROM WASTEWATER DISCHARGED FROM AN ELECTROPLATING PLANT IN CAPE TOWN, SOUTH AFRICA
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Background

The pollution of water sources is one of the major challenges municipalities face worldwide. The industrial wastewater discharged into the municipal sewers often contain toxic substances. Electroplating industries contribute to the pollution of water sources with cyanide, cyanide metal complexes and heavy metals used in their metal plating processes.

Objectives

The aim of this study was therefore to isolate and identify cyanide tolerant bacteria from wastewater discharged from an electroplating plant in the Western Cape, South Africa which was suspected of disposing of its potentially hazardous wastewater into the municipal sewers.

Methods

Bacteria were isolated from the biofilm and wastewater discharged from the electroplating plant. The pure colonies were streaked on nutrient agar supplemented with 400 mg/L cyanide to test for tolerance. To characterise and identify the cyanide tolerant isolates the following tests were performed; gram and endospore staining, biochemical tests, selective media, VITEK 2 Compact, Genomic DNA extraction, Polymerase chain reaction (16s rRNA gene amplification) and sequence analysis.

Conclusions

Two of the isolates were identified as Pseudomonas species and the other two as Bacillus species. The four identification methods used (biochemical testing, selective media, VITEK 2 and sequence analysis (16s rRNA gene) could confirm 100% identity up to the genus level even though a few discrepancies in identifying the species existed. Further studies are however needed to study other genes to differentiate the species. The isolates are further to be tested for cyanide degradation and as possible candidates for bioremediation of cyanide and metal cyanide complexes.
MYXOBACTERIA IN PEAT BOGS AND FEN – AN ASTONISHING DIVERSITY

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Background

Increasingly appearing resistances to antibiotics and the subsequent need for new bioactive substances is one of the fundamental challenges of infection research. Myxobacteria have a rich secondary metabolism, which places them among the best known natural product producers. To date more than 100 new metabolites and more than 500 derivatives were described from these soil dwelling, predatory microorganisms. In the past it turns out that in particular new families, genera and species of myxobacteria are reliable sources for new compounds.

Objectives

Promising sources for the isolation of new myxobacteria are uncommon habitats like, for example, peat bogs or fen, which are characterized by low pH values. This habitat has been neglected with regard to the isolation of myxobacteria in the past. In our study, the diversity of myxobacteria in moor-samples from the Harz-region was evaluated by standard cultivation methods as well as by cultivation independent 16S rRNA-clone bank analyses.

Methods

A total of 297 clones of myxobacterial origin were sequenced and compared to sequences of cultures, isolated from these samples, by phylogenetic analyses. It turned out that the majority of myxobacteria is only represented by clone sequences and could not be cultivated. Comparing these sequences to sequences of a public database (NCBI) revealed that most of these uncultivated myxobacteria are exclusively related to other uncultivated myxobacterial sequences from acidic soils.

Conclusions

This suggests that peat bogs and fen harbor a big diversity of new myxobacterial species, genera and even families which themselves harbor a great potential of urgently needed, new secondary metabolites.
EVOLUTIONARY HISTORY INFLUENCES THE SALINITY PREFERENCE OF BACTERIAL TAXA IN WETLAND SOILS

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Background
Salinity is a major driver of phylogenetic bacterial community composition across the globe and, given the importance of microbial community composition for ecosystem function, it is necessary to understand how salinity structures bacterial communities. If phylogenetic groups exhibit coherence in their salinity preferences, characterizing these preferences will enhance our ability to predict how changes in salinity will affect microbial communities and their associated functions.

Objectives
Consequently, we aimed to examine the response of wetland bacterial community composition and function to changes in salinity and to assess whether salinity preferences were phylogenetically conserved in bacteria.

Methods
To address these aims, we used a reciprocal transplant experiment of fresh- and saltwater wetlands soils.

Conclusions
The salinity of both the origin and host environments affected bacterial community structure (16S rRNA gene sequences, Illumina) and activity (e.g., CO₂ and CH₄ production). Phylotypes were categorized as exhibiting a preference for freshwater, saltwater, or having no salinity preference by comparing presence/absence patterns across treatments. When these preferences were related to phylogeny, a significant influence of evolutionary history was seen in all preference categories. This phylogenetic signature was corroborated by differences in the salinity preferences of high-level taxonomic groups. The majority of a-proteobacteria and g-proteobacteria phylotypes preferred saltwater, while the phylotypes of b-proteobacteria were more likely to prefer freshwater, and phylotypes within Verrucomicrobia tended to have no salinity preference. Overall, our results indicate that salinity structures bacterial communities by selecting for organisms with phylogenetically clustered salinity preferences, and these effects on community composition may have consequences for ecosystem function.
Background
Formate is an important metabolite in anaerobic environments, where it is one of the metabolic products of fermentative organisms and an energy source for many bacteria such as sulfate-reducing bacteria (SRB). SRB are anaerobic organisms that possess a high number of formate-dehydrogenases (FDHs) and hydrogenases (Hases), enzymes responsible for \( \text{H}_2 \) production from formate [1]. In sulfate-limited environments SRB grow fermentatively in syntrophy with other organisms producing \( \text{H}_2 \). Moreover, it was recently demonstrated that in the absence of sulfate, *Desulfovibrio vulgaris* displays a very high \( \text{H}_2 \) production from formate [2].

Objectives
In the present work, the electron transfer mechanism involved in formate-driven \( \text{H}_2 \) production by SRB was studied.

Methods
\( \text{H}_2 \) production by *D. vulgaris* deletion mutants of the two main FDHs (\( \Delta fdhABC \) and \( \Delta fdhAB \)) and of the four periplasmic Hases (Fe-only Hase (\( \Delta hydAB \)), two [NiFe] Hase (\( \Delta hynAB1 \) and \( \Delta hynAB2 \)), and a [NiFeSe] Hase (\( \Delta hysAB \))) was compared with the wild-type strain in order to disclose the role of each enzyme in the metabolic pathway involved in \( \text{H}_2 \) production from formate [3].

Conclusions
This work contributes to our knowledge about the electron transfer pathways involved in \( \text{H}_2 \) and formate metabolism in *D. vulgaris*, showing which of the FDHs and Hases play an important role in this process.


BACTERIAL PROFILING OF SAHARAN DUST DEPOSITION IN THE ATLANTIC OCEAN USING SEDIMENT TRAP MOORINGS – YEAR ONE RESULTS

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Background

Large quantities of dust are transported from the Sahara Desert across the Atlantic Ocean towards the Caribbean each year, with a large portion of it deposited in the ocean. This dust brings an array of minerals, nutrients and organic matter, both living and dead. This input potentially fertilizes phytoplankton growth, with resulting knock-on effects throughout the food chain. The input of terrestrial microbial life may also have an impact on the marine microbial community.

Objectives

The aim is to understand the links between dust input and the bacterial community and how this relates to ocean productivity and the carbon cycle.

Methods

The current multi-year project consists of a transect of floating dust collectors and sub-surface sediment traps placed at 12°N across the Atlantic Ocean. Sediment traps are located 1200m and 3500m below the sea surface and all are synchronized to collect samples for a period of two weeks.

Conclusions

The first set of sediment trap samples were recovered using the RV Pelagia in November 2013 with promising results. Results from 7 sediment traps (three at 1200m and four at 3500m) were obtained. In general, the total mass flux decreased as distance from the source increased and the upper traps generally held more material than those at 3500m. Denaturing Gradient Gel Electrophoresis (DGGE) was used as a screening technique, revealing highly varied profiles, with the upper (1200m) traps generally showing more variation throughout the year. Preliminary analysis of 454 sequencing from a subset of the samples supports this observation, and detailed analysis will be presented.
Background

By the recent years, it has increased the air pollution in urban environments as well as rural, due to anthropogenic activities. This has been reflected on respiratory illness or infections related to air pollutants, mainly to fine inhalable particulate matter PM2.5; nonetheless, it is rarely discussed in the aburra valley about the microorganisms associated to this sort of particles, which can cause human illness ranging from mucosa irritation and allergies to asthma and pneumonia.

Objectives

The purpose of this research is the isolation and identification of microorganisms present in PM2.5 filters of the stations MED-UNFM and MED-PJIC of the Air Quality Monitoring Network from the Aburrá Valley.

Methods

Air sampling equipment BGI PQ200 semiautomatic was used for collection of biological material in PM2.5 Teflon filters 47mm in diameter. A sample of the latter was inoculated and incubated in an enrichment broth; Serial dilutions were made and were inoculated onto selective media. Each of the strains isolated, were characterized morphologically and biochemically (VITEK® 2 Compact).

Conclusions

In this research, several microbial species from PM2.5 filters, were isolated and identified like Bacillus cereus, Acinetobacter baumannii, Enterococcus faecium and Bacillus anthracis, all those consider as pathological microorganisms. This allows inferring that air quality, besides generating problems associated to respiratory illness, can increase the possibility of contracting bacterial infections.
METAL-RICH TROPICAL SEDIMENT MICROBIOME UNVEILS A DIVERSE TAXONOMIC AND FUNCTIONAL MICROBIAL COMMUNITY

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Background
The Iron Quadrangle (Minas Gerais, Brazil) is one of the world’s largest mining regions, being historically explored for over 300 years. Since then, many toxic metals and metalloids were released into the environment leading to contamination of water bodies. It is well known that microbial community influences the bioavailability of these toxic elements and its importance in the ecological balance of various environments.

Objectives
This study investigated the taxonomic and functional diversity of the microbiome from Mina Stream sediment (MSS), historically metal-contaminated, especially arsenic.

Methods
The environmental DNA extracted was sequenced using SOLiD and Miseq platforms for the functional and taxonomic analysis, respectively.

Conclusions
Taxonomic profile obtained by Greengenes database revealed a complex microbial community, with dominance of Proteobacteria and Parvarcheota. Contigs recruited bacterial and archaeal genomes, especially Candidatus Nitrospira defluvii and Nitrosopumilus maritimus, and their presence implicated them in C and N cycling in the MSS. Functional reconstruction revealed a large diverse set of genes for ammonium assimilation and ammonification. These processes have been implicated in the maintenance of N cycle and healthy of the sediment. SEED subsystems functional annotation unveiled a high diversity of metal resistance genes, suggesting that the prokaryotic community is adapted to metal contamination. Furthermore, a high metabolic diversity was detected in MSS, suggesting that the historical arsenic contamination is no longer affecting it. Finally, the results reported herein may contribute to expand the current knowledge of the microbial taxonomic and functional composition of tropical metal-contaminated freshwater sediments.

Supported by: CNPq, CNPq/INCT-Aqua and FAPEMIG
Background
Organophosphorous Pesticides (OP) are being used for pest control in many countries. Extensive use of these compounds results in their accumulation in agricultural soil, water source, affecting insects and humans. Chlorpyriphos (CP) a broad-spectrum insecticide has been used for controlling termites, beetles on paddy fields and vegetable crops. CP acts as a neurotoxin by inhibiting acetylcholinesterase, which degrades the neurotransmission agent, acetylcholine, in the nervous system. Biodegradation of CP results in the formation of a compound TCP (3,5,6-trichloro-2-pyridinol) classified as mobile, toxic also has antimicrobial property.

Objectives
Screening, isolation and identification of the microbial isolates for Chlorpyrifos and TCP degradation

Methods
Resting cell assay for Chlorpyrifos degradation in liquid media and HPLC analysis

Conclusions
Present investigation showed microbial strains isolated and screened from agricultural soil amended with CP. Six bacterial strains (CPD-03, CPD-5C, CPD-7, CPD-18, CPD-20 & CPD-33) showed significant degradation of CP in minimal salt media supplemented with CP @ 10 µg/ml. A resting cell assay was carried out at pH 7.2, 37 °C with CP @ 10 µg/ml and monitored over a period of 48 h, and analyzed using HPLC. The degradation efficiency of these isolates varied between 65% to 67% within 48 h. The isolates CPD-18, CPD-20 and CPD-33 were able to degrade CP and metabolize TCP up to 64% - 65 % within 48 hr compared to CPD-03, CPD-5C, CPD-7 which degraded up to 65% - 67%. These six microbial strains can extend our understanding of pesticide degradation and would be beneficial in using as a consortium for cleaning up pesticide contaminated environment.
Background

Waterborne diseases are among the leading causes of morbidity and mortality in developing countries and diarrhoeagenic Escherichia coli are one of the most important etiologic agents of acute diarrhea and represent a major public health problem in developing countries like South Africa.

Objectives

In this study, we assessed the prevalence and antibiogram profiles of E. coli pathotypes in Tyume River South Africa.

Methods

Twelve water samples were collected from three sites along the rivers between August 2010 and July 2011 and E. coli was isolated by the membrane filtration method on mFC and Chromocult agar. Identification and pathotyping of the isolates were done by polymerase chain reaction technique using species/pathotype specific primers and antibiogram was conducted using the disk agar diffusion method.

Conclusions

Of the 202 presumptive E. coli isolates recovered from the river 35% were confirmed as E. coli. The pathotypes prevalence was as follows: ETEC (30%); EPEC (35%); EAEC (35%); and EIEC (16%). All five E. coli pathotypes showed high-level resistance to ampicillin, tetracycline, cotrimoxazole, and chloramphenicol but were highly susceptible to quinolones, aminoglycosides, and novobiocin. All the EAEC, ETEC, and EIEC pathotypes were resistant against ampicillin, while 87.5% of the EAEC pathotype were resistant to carbenicillin. Also, all the pathotypes were susceptibility to quinolones. TEM genes and integron conserved segments were observed in 80% of the isolates. The Tyume river appears to be a reservoir of pathogenic E. coli strains and antibiotic resistance determinants, and hence a subject of public health importance.
PHYLOGENETIC CHARACTERIZATION AND COMMUNITY DIVERSITY OF HYDROCARBON-DEGRADING BACTERIAL POPULATIONS IN SOIL MICRO COSMS ENRICHED WITH VARIOUS AROMATIC HYDRO CARBONS

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Background

High quantities of various aromatic hydrocarbons such as toluene, benzene, ethylbenzene and xylene (BTEX) are ubiquitously found in nature as a result of both natural and anthropogenic influences. Despite previous research efforts devoted to understanding the degradation of hydrocarbon pollutants in various environments, yet, there is still paucity of knowledge concerning the diversity of indigenous bacterial populations that are mostly responsible for the biodegradation of various hydrocarbons.

Objectives

The primary focus of this study was to isolate and characterize indigenous bacterial populations in soil microcosms previously enriched with various aromatic (BEX) hydrocarbons. Also, to compare the diversity of hydrocarbon degraders between the three microcosm communities examined.

Methods

Combinations of standard microbiological (phenotypic) and molecular (16S ribosomal RNA gene sequencing were used to isolate, characterize and identify 45 bacterial isolates with putative hydrocarbon-degrading capability in the three soil microcosms. Additionally, various bioinformatics and analytical tools were employed for sequence alignments and diversity measurements.

Conclusions

A total of 45 bacterial isolates belonging to 5 distinct phyla were phylogenetically characterized among indigenous bacterial populations with putative hydrocarbon-degrading in the soil microcosms. In general, bacterial members of the γ-proteobacteria {mostly Pseudomonas and Acinetobacter} dominated {between 60 to 94%} among the isolates from the three microcosms. Bacterial members belonging to the β-proteobacteria {Comamonas and Delftia sp} and Firmicutes {Bacillus sp} were also represented. Overall, the differences observed in bacterial phylotypes among the microcosms are probably attributable to the direct effects of the chemical properties of each hydrocarbon pollutant on the indigenous microbial community.
Background
Nosocomial or hospital acquired infections (HAIs) is a serious global public health issue, causing the suffering of about 1.4 million people across the world at any given time.

Objectives
This study was undertaken to ascertain the prevalence of HAI in Delta State of Nigeria.

Methods
This is a descriptive case study design in which structured questionnaire was administered to 96 doctors, 170 nurses, 24 pharmacists and 40 medical laboratory scientists from randomly selected government hospitals and 90 doctors, 180 nurses, 30 pharmacists and 30 medical laboratory scientists from randomly selected private hospitals, all located in the 25 Local Government Areas of Delta State. The study was carried out between May and July 2013 after Ethical Approval was granted by the Ministry of Health, Delta State.

The commonest HAIs encountered are Urinary Tract Infection (61.4%) followed by Hospital acquired Pneumonia (55.6%) and Staphylococcus aureus (54.4%) for the government hospitals. In the private sector, Hospital acquired Pneumonia (66.1%) is the commonest. The study also showed that the frequency of occurrence of HAI is higher in government than private hospitals. This can be attributed to the higher population of patients leading to overcrowding. The modes of transmission of hospital acquired pneumonia was observed to follow this order airborne > contact with blood and body fluids > contaminated instruments > contaminated hands > needle sticks.

Conclusions
The prevalence of nosocomial infection in this state is very high. Efforts are needed to reduce it, improve the quality of life of patients and healthcare workers and improve healthcare delivery.
BACTERIAL BIODIVERSITY IN THE RICE RHIZOSPHERE AND HIGH-SALT SOIL AND SEDIMENT ENVIRONMENTS FROM THE CAMARGUE REGION (FRANCE)

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Background

The Camargue territory in the south of France is a delta triangle of 1500 km² of the Rhône River as it flows into the Mediterranean. This zone consists mostly of salty land inhabited by a diverse community of birds, wild white horses, black bulls and waterfowl, the most famous of which are pink flamingos. Rice production covers approximately 10,000 hectares which accounts for 30 percent of France’s rice consumption.

Objectives

We are examining the microbial composition of high salinity salt ponds, soils and the rhizosphere of rice plants in the Camargue region for two consecutive years (2013-2014) in order to establish a variable-temporal comparison regarding the physical and chemical properties of the environment versus the bacterial communities present in similar (saline) environments.

Methods

Bacterial biodiversity of five rhizosphere sites, growing two varieties of rice (Arelate and Gageron), plus sixteen sites of sediments and soils from nearly saline wetlands, were studied using pyrosequencing of PCR-amplified 16S V3-V4 rDNA amplicons from total extracted genomic DNA.

Conclusions

Our preliminary results revealed bacterial populations dominated by members of the Bacteroidetes and Proteobacteria phyla. However, in sandy soils with a lower degree of salinity than the 25 ppm, we observed, members from the Acidobacteria phylum representing an important fraction. Ongoing analyses of diversity (Chao1, Shannon and Simpson index) and statistics such as principal component analyses (PCA) will permit a detailed description of the bacterial populations among the different sampling sites, and better comparisons with similar types of sites worldwide.
Background
Petroliferous soil has unique environment due to the presence of a variety of aliphatic and aromatic hydrocarbons, creating a distinctive habitat for certain microbial communities. Although hydrocarbons are constituted only of two elements, relative to their electron acceptors; they are exploited as growth substrates by numerous microorganisms. Some aerobic bacteria, especially *Pseudomonads* are one of the most frequently isolated bacteria from hydrocarbon-impacted environments due to their reported metabolic pathways.

Objectives
The aim of this study was to determine the presence, abundance and the predominant culturable aerobic heterotrophic bacteria as well as *Pseudomonads* in 4 different production wells located in the Southeast Anatolia Region.

Methods
Petroleum samples were warmed to 40-60°C for a few hours until water-phase could be observed under oil phase. Diluted water-phase samples were inoculated onto Nutrient Agar, Tryptone Soy Agar, R2A, Cetrimid Agar and Pseudomonas Agar Base with and without salt. Petri plates were incubated at 30°C for 2 days (R2A for 5 days). Genomic DNA was extracted from the pure cultures using UltraClean Microbial DNA Isolation Kit. 16S rDNA gene fragments were amplified using U968F and L1401R primers. The amplified gene fragments were subsequently analyzed using sequencing.

Conclusions
It was determined that the number of aerobic heterotrophic bacteria and *Pseudomonads* was generally low/high (min. 9 cells/ml and max. 1358 cells/ml) in the oil reservoirs. 15 pure cultures were obtained from the samples. Among the cultured bacteria, *Acinetobacter guillouiae* (99.3%), *Halomonas daqingensis* (99.7%), *Suttonella indologenes* (93.1%) and *Pseudomonas stutzeri* (99.8%) were present.
EVALUATING THE RELATIVE INFLUENCE OF BIOTIC AND ABIOTIC FACTORS ON EFFICIENCY OF BIOAUGMENTATION MEDIATED EX-SITU BIOREMEDIATION

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Background

Soil and ground water pollution with xenobiotic chemicals is a major environmental concern. Bioremediation has been regarded as an efficient and environment friendly approach for restoration of contaminated niches. Amongst different bioremediation procedures, bioaugmentation is considered as one of the most potent approach for bioremediation. However, till date very few reports have shown successful application of this approach. It is often suggested that variety of biotic and abiotic factors e.g. indigenous microbial community, physico-chemical nature of contaminating pollutant and target soil may influence the efficiency and outcome of bioaugmentation process.

Objectives

To systematically address this issue, we performed a study for determination of relative influence of biotic and abiotic factors on efficiency of p-nitrophenol (PNP) degradation during bioaugmentation mediated ex situ bioremediation.

Methods

PNP degradation was monitored in microcosms prepared with soil samples collected from seven geographically distinct Indian provinces (viz., Assam, Andhra Pradesh, Gujarat, Karnataka, Maharashtra, Rajasthan and Tamil Nadu) having diverse physico-chemical character and native microbial communities. Soil samples were spiked with 70 ppm of PNP and subjected to bioaugmentation using pre-grown cells of degradative strain.

Conclusions

Quantification of residual PNP showed apparent enhancement of pollutant degradation; however, kinetics of pollutant removal varied significantly as a function of soils properties. Results from statistical analysis of PNP degradation patterns and matrices generated with microbial community and physico-chemical characteristic of soil highlighted relatively strong influence of indigenous bacterial community on biodegradation efficiency. It was observed that soil microbial community renders such impact by differentially modulating the survival of bioaugmented strain.
AN O-NITROBENZOATE (ONB) SENSITIVE TRANSPOSON MUTANT OF ARTHROBACTER PROTOPHORMIAE STRAIN RKJ100 INDICATES FOR INVOLVEMENT OF N-ACETYLGUCOSAMINIDASE IN BACTERIAL RESISTANCE TOWARDS ITS ELEVATED CONCENTRATIONS

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Background

Microorganisms capable of thriving under extreme environments are of great significance for basic as well as applied studies. Consequently, microorganisms showing survival under extreme environments have attracted considerable interest. Several studies have shown isolation and characterization of extremophilic microorganism. However, relatively fewer studies have reported isolation and characterization of microorganisms from niches characterized by elevated concentration of anthropogenic, xenobiotic environment pollutants. Previously we had characterized Arthrobacter protophormiae RKJ100 for its ability to tolerate extremely high concentrations of o-nitrobenzoate (ONB), a toxic xenobiotic environmental pollutant.

Objectives

Main objective of present study was to characterization of gene(s)/ gene luster(s) involved in resistance of strain RKJ100 towards elevated concentrations of ONB.

Methods

Random transposon mutants of strain RKJ100 were generated and screened for ONB sensitive phenotype. A sensitive mutant was defined as one which exhibits sensitivity towards ONB at ≥30 mM. Molecular and biochemical characterization of this mutant was carried out to identify the gene disrupted due to the transposition.

Conclusions

Results from the characterization of ONB sensitive mutants showed disruption of endo-β-N-acetylgucosaminidase (ENGase) gene. ENGase is a non-essential enzyme involved in oligosaccharide processing and cell wall recycling many life forms. It has been shown to have role in cellular homeostasis. Results obtained
during this study present first evidence for its role in bacterial resistance towards elevated concentrations of toxic xenobiotic compound.
Background

Bisphenol A (BPA) is a widely used chemical in the production of plastics and resins. Tetrabromo-BPA (TBBPA), the BPA derivatives with bromide atoms, is one of the most commonly used brominated flame retardants. The broad distribution of BPA-like pollutants in the environment raises great concern because of their endocrine-disrupting characteristics and toxicity.

Objectives

Developing cheap and low environmental impact remediation methods for BPA-like pollutants is emergent. Therefore, the biodegradation abilities of diverse microorganisms toward BPA and TBBPA were investigated.

Methods

The microcosm from acclimated sediment culture was used to evaluate the biodegradation ability of diverse microorganisms and isolate pure degraders.

Conclusions

The microcosm completely removed BPA (10 mg L\(^{-1}\)) within 28 h and transformed the contaminant into several metabolic intermediates. During the degradation process, the microbial composition fluctuated and finally restricted to \textit{Pseudomonas knackmussii} and \textit{Methylomonas clara}. The four isolated BPA degraders were all genetically similar to \textit{P. knackmussii}. Although the degradation ability of mixed strains was higher than that of single strain, it was far less than that of the original microcosm. These results demonstrated the novel role of \textit{P. knackmussii} in BPA degradation, as well as the crucial role for microbial diversity in pollutant decomposition. From the acclimated sediment culture, a novel TBBPA degrader was isolated. It degraded 70 % of the pollutant (4 mg L\(^{-1}\)) within 5 days and transformed the pollutant to one degrading by-product. Our results are helpful to understand the natural attenuation processes of BPA-like endocrine disruptors, as well as to remediate those structural similar pollutants.
PRESENCE OF P. SHIGELLOIDES IN TEMPERATE CLIMATE ZONE LAKES

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Background

*Plesiomonas shigelloides* is a Gram-negative rod-shaped bacterium found in aquatic environments. Most of the reports on its isolation are from tropical or subtropical areas. Furthermore, the bacterium is considered as an emerging opportunistic pathogen.

Objectives

Aim of this study was to detect presence of *P. shigelloides* in lakes situated in temperate climate zone (Central Europe).

Methods

Total of 42 water samples taken from Vojvodina, i.e. northern Serbia and southern Hungary lakes were examined. Some samples were taken from same locations but at different time intervals, so all isolates were obtained from geographically and/or chronologically different sources. The samples were spread directly onto Inositol Brilliant Green Bile Agar and incubated at 37 °C for 24 hours. The colonies of inositol fermenting bacteria were randomly selected, subcultivated and several preliminary tests were performed (Gram staining, catalase and oxidase test, indol production, gelatin hydrolysis and citrate utilization test). *P. shigelloides* was additionally characterized and identification was confirmed by PCR using specific primers (Ref, god). Additionally, RAPD analysis was performed to estimate genetic relationships among the isolates using 5 different primers.

Conclusions

The results indicate the frequent presence of bacteria in the waters in moderate climate, since *P. shigelloides* was isolated from 81% of the samples. RAPD analysis revealed distinct DNA fingerprint patterns for each *P. shigelloides* strain. The results confirm high presence of *P. shigelloides* in temperate climate zone lakes in Central Europe, which is probably a result of global climate changes. In addition, *P. shigelloides* can represent a potential health concern in this region.
THE SOIL MICROBIOME: INFLUENCE OF THE DECADES-LASTING INDUSTRIAL PRODUCTION OF BETA-LACTAM ANTIBIOTICS

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Background

Factory sites, wastewater treatment plants or hospitals are representative environments containing high concentrations of xenobiotics. β-lactam antibiotics (BLA) in these environments could directly exert a selective pressure on bacteria, resulting in spontaneous mutations or horizontal transfer of genetic elements among them. These genetic changes could pose a threat to human health, if these bacteria become multiresistant pathogens and migrate to the clinical settings. Our knowledge about the taxonomic composition of bacterial consortia from BLA contaminated environments is scarce.

Objectives

To investigate and characterize bacterial community subjected to long-term selection pressure of BLA, soil samples from the factory site of a pharmaceutical company, which has been producing β-lactam antibiotics for more than 50 years, were taken, analyzed, and taxonomic compositions were compared to those obtained from non-polluted soils of the same area.

Methods

In this study we assessed the microbial diversity on three different sites among the factory campus and two control sites (approx. 5km from the factory) using tag-encoded 454 pyrosequencing of the 16s rRNA genes.

Conclusions

The selection pressure of BLA did not reduce the diversities of bacterial consortia compared to those in the reference samples, although composition of the dominant species was different. The data from culture dependent study showed that the overall resistome of the polluted soil communities was boosted not only in terms of
resistance to BLAs but also within the tetracycline, macrolide, chloramphenicol, or aminocyclitol class of antibiotics.

The data demonstrate a very strong selective pressure imposed by anthropogenic activities on the transfer of resistance determinants among bacteria.
Background
Laccases are multicopper oxidases widely distributed in nature and catalyze the transformation of aromatic and non-aromatic compounds with reduction of molecular oxygen to water. We cloned the syntetic optimized genes, \textit{GlLCC1} and \textit{POXA1B} from \textit{Ganoderma lucidum} and \textit{Pleurotus ostreatus} respectively in \textit{Pichia pastoris}. In expression experiments we obtained enzymatic activities of 451.08±6.46 UL\textsuperscript{-1} and 0.13±0.028 UL\textsuperscript{-1} for \textit{GlLCC1} and \textit{POXA} 1B respectively. We are planing to employ recombinant laccases for the degradation process of kraft pulping or dye removal from effluents.

Objectives
Objective was to improved the enzymatic activity of each cloned genes by improving the culture media composition.

Methods
We use a Plackett-Burman statistical design to evaluate media volume, inocula percentage, copper, glucose, NH$_4$SO$_4$, peptone and yeast extract concentration, each one of them with two levels, to detect the positive or negative influence and contribution percentage of each one.

**Conclusions**

In both cases we improved the enzymatic activity by optimizing culture media composition. In the case of *GlLCC1*; treatments 1 and 9 surpassed 1.5 UL$^{-1}$; which exceeds the maximum activity obtained in previous trials 0.13 UL$^{-1}$; being treatment 1 which achieved higher laccase activity 4.69 UL$^{-1}$ meaning an increase of 36.07 times. In the case of *POXA 1B*; treatments 4 and 11 surpassed 1200 UL$^{-1}$; exceeding the maximum activity obtained in previous trials 451.08 UL$^{-1}$; being treatment 11 which achieved higher laccase activity 1373.72 UL$^{-1}$ meaning a 3-fold increase enzyme activity. For both laccases genes the culture time of *P. Pastoris* was 168 hours.
Sequential Statistical Designs for Improvement a Recombinant Laccase Production in Pichia Pastoris Immovilized Cells

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Background

We cloned the syntetic optimized gene, POXA 1B from Pleurotus ostreatus in Pichia pastoris. In expression experiments we obtained an enzymatic activities of 451.08 ± 6.46 UL⁻¹. We are planning to employ a packed bed column, with the recombinant immobilized cells for dye removal from effluents.

Objectives

Objective was to improved the enzymatic activity of recombinant POXA 1B by improving the culture media composition in a bath culture of immobilized cells of the recombinant strain.

Methods

We use a Plackett-Burman statistical design to evaluate media volume, copper,
glucose, NH₄SO₄, peptone and yeast extract concentration, each one of them with
two levels, to detect the positive or negative influence and contribution percentage of
each one. After that, a Box-Behenken design allow us to optimize the more influent
factor by analyzing the three levels of factor interaction through a response surface.

Conclusions
We improved the enzymatic activity from 14.4U/L at 156h (first immobilized cell
assay) to 1300U/L at 168 h of immobilized cells bath culture (after statistical)
optimization, meaning a 90.3-fold increase enzyme activity at 168 hours. The
recombinant activity of the recombinant enzyme POXA 1B produced by using
immobilized cells; exceed the maximum activity obtained in previous free cells trials
451.08 UL⁻¹; meaning a 2.88-fold increase enzyme activity at 168 hours.
MICROBIOLOGICAL STUDY OF SEWAGE SLUDGE COMPOSTING USING MICRO-POREOUS LAMINATE MEMBRANE COVER SYSTEM TECHNOLOGY

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Background

Treatment of wastewater involves generation of large amounts of solid wastes worldwide, known as sewage sludge. Composting of sewage sludge from wastewater treatment plants (WWTPs) is considered a successful strategy for its sustainable recycling.

Objectives

The aim of this study was to investigate the bacterial community structure and population dynamics during composting, using both cultivation-dependent and cultivation-independent techniques, to gain knowledge contributing to the improvement of the efficiency of the process.

Methods

The composting process was conducted during 180 days in an industrial composting plant. Samples were retrieved from a full-scale pile with semi-permeable Gore-tex covers, in combination with an air insufflation system. Waste sludge from an urban WWTP was mixed with vegetal pruning wastes as bulking agent. Microbial characterization included: enumeration of viable (platables) cells, measurements of enzyme activities, phylogenetic identification of isolates by 16S-RNA gene sequencing, and characterization of bacterial communities by a next-generation, Illumina-based sequencing approach.

Conclusions

Composting of sewage sludge under microporous-laminate membrane covers enhanced the degradation process, compared to other composting technologies. Changes in enumeration of cultivable microbiota took place during the 15 first days of composting. High activities of enzymes dehydrogenase, protease and arylsulphatase were observed during the first five days, suggesting a high rate of substrate degradation. Pyrosequencing data demonstrated that bacterial diversity was strongly influenced by the stage of the process. The greatest bacterial diversity was observed between days 15 and 30. These results suggest that composting under microporous-membrane covers can improve the process performance and reduce the operating cost.
COMMUNITY COMPOSITION AND DIVERSITY OF N FIXING CYANOBACTERIA ASSOCIATED WITH MOSSES IN SUB-ARCTIC ECOSYSTEMS

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Background

Moss associated cyanobacterial communities (MAC) are thought to be the major drivers of the Nitrogen (N) budget at high latitudes. Most studies have been carried out in the boreal forest and the High Arctic, whereas biological N fixation in other moss-rich regions e.g. sub-arctic alpine ecosystems may also be largely MAC-based.

Objectives

To evaluate diversity, abundance and N fixation activity of cyanobacteria associated with the four moss species *Racomitrium lanuginosum*, *Hylocomium splendens*, *Pleurozium schreberi* and *Sanionia uncinata*, all abundant in Icelandic ecosystems.

Methods

Moss samples were collected from three moss-dominated ecosystems in Iceland. Cyanobacterial identification and quantification was carried out by phase-contrast, fluorescence and confocal scanning microscopy. Estimation of cyanobacteria relative abundance was performed by amplification and sequencing of *nifH* genes. N fixation was assessed with the acetylene reduction assay (ARA).

Conclusions

The cyanobacterial strains identified appeared to be from the orders *Stigonematales* and *Nostocales*. N fixation varied over time, also it was responsive to microclimatic/micro-topographic gradients. Simulated climate warming and grazing negatively affected N fixation activity. Moss water content and type of vegetation were the most influent parameters on potential N₂ fixation activity. Our finding about MAC in moss-widespread sub-arctic ecosystems may have substantial impact on the understanding of the N cycle in this terrestrial environment.
CHARACTERIZATION OF BACTERIAL COMMUNITY ASSOCIATED WITH THE DEGRADATION OF PIG CARCASSES

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Background
Foot-and-mouth disease virus causes a highly contagious vesicular disease of domesticated and wild ruminants and pigs. A significant volume of infected livestock carcasses should be disposed of safely, economically and environmental-friendly.

Objectives
Composting is one of strategies for the safe disposal of infected livestock carcasses. In this study, the bacterial community associated with the degradation of pig carcasses was characterized in the composting process of them.

Methods
The compost bin was constructed with concrete frames for the walls and floor. Two-layer stainless steel grids were installed to put the carcasses of 4 pigs (80~90 kg/individual) in the bin. The bin was filled with well-mature compost mixture. To collect compost sample, perforated stainless steel tubes, which have been specially designed to collect sample without disturbance, were installed in the bin. Bacterial community was analyzed using 16S rDNA-PCR and pyrosequencing.

Conclusions
The results of volatile organic compounds (VOCs) monitoring indicated that the easier degradable fractions of pig carcasses decomposed during initial 50 days, and most of pig carcasses decayed during 170 days. The VOC concentrations at the top layer of compost bin were lower than VOCs at the bottom, indicating the top layer of compost can play a role as biofilter for the removal of malodor gases. Dominant bacteria at the initial stage were Alcanivorax, Aquimaria, Atopostipes, Bacillus, Cerasibacillus, Pseudomonas, Psychrobacter, Terribacillus and Ulvibacter. At the active degrading period of pig carcasses, Alcanivorax, Bacillus, Clostridium, Ignatzschineria, Lentibacillus, Pelotomaculum, Sporanaerobacter and Tissierella were dominated. Caldicoprobacter, Clostridium, Sedimentibacter, Syntrophaceticus and Virgibacillus were followed during final composting period.
Background

Forested wetlands are interfaces between terrestrial and freshwater systems and play an important role in the hydrology and nutrient filtration, acting as carbon sinks and in biodiversity regulation and maintenance. Also, despite microbial biodiversity in these systems is high, the biotechnological potential of this microbial diversity has been almost ignored, particularly yeast communities.

Objectives

The main purpose of this study is to evaluate the potential exhibited by yeast isolates in decolorizing different azo dyes, for future use in bioremediation.

Methods

In this work, wetlands have been screened for wild yeast isolates exhibiting ability to degrade five different azo dyes, a major group of synthetic dyes highly recalcitrant to current degradation processes. From the universe of isolates selected based on different colonial morphologies, 92 yeast isolates were randomly chosen for assessing their decolorizing abilities. Hierarchical clustering, based on medium color changes during incubations, were used to evaluate differences in the performance of azo dyes decolorization.

Conclusions

Yeast isolates differed markedly in their ability to decolorize the tested dyes. Among the best eleven isolates selected and identified to the species level by molecular methods, the basidiomycetous yeasts Cryptococcus laurentii AGG729 and Cryptococcus podzolicus AGG691, showed the highest dyes removal capacity. Our
results pointed out that yeasts occurring in forested wetlands, have potential to be used in biotechnological processes, being azo dyes bioremediation a case in point.

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CHARACTERIZATION OF MICROBIAL COMMUNITIES WITHIN PETROLEUM REFINERY WASTES AND THEIR CATABOLIC POTENTIAL IN BIOREMEDIATION

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Background
Autochthonous microbial communities within the highly hazardous petroleum contaminated sites could play significant role in in situ bioremediation. Unlike oil spill and other hydrocarbon contaminated environments, microbial diversity and their remedial potential is least studied within petroleum refinery wastes.

Objectives
During the present study microbial diversity and their catabolic potential relevant for in situ bioremediation of petroleum refinery waste were studied.

Methods
Gas chromatography mass spectrometry, ICP, and other standard methods were used to characterize the waste sludge. Orion multiparameter meter was used to determine DO, pH, ORP, temperature on site. 16S rRNA gene sequences were obtained from metagenomes through Illumina (GAIIx) sequencer. Clone library based analysis was done to reconfirm the archaebacterial populations. 65 bacterial strains were isolated through plating on R2A medium. BIOLOG eco plates were used to ascertain community level physiological profiles.

Conclusions
Dominance of anaerobic, moderately thermophilic, fermentative, sulphate-, iron-, nitrate- reducing, bacterial genera like Fervidobacterium, Coprothermobacter, Thermodesulfovibrio, Deferrribacter, Nitrospira, Anaerolinea, Thermoanaerobacterium, etc. was observed. Presence of methanotrophic and methanogenic, acetoclastic, hydrogenotrophic, archaea (Methanoseata, Methanolinea, Methanocella, etc.) was confirmed by both NGS and clone library analysis. Bacterial strains isolated from the samples showed their affiliation to genera Pseudomonas, Bacillus, Burkholderia, Enterobacter, Kocuria, Microvirgula, Pandoreae etc. The isolates were screened for their ability to grow in presence of various hydrocarbons (Naphthalene, Dodecane, etc.) crude oil, oily sludge, biosurfactant production, varied temperature (15°C - 45°C), pH (3.0 - 9.0) and heavy metals. The overall study illustrated microbial community structure and their potential in survival and hydrocarbon biotransformation, which may be exploited for in situ bioremediation strategies.
Background
Freezing of water above -36°C is based on ice nucleation activity (INA) mediated by ice nucleators (IN), and biological particles are a potentially important source of IN in the atmosphere. INA of the fungal genus *Fusarium* was already described about 30 years ago and INA of *Fusarium* as well as INA of other fungal genera is assumed to be mediated by proteins or at least to contain a proteinaceous compound. However, to date the precise INA machinery of *Fusarium* remains unidentified.

Objectives
In this study we use analytical technics to identify INA protein in surface protein extract from *Fusarium acuminatum*. The ultimate Goal is to elucidate the biological and environmental impact of fungal IN.

Methods
We use liquid chromatography, customized freezing assay, SDS-page, mass spectrometry and molecular methods to subsequently analyze the fungal IN machinery.

Conclusions
Preliminary results show that the INA protein of *F. acuminatum* is contained in the early size exclusion chromatography fractions indicating a high molecular size. Moreover we could identify a single protein band from IN active fractions at 130-145 kDa corresponding to sizes of IN proteins from bacterial species. To our knowledge this is for the first time an isolation of a single protein from *in vivo* samples, which can be assigned as IN active from *Fusarium*. 
POPULATION ANALYSIS OF BACTERIA IN A LOW TEMPERATURE WATER CATCHMENT SYSTEM

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Background

Drinking water supplies are based on groundwater resources all over the world. At some locations problems with higher concentrations of iron and manganese in groundwater can occur. The principle of subsurface iron and manganese removal are periodically injections of aerated water into an anoxic aquifer through a well, resulting in the precipitation of iron and manganese hydroxides. However, the precipitation of insoluble oxyhydroxides caused by chemical and microbial processes may result in the clogging of the aquifer and pumps.

Objectives

Bacterial communities of water wells from a water catchment system with subsurface iron and manganese removal were determined to identify key bacteria. Phylogenetic diversity and relative abundance of bacteria in water samples were compared between wells already in operation to those not yet operated.

Methods

To estimate well-clogging, qPCR with universal primers was performed for all samples and specific primers targeting (i) Rhodoferax spp., (ii) Crenothrix polyspora and (iii) Gallionella spp. were applied. Furthermore, PCR-DGGE technique and 454-pyrosequencing were used to observe bacterial alterations within water samples.

Conclusions

Our results showed that wells in operation contained a higher alpha-diversity, and bacterial communities were dominated by Rhodoferax and Methylotenera. Abundant groups in not yet operated wells were Gallionella spp. and Crenothrix polyspora. Redundancy analyses were performed to determine a relationship between samples and environmental factors. The parameters nitrate, iron(III) oxide, iron(II) oxide and ammonia create differences between bacterial communities in operated and not yet operated wells.

The diversity and composition of native microbial populations in water samples differed fundamentally from those influenced by operation.
MARINE HYDROCARBONOCLASTICUS BACTERIA AS WHOLE-CELL BIOSENSORS FOR N-ALKANES

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Background

Whole-cell biosensors are useful systems for the in-situ monitoring of seawater for hydrocarbons derived from oil spills. Currently available biosensors for hydrocarbons show limitations derived from the low solubility in water of hydrocarbons or the high ionic strength of seawater.

Objectives

We compared the performance of a biosensor system for the detection of short chain alkanes in seawater, hosted in either E. coli (commonly used in many whole-cell biosensors but not optimized for alkane degradation) or different marine hydrocarbonoclastic bacteria specialized in assimilating alkanes.

Methods

A set of reporter strains containing a plasmid harbouring the reporter circuit was constructed. Fluorescence assays were carried out to study the time-dependent response of the reporter strains to different alkanes at different concentrations, and the response of reporter strains to real samples such as seawater contaminated with petrol or crude oil.

Conclusions

Some of the marine hydrocarbonoclastic bacteria tested performed better than E. coli. These strains showed very good response to seawater containing pure alkanes, petrol and crude oil in just 2 hours. Alcanivorax borkumensis proved to be the best host for the biosensor system.
HOW THE PRESENCE OF ORGANIC POLLUTANTS AND METALLOIDS CAN INFLUENCE DIBENZOTHIOPHENE -DEGRADING BACTERIAL STRAINS

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Background
In an oil polluted site, several components of environmental concern can be found including Polycyclic Aromatic Hydrocarbons (PAH), Dibenzothiophene (DBT) and metals. Studies showed that the naturally occurring soil bacteria are able to transform them to safe end products (bioremediation).

Objectives
1- To find tolerance of *Burkholderia fungorum* DBT1 (B.DBT1) and *Burkholderia fungorum* 95 (B.95) to DBT and PAH mixture and in planktonic and biofilm forms
2- Evaluating the growth and transformation ability of these strains in presence of selenium and tellurium as sample inorganic pollutants.

Methods
The growth as biofilm in 96 well plate was established for both strains using Calgary Biofilm Device (CBD) with the method described by Ceri et al., 1999. Growth curves were drawn based on CFU counting on YMA plates. Biofilm photos were taken by Confocal Microscopy. Selenite and tellurite reduction efficacy assessment was performed based on protocols described by Kessi et al, 1999 and Turner et al, 1992 respectively.

Conclusions
Both strains can tolerate high concentrations of DBT (2048 ppm) in both planktonic and biofilm forms while the mixture of PAH can only be tolerated by planktonic form for both strains. B.95 is able to tolerate the maximum used concentration of selenite (2 mM) and transform more than half amount to elemental selenium. On the other hand B.DBT1 can only convert 0.5 mM of selenite, while is able to transform completely initial 0.1 mM concentration of tellurite to elemental tellurium.
Background
Vinasse showed to be appropriate substrate for yeast growth in single culture. The use of vinasse as a substrate for microbial biomass production can be an alternative for the treatment and reuse of this waste.

Objectives
The objectives of this study were (i) to evaluate the biomass production by Bacillus subtilis and two strains of Saccharomyces cerevisiae in mixed culture using the vinasse as a substrate, (ii) investigate the nutritional quality of the product generated, (iii) to evaluate the effect of biological treatment on physico-chemical parameters and level of toxicity.

Methods
B. subtilis (CCMA 0087), S. cerevisiae (CCMA0137) and S. cerevisiae (PE2) were initially grown in 100 ml vinasse medium (49% v/v vinasse, 49% distilled water, 1% glucose and 1% yeast extract). The microorganisms grown in a consortium, two by two, and each consortium was conducted in three different cell concentration. During the incubation period were monitored the cell number and pH. After that, the dry biomass and the reduction in Chemical oxygen demand (COD) and biochemical oxygen demand (BOD) of treated vinasse was evaluated.

Conclusions
Based on these results, the best combination and concentration of microorganisms was selected for cultivation in bioreactor. During the incubation period the bioreactor same previous parameters were monitored. In addition to the COD and BOD, the treaty vinasse was evaluated for acute phytotoxicity. The data obtained showed that the two strains of S. cerevisiae in mixed culture with an initial population of $10^7$ cells/mL showed 176mg/L of biomass and reducing the power polluter of vinasse.
DISTRIBUTION AND GENOMIC ADAPTATION OF METHANOMASSILIICOCALES ECOTYPES IN WETLANDS AND ANIMAL INTESTINAL TRACTS


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Background
Methane (CH₄) is a very potent greenhouse gas, mainly produced by methanogenic archaea as an endproduct of their anaerobic respiration. Methanogenic archaea are phylogenetically diverse and living in a broad range of anaerobic environments, among others, in wetlands and ruminant animals, which represent the major natural and anthropogenic CH₄ sources. Recently, a novel order of methanogens, the Methanomassiliicoccales, was discovered.

Objectives
We aimed to assess the environmental distribution of Methanomassiliicoccales in different wetlands (arctic and acidic/neutral temperate peat) and animal intestinal tracts. Additionally, a metagenomic characterisation of Methanomassiliicoccales enrichments was intended.

Methods
Screening for Methanomassiliicoccales 16S rRNA and mcrA genes showed that Methanomassiliicoccales are widely distributed in wetlands and animal intestinal tracts. Phylogenetic analyses of 16S rRNA genes revealed two distinct animal and wetland clusters, pointing to two fundamentally different ecotypes. Ruminant enrichment cultures grow in an anaerobic medium supplemented with trimethylamine and H₂ as substrates. Methanomassiliicoccales represented one major methanogenic group in the bovine rumen performing H₂-dependent methylotrophic methanogenesis. Two partial Methanomassiliicoccales genome bins assembled from rumen metagenomes represented the two ecotypes and enabled the characterisation of the underlying genomic basis of their adaptations. Ecotype differences were reflected e.g. by the smaller genome size of the animal-associated ecotype as well as a higher abundance of genes for oxidative stress response in the soil ecotype.

Conclusions
Our findings suggest that Methanomassiliicoccales represent a novel ecologically important group of methanogens with distinct animal and soil associated ecotypes adapted to these different environments. Especially the animal-associated ecotype potentially plays an important role in global CH₄ formation.
Ozonation for the Reduction of the Microbial Load of Wastewaters


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Background

Treated municipal wastewater contains microbial densities up to $10^6$ CFU/100mL. Ozonation is an oxidation process widely used to disinfect water due to the strong ozone biocidal properties.

Objectives

This work aimed at assessing the effectiveness of ozonation on the reduction of the microbial load of treated wastewater.

Methods

A synthetic wastewater containing a mixture of fungi and bacteria, including antibiotic resistant and endospore forming strains, was submitted to ozonation assays performed in a 1L lab-scale reactor, with contact periods of 15, 30 and 60 min. The treatment efficiency was assessed based on the microbial enumeration and quantification of 16S rRNA, and selected indicator genes, before and after ozonation and after three days of storage at room temperature.

Conclusions

Ozonation for 15 min reduced the total heterotrophic bacteria and fungi by 3 log and 1 log, respectively. Coliforms and enterococci were extensively removed, being detected at densities of about 5 CFU/100mL. However, 3 days after water ozonation, a strong recovery was observed, with total heterotrophs reaching $10^9$ CFU/100mL and fungi $10^2$ CFU/100mL. Total coliforms and enterococci were still detected after 3 days. After 30 and 60 min of ozonation, viable cell counts were below the detection limit (1 CFU/100mL) and no recovery was observed after 3 days. Using culture independent methods, it was possible to confirm those results, being observed significant reductions of selected indicator genes after 15-30 min treatments. However, both 16S rRNA and $bla_{TEM}$ genes, but not $vanA$, were detected after 3 days recovery.
MIGRATORY HABITS, AREA AND HABITAT INFLUENCE THE MICROBIOTA OF BIRDS IN THE DANUBE DELTA NATURAL RESERVE

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Background
Wild birds from aquatic habitats could, as reservoirs and vectors for antibiotic resistant bacterial pathogens, directly affect the health of animals and pose serious risks to residents and tourists, the microbial load and its variety increasing with the broadening of the habitat and depending on the migration area.

Objectives
The research aimed to monitor the variation and distribution of pathogenic bacteria in birds with different migratory habits, areas and habitats in the Danube Delta.

Methods
A total of 112 rectal and oro-pharyngeal swabs from 26 species of migratory and sedentary birds from the Danube Delta Natural Reserve were collected and subjected to classical cultivation or enrichment techniques. 113 strains were identified on Chromogenic UTI medium BrillianceTM and TCBS Cholera medium (Oxoid) as belonging to Vibrio, Pseudomonas, Proteus, Escherichia, Staphylococcus and Enterococcus genera. Pathogenic bacteria were prevalent in migratory versus sedentary birds, with alarming proportions of Vibrio spp. (23.89%), followed by E.coli/coliforms (21.26%) (table 1):

Bacterial pathogens (%) isolated from cloacal and oro-pharyngeal specimens collected from wild bird species in the Danube Delta Natural Reserve (Table 1).
Conclusions

Thus, the bacterial load but not the spectrum of isolated species is influenced by migration site and habits rather than habitat.
BIODEGRADATION OF PERCHLOROETHYLENE AND TRICHLOROETHYLENE UNDER AEROBIC CONDITION
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Background

Tetrachloroethene (also known as perchloroethylene; PCE) and trichloroethylene (TCE) are highly chlorinated hydrocarbons, are the common groundwater contaminates in industrial areas. Dechlorination of hydrocarbons is a complex process and biological dehalogenation under aerobic condition also required more clarification.

Objectives

The hydrocarbon degrading bacterial communities produces mono/di oxigenase enzymes and has the potential to degrade the chlorinated hydrocarbons with co-metabolic pathways. Soil samples were collected from contaminated site and enrichment of specific microbial communities were obtained using methanol and toluene as sole carbon source.

Methods

The enrichment cultures Ui-mix and MEOH-1 were examined for the ability to degrade TCE and PCE under aerobic condition. Growth and degradation rate are examined using spectrophotometer and gas chromatography equipped with ECD. The colonies on the enrichment cultures are isolated, purified and identified based on the 16S rRNA gene sequencing.

Conclusions

We have developed two consortiums which can degrade the PCE and TCE completely in aerobic condition. Ui-mix and monocultures from the consortium showed highest degradation effectivity to co-metabolize TCE along with toluene as sole carbon source. Similarly, the MeOH-1 and monocultures degraded PCE under aerobic condition. The consortium Ui-mix contain diverse group of bacteria incudes Chryseobacterium, Pseudomonas, Enterobacter and Microbacterium; similarly Pseudomonas, Hyphomicrobium, Cupriavidus, and Microbacterium are isolated from MeOH-1. The strains M3-1, M3-3 and M3-7 showed 98.4%, 95.3% and 94.5% removal efficiency of TCE and PCE within 20 days, respectively. Further studies have to be performed to optimize and enhance the removal efficiency and it gives a gateway for the bioremediation of chlorinated ethane.
METHANOGENIC ACTIVITY AND DISTRIBUTION OF THE METHANOMICROBIALES IN TRANSITIONAL BOG AND SWAMP FOREST PEAT PROFILES (POLESKI NATIONAL PARK, POLAND)

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Background

Peatlands are the largest natural carbon storage. Under good aeration conditions carbon is released as a CO₂ but when there is lack of oxygen, anaerobic brake down of organic matter take place and CH₄ release occur. In most peatlands with neutral pH, the main substrate of CH₄ production is acetate while in acidic, the main precursors are CO₂ and H₂. CH₄ production is attributed to the activity of methanogenic Archaea, which, in peat soils are represented in majority by the members of the Methanomicrobiales.

Objectives

The aim of presented work were: (1) to examine which layers of the peat soil profiles (from the surface top to a mineral base) in transitional peat bog and swamp forest surrounding eutrophic Lake Moszne in Poleski National Park (East of Poland) show the highest methanogenic activity; (2) to test the presence of the Methanomicrobiales in investigated peat materials.

Methods

Methanogens were detected in fresh samples via FISH technique using MG1200 probe. Soil samples were further incubated anaerobically (at 5-30°C) and CH₄ production (gas chromatography technique) was controlled.

Conclusions

The highest methanogenic activity (up to 39.46 mg CH₄ kg dw⁻¹ d⁻¹) below layers 45 cm (transitional bog) and 150 cm (swamp forest) were found. In all of the investigated samples the presence of coccus, bacillus and curved rods from the Methanomicrobiales were confirmed. Among investigated, morphologically diverse order, the dominant role plays coccus (up to 100%) while the rarest were representatives of curved rods (up to 9%).
RESPONSE OF THE COALBED ROCK METHANOTROPHIC COMMUNITY TO TEMPERATURE CHANGES

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Background

Methanotrophic bacteria are ubiquitous in the environment and play an important role in the global carbon cycling. Recently published research revealed that in spite of severe oxygen limitation, aerobic methanotrophic species are also found in deep subsurface geological formations such as coalbed rocks. These rocks are excavated in huge amounts during coal exploitation and comprise a troublesome waste. The presence of the methanotrophic community creates an opportunity to reuse these materials for the purpose of methane biofiltration; however surface conditions vary much from these in situ, especially according to temperature, which in geological formations is stable (c.a. 30 °C).

Objectives

In the current study we investigated methanotrophic communities of the rocks originating from Upper Silesian (Poland) coal mines in terms of identity (16S rRNA sequences) and response to the temperature changes.

Methods

Assays were performed in hermetic microcosms where samples of 15 g of rock were incubated under mixtures of methane and air. Headspace gases were measured by means of gas chromatography. DNA was isolated using PowerSoil® DNA Isolation Kit (MoBio) and amplicons obtained with methanotroph-specific IF/IR, IIF/IIR primers.

Conclusions

The investigated microbial communities were heterogeneous and consisted of both type I and II methanotrophs. Their ability of to oxidize methane decreased along with temperature and in majority, yet not all, of the samples was inhibited at 10 °C. It was hypothesized that the potential of methane turnover in the coal bed rocks is related with identity of the methanotrophic inhabitants with type II methanotrophs (α-Proteobacteria) being responsible for methane oxidation at low temperatures.
UTILIZATION OF RHAMNOLIPIDS DURING BIOSURFACTANT-MEDIATED BIOREMEDIATION OF HYDROCARBON-POLLUTED SOIL

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Background

It was proven that rhamnolipids are environmental-friendly biosurfactants produced by Pseudomonas species. There are many studies dealing with rhamnolipids-mediated bioremediation of hydrocarbons. However, it must be mentioned that both the environmental fate and the influence of rhamnolipids on microbial communities remain unexplained. We hypothesize that rhamnolipids could be more readily utilized by soil microbial communities than hydrocarbons.

Objectives

1. to investigate and compare biodegradation rates of rhamnolipids and hydrocarbons.
2. to investigate the effect of rhamnolipids on quantitative and qualitative composition of microbial community

Methods

Biodegradation rates – HPLC-MS, structure of the microbial community – Illumina.

Conclusions

In most cases at least 80% of monorhamnolipids and dirhamnolipids were degraded during field experiment. The biodegradation rates of hydrocarbons was significantly lower compared with rhamnolipids. Rhamnolipids are more preferentially degraded by soil microorganisms than hydrocarbon compounds. Thus, we suggest that the role of rhamnolipids during bioremediation processes may be negligible.

Ławniczak Ł, Marecik R, Chrzanowski Ł, Contributions of biosurfactants to natural or induced bioremediation, Applied Microbiology and Biotechnology (2013), 97. 2327–2339.
CHARACTERIZATION OF A PSEUDOMONAS SP. ISOLATED FROM BRAZILIAN SOIL AND ITS ABILITY TO DEGRADE ATRAZINE.

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Background

Atrazine is a selective and systemic herbicide used for controlling weeds in several crops. It is considered an environmental pollutant due to its high potential to contaminate the surface water and groundwater. Pseudomonas sp. ADP, the model strain for atrazine biodegradation, mineralizes this herbicide using it as a nitrogen source.

Objectives

Characterization of a strain isolated from a Brazilian soil sample and its ability to degrade atrazine.

Methods

The soil sample was collected in Ribeirão Preto, São Paulo, and processed according to the method describe by Gargouri et al. (2013), with modifications. The strain was selected from ATZ-R medium, in accordance with the formation of clear zones around the colonies. The identification was performed by 16S rRNA sequence analysis. PCR reactions for detection of atz genes (atzA, atzB, atzC, atzD, atzE and atzF) was carried out according to the methodology of Devers et al. (2004) and, then, sequenced. Plasmidial DNA extraction was performed according to the alkaline lysis technique, previously described by Birboin & Doly (1979). The degradation test was perfomed in solid and liquid ATZ-R medium.

Conclusions

The strain was identified as Pseudomonas sp., presented all atz genes and a 35 MDa plasmid, however, only the atzA gene was detected in this plasmidial DNA. The strain was capable to mineralize this herbicide after 48 hours in solid medium, indicated by the clear zones around the colonies. The analysis of the ATZ-R liquid medium in spectrophotometer (225 nm) showed a significant decrease of atrazine after 48 hours of incubation.
THE MICROORGANISM THAT NEVER READ THE LITERATURE - FASTEST GROWING PHOTODAMAGE TOLERANT ALGA ISOLATED FROM DESERT CRUSTS, DEPENDS ON PIONEER FILAMENTOUS CYANOBACTERIA SPECIES TO SURVIVE DESICCATION

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Background
With the premise that unique capabilities can be found in microorganisms facing extremely harsh conditions, we focused our efforts on desert biological sand crusts (BSC), one of the harshest environments to support life. Recently, we have isolated a small green alga (named *Chlorella ohadii*), which does not conform to some of the basic fundamentals in microbial ecology and photosynthesis:

Objectives

1. Acquired ability to acclimate to extreme environments is usually accompanied by reduced performance under optimal conditions. We intend to show that *C. ohadii* does not obey this rule. In its natural habitat, *C. ohadii* is facing diurnal desiccation-hydration cycles, vast temperature amplitudes and extremely high illumination intensities, yet when grown under optimal laboratory conditions it exhibits the fastest growth rates ever reported for an alga.

2. After many years of research, some feel that we have elucidated the functioning of the photosynthetic machinery, and what sets the upper limit for algal growth. However, the unparalleled fast growth, extremely high photosynthetic rates and resistance to photodamage, suggest this may not be the case. Namely, *C. ohadii* has been shown to be completely resistant to photoinhibition, and its productivity was unaffected by irradiances as high as twice full sun light.

3. Survival of *C. ohadii* in its BSC habitat depends on close association with filamentous cyanobacteria; a unique and novel mode of interspecies interaction.

Methods

We wish to present detailed physiological analysis of the unique properties supporting this phenomenal growth and resistances, combined with insights from their genomic, transcriptomic and metabolomic characterization.

Conclusions
DETERMINING THE EFFECT OF SAMPLING VOLUME AND FLOW RATES ON INVESTIGATIONS OF BACTERIAL COMMUNITY DIVERSITY IN A LOW BIOMASS AQUATIC ENVIRONMENTS

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Background
As water travels from treatment plant through the pipelines to consumer’s taps, the diverse drinking water microbiome varies in abundance and in composition as a result of a large number of variables. A quantitative understanding of relevant variables is essential towards ensuring better control of drinking water quality. Increasingly, DNA sequencing approaches such as 454 pyrosequencing and Illumina MiSeq sequencing are being utilised to this end. Yet, little has been done to understand the influence of sample collection protocols on the data collected through DNA sequencing approaches. The ability to accurately understand the factors that affect the change in the drinking water microbiome is critically reliant on the robust approaches for sample collection.

Objectives
The goal of this study was to understand the effect of sample volume and sampling flow rate on the structure and membership of drinking water bacterial communities. Understanding these effects is especially critical in drinking water systems, which represents a low biomass but high diversity aquatic environment.

Methods
To do this, we sampled at five different residential sampling locations in the City of Glasgow, UK. This sampling was conducted such that five different sample volumes, ranging from 1 to 20 litres, were filtered to harvest microbial cells at each sampling location and this exercise was repeated at two different flow rates at the faucet (laminar and turbulent flow regimes).

Conclusions
We will provide a detailed overview of these two variables on the reproducibility and reliability of DNA sequencing based investigations of the drinking water microbiome.
PHYSIOLOGIC AND GENOMIC CHARACTERIZATION OF A NOVEL NITROSPIRA SPECIES ENRICHED UNDER ANAEROBIC, DENITRIFYING CONDITIONS
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Background
Members of the genus Nitrospira are dominant in many natural habitats and of vital importance for wastewater treatment. They are chemolithoautotrophic organisms capable of growth with nitrite and CO₂ as sole energy and carbon source. It further has been demonstrated that some Nitrospira can utilize simple organic carbon compounds and molecular hydrogen as alternative substrates and can switch to nitrate reduction under oxygen limitation. However, this metabolism has been assumed to be a survival strategy and in most systems studied so far Nitrospira was outcompeted by denitrifying organisms when anoxic conditions prevailed.

Objectives
Here, a community of nitrogen cycle bacteria was enriched from the anaerobic compartment of a biofilter connected to a recirculating aquaculture system.

Methods
The culture was fed with filtered water from the aquaculture system, supplemented with ammonium, nitrite and nitrate.

Conclusions
A stable enrichment culture was obtained which anaerobically converted ammonium, nitrite and nitrate into dinitrogen gas. The culture was dominated by anaerobic ammonium-oxidizing Brocadia species, a denitrifier (Denitratisoma), and a Nitrospira species. Anammox bacteria and Nitrospira co-aggregated, while the denitrifiers formed separate clusters. This culture shows that anaerobic ammonium oxidizers and nitrite oxidizers, who were considered to be mutually exclusive, can be grown as a stable co-culture. Furthermore, this shows that some Nitrospira species can be competitive under nitrate-reducing conditions. This study demonstrates another unexpected lifestyle for an organism believed to be only competitive under aerobic lithoautotrophic conditions. It further indicates that Nitrospira in combination with anammox can be of interest for anoxic wastewater treatment systems.
Background
Oxalate-degrading bacteria are known from a wide variety of ecological niches and different taxonomical origins. They play important roles in local and global carbon-cycling and, when present in the guts of mammals, they seem to provide protection against excess oxalate uptake and renal stones.

Objectives
In the present study, we aim to investigate the ability to grow at minimal medium with Ca-oxalate or Na-oxalate as a sole carbon source of 30 diazotrophic Azospirillum strains isolated from the rhizosphere of various field grown gramineous plants species grown at different geographical Greek locations, molecular characterized at our previous research works, and reference type strains. Most of these strains tested for the ability to grow at minimal medium with oxalate crystals isolated from Amaranthus blitum (family Amaranthaceae) as a sole carbon source. Additionally, we investigated for the presence of formyl-CoA transferase (frc) gene a molecular marker used for the specific detection and identification of oxalotrophic bacteria involved in oxalate degradation.

Methods
Minimal media with Ca-oxalate/Na-oxalate or oxalate crystals from plant were used as a sole carbon source for bacterial growth. Molecular biology techniques and phylogenetic analysis for the frc genes.

Conclusions
The majority of the examined strains display an ability to use oxalic acid as a sole carbon source. This result and the presence of the frc genes shows that these strains can metabolize oxalate via the oxalyl - CoA - decarboxylase pathway to produce formate and incorporate it in their metabolism.
DETOXIFICATION AND BIODEGRADATION OF ENVIRONMENTAL POLLUTANTS BY MARINE MICROBIAL CONSORTIA

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Background
The high industrial activity causes several environmental problems, mainly due to the discharge of wastes. Marine environments are susceptible to contamination by industrial waste and also may represent a target niche for microbial prospecting for bioremediation. Marine microorganisms are adapted to saline conditions and have potential for being used in many processes.

Objectives
To evaluate the potential of microbial consortia in detoxify and degrade environmental pollutants.

Methods
Microbial consortia were structured in different combinations using four ligninolytic fungi from marine environments, two bacteria from oil reservoir and two lipolytic yeasts from Antarctica, previously selected based on their capacity to produce enzymes and to degrade hydrocarbons. Erlenmeyer flasks containing 50 ml of mineral medium, the microbial consortium and the pollutant (RBBR 500 ppm and Diesel oil 1% vv) were kept in incubators for 7 (RBBR), 14 and 21 (diesel oil) days at 140 rpm and 28 °C. Samples were analyzed in Microtox for acute toxicity and ligninolytic enzymes (Lac, MnP, LiP) were quantified.

Conclusions
Consortium 7 showed promising results in the studies with RBBR dye, presenting discoloration up to 70% and a very low rate of mycelial adsorption (11%). Enzymatic production of Lac was up to 141 U/L. LiP and MnP producion were very low. All samples containing RBBR were detoxified. Samples containing diesel oil presented no detoxification and the production of ligninolytic enzymes was higher for MnP (114 U/L). A longer period of incubation and/or a lower concentration of oil will be tested, since this compound is highly complex.
EFFECTS OF DUST STORM EVENTS ON TUBERCULOSIS INCIDENCE RATE IN NORTHWEST OF CHINA

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Background

Tuberculosis (TB) is a major public health problem in China. Gansu has very high annual attendance rate of TB in China, and the province is also famous because of its severe dust storms. The epidemic timing starts in February and ends in July, and the dust storm mainly distribute throughout the spring and early summer, which strongly indicate a close linkage between causative agent of TB and dust storm events.

Objectives

We investigated the general impact of dust storms to TB across time by analyzing variation in weekly clinic visits in Gansu during 2005-2012 in relation with the climatic event.

Methods

We applied Mann-Whitney-Pettitt test and regression model to detect the seasonal periodicity of TB and dust storm in a time series, and assess relationships between the meteorological variables and weekly TB clinic visits.

Conclusions

By comparing the information on cases of TB of Gansu weekly reported with dust storm events, we found a clear link between population dynamics of the disease and the climate disaster: the onset of epidemics and the dust storm shared the almost same mean week. Particulate matter might be the direct reason for the outbreak of TB in dust storm days. To our knowledge, this is the first population-based study that provides a clear demonstration that epidemic of TB was affected by dust storm in China, which will help understanding the association between this environmental problem and the evolution of epidemic disease.
GENETIC INSIGHTS INTO THE SULFUR OXIDATION PATHWAY OF SULFUR CHEMOLITHOTROPHS LIVING IN FRESHWATER LAKES

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Background
Chemolithotrophic sulfur-oxidizing bacteria are capable of oxidizing inorganic sulfur compounds as sources of energy for growth. A number of genomic studies have been taken place focusing on sulfur oxidizers living in habitats where a sufficient amount of reduced inorganic sulfur compounds are available. These studies revealed that taxonomically diverse sulfur chemolithotrophs use different enzymes for the oxidation of various sulfur compounds. On the other hand, sulfur chemolithotrophs living in freshwater lakes, where the availability of reduced inorganic sulfur compounds is generally low, have been overlooked. Recently, we revealed the dominance of betaproteobacteria in the sulfur-oxidizing bacterial community of freshwater lakes, and isolated several of them in pure culture.

Objectives
The aim of this study is to gain genetic insights into the sulfur oxidation pathway of sulfur chemolithotrophs isolated from freshwater lakes.

Methods
The genomes of the new isolates were sequenced and then analyzed focusing on the composition and phylogeny of genes for sulfur oxidation. In addition, their genomes were compared with available complete genomes of other sulfur oxidizers.

Conclusions
As the first comprehensive comparative genomic analysis focused on sulfur chemolithotrophs from freshwater lakes, the results of our study revealed that they possess common sets of genes putatively involved in sulfur oxidation. One of these gene sets was shared only by freshwater sulfur-oxidizing betaproteobacteria.
Background

*Candidatus* Nitrotoga arctica is a cold adapted nitrite-oxidizing bacterium (NOB), which was cultivated from the Siberian Arctic. Since 2007, *Nitrotoga*-like NOB could be detected in different natural and technical environments e.g. beneath the Antarctic ice sheet, in cave systems, in wastewater treatment plants and in aquaculturing facilities. Thus, they are more distributed than previously assumed and of importance for the global nitrogen cycle as well as for a successful nitrification in wastewater processing.

Objectives

The aim of this project is to gain a deeper understanding of the phylogenetic diversity within the genus *Nitrotoga* belonging to the *Betaproteobacteria* and to compare representatives derived from natural and artificial ecosystems.

Methods

The 16S rRNA gene was analysed combining specific and semi specific primer pairs for Eubacteria and *Nitrotoga*. Additionally, the beta subunit of the key enzyme of nitrification in *Nitrotoga* and other NOB, the nitrite oxidoreductase B (*nxrB*), was sequenced.

Conclusions

So far, *Nitrotoga*-like bacteria from widespread habitats do not differ noticeably in their 16S rRNA gene sequence from each other, not even those from extreme or technical origin (similarity > 98%). Although they might possess various physiological features, different species cannot be recognized on basis of the 16S rRNA gene. A higher resolution was obtained by comparing the sequences of the gene *nxrB*. In contrast to the ubiquitous and phylogenetically ancient NOB genus *Nitrospira*, *Nitrotoga* does not seem to be very diverse so far. However, its high abundance in various environments makes it an important player in nitrification.
NAPHTHALENE DEGRADATION BY SULFATE-REDUCING MARINE COMMUNITIES

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Background
Polycyclic aromatic hydrocarbons (PAHs) are ubiquitously distributed to the environment through the extensive petroleum and chemical industries. These toxic chemicals are of concern because of continual, widespread release.

Objectives
The bicyclic hydrocarbon naphthalene undergoes aerobic and anaerobic transformations, however our understanding of the details of anaerobic naphthalene degradation lags behind that of aerobic processes.

Methods
Sediment was obtained from Tuckerton, NJ, where PAHs are introduced through nonpoint deposition to an otherwise relatively pristine ecosystem. Anaerobic enrichment cultures were established with 10% anoxic estuarine sediment under sulfate reducing conditions and amended with 500μM naphthalene as the sole carbon source. Naphthalene concentrations were monitored using GC/FID.

Conclusions
Naphthalene loss was observed in 139 days in primary enrichments, followed by 48 days in transfer cultures. Subsequent transfers receiving 30-50% active cultures are sediment free and degrade naphthalene within 21 days. Stoichiometric loss of 3mM sulfate per 500μM naphthalene is observed. In the presence of molybdate, which inhibits sulfate reduction, no naphthalene degradation occurs, linking sulfate reduction to naphthalene metabolism. Distinct bands unique to naphthalene degrading cultures are seen in 16S rDNA analyzed by DGGE, indicating the enrichment of naphthalene degrading bacteria. By incorporating ¹³C-Naphthalene into DNA and RNA via stable isotope probing (SIP), the active microbes responsible for naphthalene degradation can be determined. Additionally, the recovery of 2-naphthoyl-CoA-reductase from ¹³C-DNA as well as identification of ¹³C intermediates can help to describe the metabolic pathway. This investigation of a robust sulfidogenic marine enrichment culture advances our understanding of the diversity of microbes able to anaerobically degrade naphthalene.
BACKGROUND

Agriculture in Poland has been an important part of the country's economy (about 60% of Poland’s total area is used for crop cultivation). It is known, that agricultural types of soil contain much smaller number of microorganisms, in comparison with natural soils. Thus we hypothesised that agricultural soils are biologically degraded and not being able to become naturally regenerated which may lead to their inability of regaining the satisfactory level of fertility.

OBJECTIVES

The aim of the study was to indicate which type of crop: oat or triticale is more favourable for sustaining soil microbiological activity.

METHODS

Eight different soil units agriculturally used, and the same number of control soils not agriculturally exploited were studied. Soils were collected in April 2014, using Egner’s bow from the surface layer. Under laboratory conditions the following analyses were performed: pH, electric conductivity, total carbon, phosphorus and nitrogen content, microbial biomass (chloroform fumigation), respiration (GC), soil dehydrogenase activity (TTC) and DNA isolation (chloroform extraction method with CsCl₂ gradient). Spearman's ρ correlation coefficient was used to assess relationships between chemical and microbiological soil properties.

CONCLUSIONS

It was demonstrated that cultivated soils are biologically degraded, what was evidenced by lower values of all microbiological parameters in relation to control sites. Comparing crop type we found that triticale is more favourable for sustaining soils microbiological activity rather than oat. Determined positive correlations proved that pH, carbon, phosphorus and nitrogen content are the most important factors determining soil microbiological activities.

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ENVIRONMENTAL FACTORS INFLUENCED ON SOIL MICROBIOLOGICAL ACTIVITY*

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Background

Many of recent studies have focused on documenting how soil bacterial communities are affected by specific environmental changes or disturbances. It was indicated that vegetation type, carbon and nutrient availabilities, and soil moisture may influence microbial activity at local scales, whilst soil pH seemed to be a better predictor at the continental scale.

Objectives

The aim of the study was to determine effect of selected environmental factors: pH, redox potential (Eh) and total carbon (TC) on soil microbiological activity, expressed as microbial biomass (MB), DNA content, potential ammonification (PA) and most probable number (MPN) of ammonifying bacteria.

Methods

Fifth representatives of *Eutric Cambisols* collected in April 2014 from the surface layer of agricultural sites were investigated. Spearman’s rho correlation coefficient was used to determine relationships between environmental and microbiological soil properties.

Conclusions

Positive correlations among pH, TC and all microbiological properties were reported. pH was found to be proportionally related with MB (0.73**), DNA (0.84***), PA (0.65**) and MPN (0.73**). Analogical trend demonstrated TC, what was confirmed by values of r coefficients, as follows: MB (0.64**), DNA (0.78**), PA (0.76**), MPN (0.83***). In the case of Eh it was noted that its lower values have stimulating effect on microbiological activity in *Eutric Cambisols*, as evidenced by following assigned rho: MB (-0.69**), DNA (-0.83**), PA (-0.75**) and MPN (-0.82**). Among investigated environmental factors only impact of moisture have insignificant character (p>0.05) in relations to tested soil microbiological properties.
*Project was financed by the National Science Centre (Poland), granted on the basis of decision DEC-2013/09/D/NZ9/02482.
THERMOPHILIC METHANOGENS REDUCE AMORPHOUS Fe(III) OXIDES AND GENERATE CRYSTALLINE MAGNETITE ON CELL SURFACES

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Background
Methane production is affected by various environmental stimuli including the presence of Fe(III) oxides [1,5]. Inhibitory effect of Fe(III) oxides and Fe(III)-reducing ability in mesophilic methanogens has been reported [2,3]. There have been only limited the information about thermophilic methanogens.

Objectives
Thermophilic methanogens were investigated for their ability to reduce poorly crystalline Fe(III) oxides (ferrihydrite) and the inhibitory effects of ferrihydrite on their methanogenesis.

Methods
Methanothermobacter thermautotrophicus strain ΔH⁰, Methanosaeta thermophilia strain PT⁰, and Methanosarcina thermophilia strain TM-1⁰ and strain FE-1 were anaerobically cultivated in various methanogenic substrates supplemented media at 55 °C in the presence or absence of ferrihydrite. The amounts of methane and concentrations of Fe(II) were measured by gas chromatography and ferrozine method, respectively.

Conclusions
Fe(III) reduction was observed by the thermophilic methanogens only in the presence of H₂ as the reducing source [4]. While addition of ferrihydrite resulted in inhibition of methanogenesis, ferrihydrite reduction by the methanogens partially alleviates the inhibitory effects. Methanosarcina thermophilia generated magnetite particles on its cell surfaces through ferrihydrite reduction. These findings suggest that interaction between Fe(III) minerals and methanogens has significant impacts on materials and energy cycles in anoxic environments.
References
OPPORTUNISTIC PATHOGENIC YEASTS ON THE SURFACE OF FRUITS

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Background
Infections caused by treatment-resistant non-albicans \textit{Candida} species, such as \textit{C. tropicalis}, have increased. It has become an emerging challenge in managing fungal infections. Previous researches have showed that \textit{C. tropicalis} are opportunistic yeasts exist in environment.

Objectives
We are interested in whether the opportunistic yeasts may spread from environment into human body.

Methods
We isolated and characterized pathogenic yeasts on the surface of fruits from supermarkets. A total of 291 isolates of 83 species from 24 different types of fruits were recovered. We have determined their species and drug susceptibility.

Conclusions
Of the 83 species, 7 common pathogenic \textit{Candida} species were detected. They included 16 \textit{C. guilliermondii}, 15 \textit{C. famata}, 3 each of \textit{C. parapsilosis} and \textit{C. tropicalis}, 2 each of \textit{C. krusei}, \textit{C. lusitaniae} and \textit{C. orthopsilosis}. The drug susceptibilities of 162 of the 291 isolates were determined. Totally, 158 (97.5%), 104 (64.2%), and 102 (63%) isolates were susceptible to amphotericin B (MICs ≤ 4 mg/l), fluconazole (MICs ≤ 8 mg/l), and triadimenol (MICs ≤ 8 mg/l), respectively. One \textit{C. tropicalis} isolate (F91) from wax apple had MICs at 64 mg/l for both fluconazole and triddimenol. It belongs to diploid sequence type (DST) 149, a genotype found in isolates from human as well as soil. Hence extra caution shall be taken when providing fruits or juice to severely immunocompromised patients since drug resistant pathogenic yeasts may be on the surface of fruits.
Background

Microbial reduction of chalcogen oxyanions such as tellurite and selenite is promising for development of innovative biotechnological processes for applications in bioremediation, wastewater treatment and production of functional nanomaterials.

Objectives

Here, we demonstrate rapid and stable bioreduction of tellurite, for the first time, using unaclimated anaerobic granular sludge, obtained from an operating upflow anaerobic granular sludge blanket reactor treating paper mill wastewater. In addition, bioreduction of tellurite in the presence of selenite was determined.

Methods

Bioreduction experiments were performed in serum bottles under different growth conditions using anaerobic granular sludge as source of microorganisms. Bioreduction of selenium and tellurium oxyanions profiles were determined at regular time intervals. Biochemical and microbial characterization of selenium and tellurium oxyanions reducing granular sludge was determined.

Conclusions

Tellurite was rapidly reduced to brown and then to black colored elemental tellurium by anaerobic granular sludge. The reduction of tellurite occurred at a much faster rate than the selenite when supplied individually. Complete reduction of 1 mM of tellurite and selenite was observed in 8 and 96 h, respectively. When both tellurite and selenite were present in the medium, the reduction of tellurite was not significantly affected. But, selenite reduction was found to be slower in the presence of tellurite. It is interesting to note an overlap between the reduction of both tellurite and selenite, which is needed for production of Se-Te functional nanomaterials. Repeated bioreduction of 1 mM of tellurite was sustainable during multiple cycles of feeding in a fed-batch experiment, indicates the applicability in biological treatment and formation of Te based nanomaterials.
MICROBIAL ASSOCIATIONS RELATED TO METHANE PRODUCTION IN AN UP-FLOW ANAEROBIC SLUDGE BLANKET TREATING MOLASSES WASTEWATER

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Background
Anaerobic digestion of organic wastes involves complex microbial interactions. The understanding of microbial interactions in anaerobic digestion can be utilized to construct strategies for methane production stably and effectively.

Objectives
In this study, methane production was evaluated in the up-flow anaerobic sludge blanket (UASB) reactor using molasses wastewater, and the active bacterial and archaeal communities were characterized using rRNA-based ion torrent sequencing.

Methods
The UASB reactor achieved a stable process performance at an organic loading rate of 1.7~13.8 g-COD·L\(^{-1}\)·d\(^{-1}\) (87–95% COD removal efficiencies), and the maximum methane production rate was 4.01 L-CH\(_4\)·L\(^{-1}\)·d\(^{-1}\) at 13.8 g-COD·L\(^{-1}\)·d\(^{-1}\). Community analysis revealed that bacterial and archaeal communities shifted along with OLR, and \textit{Lactococcus} and \textit{Methanosaeta}, comprising up to 84% and 80% of the respective communities, drove the changes. Microbial network analysis was applied to identify associations among the relative abundances of bacterial and archaeal operational taxonomic units, environmental parameters, and reactor performance. The constructed network (average shortest path, 2.261; clustering coefficient, 0.514) revealed the prevalence of bacteria–archaea associations (50% of microbe–microbe associations) and contained seven microbial hub nodes including both bacterial and archaeal nodes. Interestingly, the \textit{Lactococcus} and \textit{Methanosaeta} were network hub nodes and positively correlated. In addition, they shared the other microbial hub nodes as neighbors and were positively correlated with methane production.

Conclusions
The results indicate that the close association between \textit{Lactococcus} and \textit{Methanosaeta} is responsible for the stable production of methane in the UASB reactor using molasses wastewater.
INFLUENCE OF SOIL PHYSICAL AND CHEMICAL PROPERTIES ON FUNGAL AND BACTERIAL COMMUNITIES IN 12 VINEYARD SOILS FROM NORTHERN ITALY

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Background

Soil chemical and physical properties, which are determined both by pedo-climatic factors and by management, can significantly influence microbial communities.

Objectives

Scope of this work was to assess whether given features can specifically influence defined microbial taxa in a man-managed environment such as the vineyard.

Methods

Soil samples were collected in 12 vineyards located in two neighbouring groups of hills, both renowned in Italy for their wines: the Euganean Hills, whose soils are of prevalent volcanic origin, mainly from trachyte and riolite rocks, and the Berici Hills, with a marine sedimentary genesis, mainly from clay-limestone. Soil bacterial and fungal communities were determined through 16S and ITS 454 pyrosequencing analysis, their relative taxa abundances were calculated and their levels of correlation with soil characteristics were tested.

Conclusions

In order of abundance, the dominant bacterial taxonomic groups across all samples were Actinobacteria, Alphaproteobacteria, Acidobacteria, Bacteroidetes, Betaproteobacteria, Chloroflexi, Gemmatimonadetes, Planctomycetes,
Deltaproteobacteria, Gammaproteobacteria. Among fungal phyla, Ascomycota largely dominated followed by Basidiomycota. Prevailing fungal classes were Dothideomycetes, Sordariomycetes, Agaromycetes, Leotiomycetes, Eurotiomycetes and Tremellomycetes. Some of these taxa and less abundant microbial groups were found to be significantly influenced by soil pH and texture. Moreover, a considerable number of significant correlations were found between the relative abundance of microbial taxa and the amount of several different chemical parameters such as total carbon, nitrogen, phosphorus, exchangeable bases and microelements.

This study provides novel insights into how soil structure and management can affect soil microbial community composition.
EFFECT OF BIOAUGMENTATION WITH BACTERIAL STRAINS ISOLATED FROM THE SOIL OF RICE FIELDS ON COMPOSTING PROCESSES USING RICE STRAW AND SEWAGE SLUDGE

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Background
Composting is a biotransformation process in which solid organic matter turns into mature and stabilized material by the action of microorganisms in an aerobic process. Microorganisms play a key role in composting; the performance of the process and the quality of the final product depends on the quantitative and qualitative composition of microbial communities associated to the different stages of the process.

Objectives
In this work, we describe the use of two bacterial strains, Paenibacillus barcinonensis and Bacillus sp. BP7, isolated from the soil of rice fields in the Ebro river delta in Spain, as bioaugmentants in composting processes using rice straw and sewage sludge as starting material.

Methods
Composting piles were created by blending sewage sludge and rice straw at a ratio of 2.6:1 (w/w fresh weight) with a moisture content of 60%. To test the effect of the two strains, individual piles were seeded with 10⁸ ufc/gram of either P. barcinonensis or Bacillus sp. BP7, and a series of parameters were determined during the composting process.

Conclusions
Among the parameters determined, the humification index after 25 days had increased 40% in the piles supplemented with P. barcinonensis and 20% in those supplemented with Bacillus sp. BP7, a clear indicator of the efficiency of the bioaugmentation. This was also accompanied, in the case of P. barcinonensis, by a change in the humic/fulvic acids ratio that went from 3.2 after 25 days in the control pile to 5.2 in the case of P. barcinonensis. These results suggest the potential application of bioaugmentation in composting.
Background
Pharmaceutical and personal care products (PPCPs) discharged with wastewater treatment plant effluents are an emerging surface water quality concern. Ibuprofen is the most widely used member of a diverse class of pharmaceuticals termed non-steroidal anti-inflammatory drugs (NSAIDs), many of which share a phenylacetic acid (PAA) core. Ibuprofen has been detected in bodies of water worldwide. Investigations into its environmental impact have found that ibuprofen induced changes on fish, plants, algae and microbial aquatic communities at environmentally relevant concentrations.

Objectives
The aim of this research was the isolation of bacteria with characteristics for potential bioaugmentation to enhance ibuprofen degradation in wastewater treatment plants an in the sewage sludge resulting from the activity of these plants.

Methods
Composting piles spiked with ibuprofen were created by blending sewage sludge and rice straw at a ratio of 2.6:1 (w/w fresh weight) with a moisture content of 60% to which a concentration of 1g/l of ibuprofen was added. Samples were taken at different days in the different phases, up to 42 days when ibuprofen had been completely degraded. Microorganisms extracted from these samples were tested in minimal medium supplemented with 500 mg/ml of ibuprofen and incubated at 24°C or 55°C, to discriminate mesophilic from thermophilic microorganisms.

Conclusions
Ibuprofen degradation occurred only in the samples incubated at 24°C. Plating of microorganism from these samples allowed the isolation of some 30-40 different colony morphologies that were again tested for ibuprofen degradation. Two of the isolates were confirmed to degrade ibuprofen and were partially characterized as belonging to the genus *Klebsiella*. 
DEVELOPMENT OF A TEST FOR THE DETECTION OF BACILLUS THURINGIENSIS VAR KURSTAKI IN ENVIRONMENTAL SAMPLES

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Background

*Bacillus thuringiensis* was isolated for the first time in 1902 from diseased silkworm larvae and its commercial use as an ecological insecticide started in 1958. *B. thuringiensis var kurstaki* (*Btk* for short) is routinely used to control caterpillar pests both at large extensions and at small gardens. The crystal toxin is only active when ingested by the caterpillars and absorbed in the alkaline digestive tract. The toxin has a relatively narrow spectrum of action and does not affect mammals, birds, or other insects and besides, the persistence of Btk on foliage is of around five days and does not accumulate on the environment. Because of its relative safety Btk has also been in use as a simulant by armed and security forces in exercises of response to biological or bioterrorist attacks.

Objectives

In this work we have developed rapid tests that can be used for the detection of the presence of *Btk* on air or surface samples after controlled release, and to measure the efficiency of decontamination measures.

Methods

Polyclonal antibodies were raised in rabbits by injecting them with autoclaved commercial *Btk* spores.

Conclusions

Immune response was confirmed by Western-Immunoblot and immunofluorescence assays and the specific IgGs, after purification by affinity chromatography using Btk spores, were coupled to polystyrene beads, to develop a slide agglutination test, colloidal gold, as part of lateral flow immunochromatography tests, or polystyrene ELISA plates, to develop ELISA based assays. Preliminary results confirm the validity of slide agglutination test whilst lateral flow and ELISA are under development.
MOLECULAR IDENTIFICATION OF MICROORGANISMS USED FOR "BACTERIOGRAPHIE"

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Background

The knowledge that bacteria use pigments to guard against UV radiation gave Erich Schopf the idea of using bacteria as unique tool for creating colors. Instead of painting artists’ colors on different painting supports, he applies bacteria on agar-based media. Initially invisible, the picture gets visible due to the growth of the bacteria used. Erich Schopf has called this technique “Bacteriographie” (www.bacteriographie.com).

Meanwhile the ensemble of bacteria used for “Bacteriographie” comprises around 900 microorganisms from different regions of the world (e.g. Austria, Germany, Greenland, Iceland, India, Indonesia, Ireland). They were collected from air, food, water, soil and recently also from holy water. Next to their ability to form pigments a general criterion is their growth at room temperatures within a foreseeable time period. Additionally, they have to undergo strict screening tests to perceive their suitability.

Objectives

Although some classical microbiological tests were done to get more information about the microorganisms themselves, the identity of the isolates is generally not known. Thus, this study focused on their identification at species-level using molecular biological methods.

Methods

A subset of the most important isolates (n = 120) was identified by 16S rRNA gene sequencing.

Conclusions
About 40 different genera could be identified. The relation between gram-positive and gram-negative species was nearly balanced. However, also a few fungal species (e.g. *Pseudozyma* spp., *Cryptococcus* spp.) were identified. Different species of the genera *Pseudomonas, Flavobacterium, Microbacterium, Rhodococcus, Kocuria, Chryseobacterium* and *Arthrobacter* were frequently detected, which form yellow, orange and red pigments.
RAPID RECOVERY OF CYANOBACTERIAL PIGMENTS IN DESICCATED BIOLOGICAL SOIL CRUSTS FOLLOWING ADDITION OF WATER

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Background

Cyanobacteria in biological soil crusts were shown to move upwards in to track water, however this it is not known how widespread this process is in other crusts.

Objectives

To examine soil surface colour change to green and hydrotaxis following addition of water to crusts

Methods

We used pigment extraction, hyperspectral imaging, microsensors and ¹³C labelling experiments coupled to matrix-assisted laser desorption and ionization time of flight-mass spectrometry (MALD-TOF MS)

Conclusions

The topsoil colour turned green in less than 5 min following water addition. The concentrations of chlorophyll a, scytonemin and echinenon rapidly increased in the top <1 mm layer while in the deeper layer their concentrations remained low. Hyperspectral imaging showed that, in both wet and dehydrated crusts, cyanobacteria formed a layer at a depth of 0.2-0.4 mm and this layer did not move upward after wetting. ¹³C labelling experiments and MALDI TOF analysis showed that Chl a was already present in the desiccated crusts and de novo synthesis of this molecule started only after 2 days of wetting due to growth of cyanobacteria. Microsensor measurements showed that photosynthetic activity increased concomitantly with the increase of Chl a, and reached a maximum rate approximately 2 hours after wetting. We conclude that the colour change of soil crusts to green upon water addition was not due to hydrotaxis but rather to the quick recovery and reassembly of pigments.
Background
Sabkha is a unique environment of economic and geologic importance with a valuable resource of distinctive features. Saudi Arabia possesses coastal Sabkhas which are extensively distributed along both the eastern and western areas.

Objectives
Therefore, samples (I and II) with depth of 50 cm were collected from a remote salt marsh at the southwest of Saudi Arabia, which is called Jazan Sabkha, to unveil the bacterial community that survive in such extreme environment.

Methods
Each sample was cut horizontally at interval of 1 cm for the first 10 cm and at 5 cm for the next 20 cm. The samples are considered salty and sulphurous, where sodium and soluble chloride at the range of 11,000-34,000 ppm and 11,000-99,000 ppm, respectively, while sulfate varied from 2,070 to 18,820 ppm. Next generation sequencing (454 pyrosequencing) was applied to determine the bacterial communities inhabiting each layer.

Conclusions
Overall, Pseudomonas Genus was dominated in both samples with 46% and 39% for I and II, respectively. Pseudomonas putida was identified in each cross-section at 1 cm with variable percentages. In addition, P. veroniim, P. cannabina and Herbaspirillum rubrisubalbicans were presented with less percentages. Leptospirillum spp. were detected at lower layers and at they were optimized between 15-20 cm while Methylobacterium tardum appeared at their maximum around 7-9 cm. Most of genera saline species of Halobacteriaceae family like Halorhabdus spp., Halonotius spp., Halobaculum spp., Halomicrobium spp., Halorubrum spp., Halogranum spp., Haloplanus natans, Salinibacter spp. and Natronomonas spp. were detected within 1-2 cm near to the salty crusty surface of Sabkah.
CONSTRUCTION OF PUC19 PLASMID METAGENOMIC LIBRARY FOR EXPLORING NOVEL PROTEASE ENZYMES FROM LAKE ACIGOL, A HYPERSONAL LAKE IN SOUTHERN, TURKEY

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Background
Investigations of extreme environments are important for the study of evolution relationships, discovery of new species and various ecological relations among organisms. Besides, new metabolites and novel enzymes, metabolic pathways of organisms' can be discovered from extreme environments.

Objectives
In this study, metagenomics approach is used to isolate potential biotechnological novel enzymes from Lake Acıgöl. Especially we have focused on proteases which are ubiquitous enzymes and they have crucial roles in different industrial applications. Research area Lake Acigol is located between Afyon, Denizli and Burdur city boundaries in Aegean region, Turkey. It is a good example for extreme environments due to its high salinity (about 200g/L NaCl) which makes the lake suitable for halophilic microorganisms.

Methods
Bacterial population of Lake Acıgöl have already been identified and Lake bacterial population consist of 38.8% Uncultured Bacterium, 30.8% Firmucutes, 15.3% Bacteriodetes, 7.6% Gammaproteobacterium and 7.6% Deltaproteobacterium. Because of the high population of uncultured microorganisms Lake Acigöl is a potential candidate for novel enzyme exploration. To isolate extremophilic protease enzymes from Lake Acigol sediment samples were collected from different part of the Lake and Metagenom (DNA) isolation was carried out by manually according to Zhou et. Al., 1999 [1].

Conclusions
Metagenom library was constructed by using pUC19 plasmid. Screening studies for possible protease enzymes will be carried out using 1% of skim milk LB agar plates and Salt Tolerance Screening Method according to Culligan et. Al., 2013 [2].
Background
Microbe-mineral interactions have become of interest for space exploration as microbes can biomine useful elements from planetary regolith, which could serve as nutrients in a life support system.

Objectives
Therefore, this research aimed to assess the impact of space conditions on physiology of Cupriavidus metallidurans CH34 and identifying the molecular mechanisms behind microbe-mineral interactions on basalt, a lunar-type rock.

Methods
Survival of C. metallidurans CH34 was monitored after a 3-month period in mineral water with or without basalt by plate counts, flow cytometry, ICP-MS and microscopy. The effect of space conditions was studied via a flight experiment on board the Russian PHOTON-M4 capsule.

Conclusions
The results obtained from ground experiments showed that the cultivable fraction dropped to 10% indicating a transition to a more dormant state. In the presence of basalt, CH34 remained viable, attached and formed a biofilm. The space flight experiment indicated more viable cells compared to the ground experiment both in the absence and presence of basalt, indicating a positive effect of space flight on survival.
Additional physiological and molecular analyses are on-going to confirm these observations and to determine the molecular processes.

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MEMBRANE-BOUND AMYLOPULLULANASE IS ESSENTIAL FOR STARCH METABOLISM OF SULFOLOBUS ACIDOCALDARIUS DSM639

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Background
The growth of Sulfolobus shows that these species prefer starch or long-chain maltooligosaccharides to maltose as a carbon source and have both α-amylase and α-glucosidase activities. The annotation of the genome sequence of S. acidocaldarius assume that the gene encoding amylolytic enzyme, Saci_1162, within the putative maltose/maltodextrin ABC transporter operon (Saci_1160 to Saci_1166) is involved in the degradation of starch or α-linked polymers into small maltooligomers.

Objectives
A putative amylolytic enzyme, Apu (Saci_1162), was characterized whether it is involved in the starch metabolism in S. acidocaldarius.

Methods
The physiological role of Apu in starch metabolism was investigated by the growth and starch degradation pattern of apu disruption mutant as well as biochemical properties of recombinant Apu.

Conclusions
The Δapu mutant lost the ability to grow in minimal medium in the presence of starch, and the amylolytic activity observed in the membrane fraction of the wild-type strain was not detected in the Δapu mutant when the cells were grown in YT medium. The purified membrane-bound Apu initially hydrolyzed starch, amylopectin, and pullulan into various sizes of maltooligosaccharides, and then produced glucose, maltose, and maltotriose in the end, indicating Apu is a typical endo-acting amylopolullulanase which belongs to glycosyl hydrolase family 57. The maltose and maltotriose observed in the culture medium during the exponential and stationary phase growth indicates that Apu is the essential enzyme to initially hydrolyze the extracellular starch into small maltooligosaccharides to be transported into the cell.
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Extreme environments

MICROBIAL COMMUNITY PROFILE OF THE IRON-SULPHATE RICH WATERS OF RIO SUCIO (BRAULIO CARRILLO NATIONAL PARK, COSTA RICA)
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Background
Río Sucio (‘Dirty river’) in Braulio Carrillo National Park (Costa Rica) is a river of volcanic origin that possesses a characteristic yellow color due to the presence of high amounts of mineral deposits from Irazú Volcano.

Objectives
To perform a physicochemical characterization of the Rio Sucio water and to analyze the impact of these parameters on the microbial community that inhabits the river.

Methods
Physicochemical analysis was performed by ion-exchange chromatography (IC), inductively coupled plasma mass spectrometry (ICP-MS), scanning electron microscopy with electron dispersive spectrometer (SEM-EDS) and X-ray diffraction (XRD). Analysis of the microbial community was performed by analysis of the V5-V6 hypervariable regions of the 16S rRNA gene. PCR amplicons were sequenced on an Illumina MiSeq instrument and the sequences compared against the RDP database (Ribosomal Database project – http://rdp.cme.msu.edu/).

Conclusions
Physicochemical analysis revealed that Río Sucio is a slightly acidic river (pH 5.0) containing high concentrations of sulphate (502 ± 29 mg/L), calcium (96.6 ± 2.2 mg/L), magnesium (27.36 ± 0.16 mg/L), aluminium (17.1 ± 1.9 mg/L) and iron (5.20 ± 0.11 mg/L). The precipitated material in the bottom of the river was composed mainly of iron oxides, as indicated by SEM-EDS and XRD. Microbial diversity analysis showed that the most abundant organisms are closely related to sulfur and iron oxidizing bacteria such as \textit{Sulfuritalea spp.}, \textit{Ferrovum spp.}, \textit{Acidithiobacillus ferrooxidans}, \textit{Leptospirillum ferrooxidans} and \textit{Ferrithrix spp}. These observations suggest that Río Sucio has an acid rock drainage environment where the chemical composition is biologically driven by sulfur and iron oxidizing bacteria.
Background
Biological systems are frequently exposed to microwave radiation. Many studies have investigated the influence of microwaves on these systems, but controversy over methods to distinguish between thermal and non-thermal microwave effects remains.

Objectives
To differentiate between non-thermal and thermal microwave effects on a physiology of a microorganism, a thermophilic bacterium was grown in a constant-temperature microwave or a convection oven. Comparing the growth properties of the thermophile in these conditions will reveal non-thermal microwave effects on cell growth and
physiology. Biophysical and biochemical analysis will demonstrate changes in morphology and chemical composition arising from microwave exposure.

**Methods**

Cell growth was analyzed by optical density (OD) measurements (supported by independent quantitative DNA analysis), and cell morphologies were characterized using electron microscopy imaging (SEM, TEM), dynamic light scattering (DLS), and atomic force microscopy (AFM). AFM was also used to probe the biophysical characteristics of the cells, in conjunction with nano-infrared spectroscopy (Nano-IR). Attenuated total reflectance infrared spectroscopy (ATR-IR) and fatty acid methyl ester (FAMEs) analysis were used to determine biochemical differences between cells grown in microwave and oven conditions.

**Conclusions**

Thermophilic bacteria were grown in a synthetic microwave such that thermal effects and microwave effects were distinguishable. These data demonstrate that there are physiological differences between cells cultured in a dielectric field and a convection oven, and that microwaves induce non-thermal changes to the structure, physiology, and chemical composition of the organism.
Background
Deep-sea is a fascinating environment, which despite the extreme conditions of pressure, temperature and nutrients, is home to many life-forms. In order to survive here, the organisms need to develop mechanisms to counter these conditions. Fungi, one of the most ubiquitous eukaryotic forms of life, have also been reported from deep-sea sediments. In order to survive, they undergo both morphological and biochemical adaptations. Induction of proteins is one such response elicited by fungi for survival.

Objectives
In our study to understand the mechanism of survival by fungi occurring in deep-sea conditions, we are looking for proteins expressed by fungi and their role in tolerance to high hydrostatic pressure using LCMS QToF.

Methods
To understand the mechanism of survival of fungi, in deep-sea conditions, we examine tryptic digests of proteins expressed under various hydrostatic pressure using LCMS QToF.

Conclusions
The results showed that some proteins were up-regulated whereas some were down-regulated at higher pressures. Some over-expressed proteins, probably playing a role in pressure tolerance were 30 KDa heat shock protein and heat shock protein SSC1 mitochondrial. Unfortunately, none of the up-regulated proteins could be considered as ones produced exclusively in response to high hydrostatic pressure as all are reported in response to other stresses also. Along with these proteins, there were several hypothetical proteins to which no function has been assigned so far. These could well prove to be the ones limited to pressure stress, which needs to be investigated further by either inactivating the proteins or using knock-down mutations.
Background
Urmia Lake in the Northwest of Iran is the second largest hypersaline lake in the world which has been explored with great haloarchaeal diversity. Extreme halophilic Archaea are widely distributed in hypersaline habitats.

Objectives
During the course of biodiversity studies in Urmia Lake, several new extreme halophilic Archaea were isolated. Amongst them we chose strain DC8\textsuperscript{T} for further characterization.

Methods
Strain DC8\textsuperscript{T} has been characterized taxonomically using polyphasic approach. According to minimal standards we performed Molecular, biochemical, morphological and physiological tests for characterization.

Conclusions
16S rRNA gene sequencing showed that strain DC8\textsuperscript{T} is a member of the family Halobacteriaceae; however, its similarity was as low as 90.1% and 89.3% to haloarchaeal taxa including Halosimplex and Halobaculum type species, respectively. The cells of strain DC8\textsuperscript{T} were non-motile and pleomorphic and needs at least 2.5 M NaCl and 0.02 M MgCl\textsubscript{2} for growth. Optimal growth was at 4.0 M NaCl and 0.1 M MgCl\textsubscript{2}. The optimum pH and temperature for growth were pH 7.5 and 45°C. The G+C content of its DNA is 68.1 mol%. Polar lipid analyses revealed that strain DC8\textsuperscript{T} contains phosphatidylglycerol, phosphatidylglycerol phosphate methyl ester and phosphatidylglycerol sulphate. The only quinone present was MK-8 (II-H\textsubscript{2}). The physiological, biochemical and phylogenetic differences between strain DC8\textsuperscript{T} and other previously described genera of extremely halophilic archaea suggest that this
strain represents a novel species in a new genus within the family *Halobacteriaceae*, for which the name *Halositis urmiana* gen. nov., sp. nov. is proposed.
BACKGROUND

Despite the crucial role of microorganisms in lake ecosystem function, they are typically poorly studied in high altitude ecosystems. In general, there are few reports of microbial diversity in lakes located at altitudes >5000 m. Lake Llullaillaco is a previously undescribed lake located at 6170 m on the Chilean side of the Llullaillaco volcano, the second highest volcano in the world (6739 m).

OBJECTIVES

The aim of this study was to describe the basic morphology of Lake Llullaillaco and the microbial diversity from water samples taken from the lake in February 2013.

METHODS

Bacterial cultures were obtained using different culture media and microbial diversity was analysed by the pyrosequencing of 16S rRNA genes of Bacteria and Archaea. At the time of sampling, the lake had an area of 0.9 ha and maximum depth of 6.8 m. Water temperature ranged between 2 and 5°C and pH was 6.5. 24 bacterial isolates were obtained belonging to the Alpha-, Beta-, Gammaproteobacteria and Actinobacteria. Bacterial diversity was dominated by Proteobacteria (86.5%), Actinobacteria (11.9 %) and Bacteroidetes (1.4%), with Beta-, and Alphaproteobacteria being the most abundant taxa. Archaeal diversity was dominated by Euryarchaeota and the Halobacteriaceae, with a high contribution of unidentified sequences.

CONCLUSIONS

This study is the first description of microbial diversity in Lake Llullaillaco: diversity was low and characterized by a combination of phylotypes previously described from cold environments and a series of undescribed microorganisms.

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MICROBIAL DIVERSITY IN ARMENIAN SALINE-ALKALINE SOILS AND SUBTERRANEAN SALT DEPOSITS ANALYZED BY MOLECULAR AND CULTURE-BASED METHODS

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Background

One of the main directions of microbial ecology of extremophiles is the study of halophilic microorganisms' biocenoses, which are mainly found in saline lakes and other water systems as well as saline soils and salt mines. Microbial biodiversity of salt mines and saline-alkaline soils distributed on the territory of Armenia is still poorly investigated.

Objectives

The aim of the present study was to investigate the microbial composition of saline-alkaline soils of the Ararat Plain and Avan subterranean salt deposits in Armenia.

Methods

For this study, clone-library construction of PCR-amplified 16S rRNA genes, DGGE-PCR fingerprinting and cultivation-dependent methods were used. 16S rRNA gene clone libraries and PCR-DGGE fingerprinting sequences were generated from total DNA extracts using universal archaeal and bacterial oligonucleotide primer sets.

Conclusions

Sequence analysis of bacterial and archaeal clone libraries and DGGE-PCR products from the samples indicated a dominance of Firmicutes and Euryarchaeota from the bacterial and archaeal domains, respectively. Several aerobic chemoorganotrophic endospore-forming bacteria were isolated from the salt samples and identified as representatives of the genus Halobacillus, Piscibacillus, Virgibacillus and Streptomyces. Five halophilic archaeal strains were isolated from salt stone samples. Four of the archaeal strains were most closely related to members of the genus Haloarcula (97-99% similarity) and one strain most closely to the genus Halarchaeum (<97% similarity), indicating that Avan salt mine harbors a unique community of possible novel species.
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Extreme environments

COPQ, A NOVEL FAMILY OF SMALL SECRETED PROTEINS WITH PUTATIVE RADICAL-SCAVENGING AND METAL-DETOXIFYING ACTIVITIES, ARE STRICTLY CONFINED TO THE GENERA RALSTONIA AND CUPRIAVIDUS
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Background
The soil bacterium Cupriavidus metallidurans CH34 is tolerant to a wide range of metals (1). Whole-genome transcriptomics demonstrated a common response in this organism for metal and oxidative stress (2). Some of the highly induced genes are well conserved within 14 C. metallidurans strains (3) and encode short proteins (coined the COPQ family) that possess a distinctive signal peptide and up to ten repetitive motifs [GSRD]XXD[PV][YF]T[DE]G[ASG].

Objectives
Bioinformatic analysis of the COPQ family.

Methods
Transcriptomic procedures are in (2). Structural predictions were performed with I-TASSER and PHYRE-2. Sequence similarity analysis was done by iterative BLAST against the non-redundant protein sequence database (nrdb – version 2014/09/10). Motif detection was done using BioGrep against 2,616 bacterial proteomes (refpep – version 2014/01/09).

Conclusions
BLAST analyses and BioGrep motif searches against reference protein databases showed that the CopQ family of proteins is strictly confined to the highly related species of Cupriavidus and Ralstonia. COPQ proteins are predicted to form straight, slightly helical polypeptides, probably owing to the abundance of chain-disturbing and evenly spaced glycines and prolines. The aromatic rings of the Tyr and Phe residues at the center of the motifs are fully exposed allowing easy access to reactive oxygen species (ROS).
We propose that COPQ-family proteins have a radical-scavenging function shielding cells from oxidative attack and/or metal toxicity. Highly purified COPQ proteins in single or mixed fashion are to be tested for their anti-oxidant and metal-binding
properties.
(2) Monsieurs et al. (2011). Biometals, 24(6) 133-1151
BIODEGRADATION OF AROMATIC HYDROCARBONS BY HALOPHILIC ARCHEAE ISOLATED FROM ALGERIAN SEBKHAS
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Background
Actualy, the biodegradation of hydrocarbons (specialy PAHs) has recieved great attention, however, such high and fluctuating salinity promotes the loss of cell wall integrity, protein denaturalization, and changes in osmotic pressure, the biological treatment of industrial hypersaline waste-waters and the bioremediation of polluted hypersaline environments are not possible with conventional microorganisms.

Objectives
The aim of our study is the identification of microorganisms that could answer these criteria for the biodegradation of extreme environments.

Methods
Three strains halophilic archaea Haloarcula sp.D21(AM982816), Halovivax sp.A21(AM982815) and Natrialba sp.C21(HG423210) were isolated from the salt lake close to Ain Salah, Algeria. These strains show good growth on extreme saline culture media (up to 25%NaCl) in the presence of diesel, naphthalene and pyrene as the sole carbon source. The strain Natrialba sp.C21 has a better growth on these media even at 35% of salt. However, the highest rate of biodegradation of these three hydrocarbons is labeled in the presence of extreme halophilic consortium containing the three strains. This biodegradation is marked by the high rate of growth of the strains measured by the optical density, the production of biosurfactans estimated by the emulsifying index and by the lowering of the surface tension of the culture media (below 40mN/m). In addition, the biodegradation of these hydrocarbons in the presence of the halophilic consortium is correlated with the rate of the concentration of NaCl and Mg²⁺ in the medium.

Conclusions
Thus, our halophilic haloarchae strains, specialy Natrialba sp.C21, are potential candidates for the degradation of pollutants at high salt concentrations.
Background

Cyanobacteria produce a large variety of toxic secondary metabolites. In the Arctic and Antarctic, many unusual variants of microcystins (MCs) - the most common type of cyanobacterial freshwater toxins - have been detected. These variants are generally difficult to detect by standard methods in background-rich samples.

Objectives

Therefore, there is a need to develop a screening method that can be used for a large number of environmental samples.

Methods

In this study, 25 cyanobacterial samples from diverse biotopes in Svalbard (Arctic) were analyzed for the presence of MC. In a preliminary ADDA-MC specific ELISA, 18 of 20 samples were tested positive, an unusually high percentage. In contrast, a liquid chromatography-tandem mass spectrometry (LC-MS/MS) precursor-screening, newly designed to identify candidate MCs from the ADDA fragment ions, suggested the presence of MCs in nine of the 25 samples. In three of the latter, MC variants could be subsequently confirmed and identified by complete MS/MS analysis as variants of MC-RR and MC-LA, similar to those previously reported for polar habitats. In five of the same nine samples, a gene involved in toxin production - mcyE - was successfully amplified, validating the analytical data. The amplified gene sequences were 93-98% similar to mcyE genes of Nostoc, indicating that this genus could be responsible for MC production in these samples.

Conclusions

The presence of the unusual variants in the polar regions could help to understand the evolution and ecological function of the toxins. Moreover, the toxicological risk of unusual toxins is not yet known and might be an undetected threat.
‘EX-SITU’ PRESERVATION AND CHARACTERIZATION OF ANTARCTIC CYANOBACTERIA IN THE BCCM/ULC COLLECTION

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Background
The BCCM/ULC public collection of (sub)polar cyanobacteria is funded since 2011 by the Belgian Science Policy Office. An ISO9001 certificate was obtained for the public deposition and distribution of strains, as part of the multi-site certification for the BCCM consortium. BCCM/ULC is currently holding 160 public cyanobacterial strains and the catalogue is available on http://bccm.belspo.be/catalogues/ulc-catalogue-search.

Objectives
Continuous maintenance of living cultures, some of which are also cryopreserved, ensure the preservation and the possibility to rapidly deliver strains to clients for fundamental and applied research.

Methods
The main holding of the collection concerns (sub)polar strains isolated from different biotopes and representative of a large taxonomic diversity. The molecular characterization is underway, on the basis of 16S rRNA and ITS sequences, but also Multiple Locus Sequence Analysis and genome sequencing.

In addition, cyanobacteria are known to produce a range of secondary metabolites (e.g. alkaloids, cyclic and linear peptides, polyketides) with various bioactivities. The potential of the polar strains to produce cyanotoxins and other secondary metabolites is currently studied by ELISA, LC-MS and the detection of genes involved in their production.

Conclusions
Due to the geographic isolation and the strong environmental stressors of the habitat, the exploration of these metabolites in Antarctic cyanobacterial strains seems especially promising for biotechnology or biomedical applications.
THE CRENARCHAEON IGNICOCUS HOSPITALIS IS A POLYEXTREMOPHILIC ORGANISM WITH UNUSUAL HIGH RADIATION TOLERANCE

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Background
The hyperthermophilic crenarchaeon Ignicoccus hospitalis was isolated from deep-sea hydrothermal vents living there strictly anaerobic and chemolithoautotrophic. From an astrobiological point of view, hyperthermophilic organisms are promising candidates for early Earth inhabitants, when the absence of oxygen, occasional meteorite impacts followed by heated up oceans, and the missing UV-absorbing ozone layer enabled higher radiation dose of short wavelength to penetrate Earth’s surface.

Objectives
To ensure DNA integrity, I. hospitalis needs proper DNA repair mechanisms to cope with today’s harsh environmental conditions in its natural habitat. These highly efficient DNA repair mechanisms might result in a tolerance to other yet untested harmful environmental impacts of astrobiological relevance. Therefore, I. hospitalis’ radiation tolerance regarding ionizing and non-ionizing radiation has extensively been studied, and the DNA damage visualized on a molecular level. Future experiments will focus on repair kinetics and on the identification of proteins involved in DNA repair.

Methods
The survival after radiation exposure was determined by the most probable number technique (A), and PCR-based assays like RAPD (randomly amplified polymorphic DNA) analysis qualitatively confirmed changes in the genomic DNA after irradiation treatment (B).

**Conclusions**

*I. hospitalis* cells showed a remarkable radiation tolerance although never exposed to it in its natural habitat. An exposure to 1000 J/m² monochromatic UV-C (254 nm) resulted in a reduction by 2-3 orders of magnitude which is in the same order as shown by *Deinococcus radiodurans*. An exposure to 24 kGy of ionizing radiation was survived (A); the DNA integrity was only slightly affected (B).

References


Background

Ferroplasma acidarmanus dominated the acid mine draining biofilm community in accordance with metagenomic metabolic modelling (Chen et al., 2012). One of the most impressive features of F. acidarmanus was its ability to survive at such an extreme proton gradient with only a cell membrane. The S-layer protects the cell against mechanical and osmotic stresses of extreme pH conditions. A rigid cell walls or S-layer were not documented neither in F. acidarmanus Fer1 nor T. volcanium as yet.

Objectives

Here we present an evidence for function of a gene coding for S-layer and the cellular localization of the protein in F. acidarmanus Fer1,

Methods


Conclusions

The gene 638394352 was identified at 66-1422 (+) and coded for 452 amino acids with an isoelectric point of 10.08. The S-layer protein possessed a high content of acidic and hydrophobic amino acids. Protein played a structural role for the cell envelope and its biogenesis. We predicted two transmembrane domains of about 20 aa each anchored within the membrane while the majority of amino acids were located extracellular. The gene shared sequence homology with S-layer domain protein from Sulfolobus islandicus. The cellular localization model of 638394352 agreed with the cellular structure and localization of S-layer protein of orthologues Sulfolobus, which formed extracellular monomolecular crystalline mushroom-like
arrays of proteinaceous subunits on the cellular surface.

Figure 4. Model of cellular localization of protein product of OID 638394352 from *F. acidarmanus* fer 1 produced based on combined results of SOSUI, Phobius, SignalP, TMHMM and PSORT.

The peptide statistics, cellular localization, and protein homology, indicated that the gene was coding for S-layer domain..
Background
Temperature exerts multiple effects on the structure and function of enzymes which are still poorly understood at the molecular level. The adaptation of microorganisms to extreme temperatures includes the evolutionary development of biocatalysts with a high catalytic efficiency under these conditions. Nowadays, such extremophilic enzymes are used as valuable tools to study relationships between protein stability, dynamics, and function.

Objectives
Here, we report on a comparative biochemical and molecular dynamics analysis of four homologous esterases belonging to the hormone sensitive lipase family which were isolated from bacteria living at temperatures ranging from 10°C to 70°C.

Methods
The recombinant esterases showed highly similar substrate specificities. Furthermore, their optimal temperatures and thermostabilities resembled the temperatures of the respective bacterial habitats. Apparently, the thermal properties of these esterases were optimized to sustain the temperatures of their respective habitats. Therefore, we have used this set of enzymes for the analysis of molecular determinants beyond thermal adaptation.

Conclusions
The structural analysis revealed significant differences between structures arising from surface exposed loop regions. The high flexibility of these structural domains was confirmed by molecular dynamics simulations performed at temperatures ranging from 15°C to 90°C. The results led us to propose four highly flexible loops (L2, L4, L10 and L12) distant from the active site as the structural regions most relevant for thermal stability. The link of such surface exposed loop-structures to thermal stability may indicate that natural thermal adaptation is achieved by tuning atomic interactions in “non-active site” loops without interfering with the catalytic function of an enzyme.
ANALYSES OF THE ANTIOXIDANT EFFECT OF COBALAMIN IN LEPTOSPIRILLUM GROUP II STRAIN CF-1 USING TRANSCRIPTOME AND PROTEOME APPROACHES

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Background
Acid bioleaching environments are characterized by high levels of iron and heavy metals, which can increase the generation of reactive oxygen species and induce oxidative stress in microorganisms. Metaproteomic studies on Leptospirillum spp., a member of the bioleaching consortium, have shown that it contains high levels of cobalamin biosynthesis proteins. We have found that cobalamin exerts an antioxidant effect in Leptospirillum group II strains.

Objectives
The aim of this study was to clarify the possible mechanisms involved in cobalamin effect in Leptospirillum group II strain CF-1 through transcriptomic and proteomic approaches.

Methods
The sequencing and annotation of the genome were carried out using PacBio RS II technology and RAST software, respectively. Transcriptomic profile was obtained by RNA-seq using Illumina Hiseq 2500 sequencing. The mRNA abundance was calculated using Blast and expressed as RPKM (reads per kilobase per million) data. Proteomic assays were carried out using 1D-PAGE LC-MS/MS, and the LFQ intensities were normalized by MaxQuant software.

Conclusions
The genome analysis showed one circular chromosome with 2,709,324 bp and 3,070 coding sequences. Transcriptomic and proteomic analyses showed that cobalamin produces up-regulation of pathways involved in biosynthesis and repair of proteins, biosynthesis of iron sulfur clusters and amino acids, biofilm formation and energy metabolism proteins. Most of up-regulated pathways use S-adenosylmethionine or folate as cofactors which are products of cobalamin-dependent methionine and folate cycles. These data lead us to suggest that cobalamin could activate the central metabolism, improving the response to oxidative damage and restoring redox balance of the cell.
Acknowledgement: Fondecyt Grants 1120746, 1110203.
THE FE-S CLUSTER ASSEMBLY (ISC) SYSTEM IS FUNCTIONAL UNDER OXIDATIVE STRESS AND IRON STARVATION CONDITIONS IN THE ACIDOPHILIC BIOLEACHING BACTERIUM LEPTOSPIRILLUM GROUP II CF1

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Background
The synthesis and assembly of Fe-S clusters is a highly complex and coordinated process in living cells. The ISC system is the housekeeping [Fe–S] cluster assembly system whereas the SUF system is functional under harsh environmental conditions such as oxidative stress and iron starvation. Members of Leptospirillum genus are acidophilic iron-oxidizing bacteria that belong to the consortium of microorganisms that participate in the bioleaching of ores and metal recovery. Interesting, despite to inhabit in a highly oxidizing environment with high concentrations of metals, these microorganisms have only one [Fe-S] cluster assembly system, ISC

Objectives
In this work we evaluated the functionality of the [Fe-S] cluster biogenesis system ISC of Leptospirillum Group II CF-1.

Methods
Bioinformatic analysis and RT-PCR experiments were used to characterize the genetic clusters that contain isc genes and to evaluate the co-transcription of genes. The expression level of key genes iscR, iscS and hscB was determined by quantitative RT-PCR analysis when cells were exposed to oxidative stress and iron starvation. The functionality of the ISC system was evaluated measuring the activity of cysteine desulfurase enzyme (IscS).

Conclusions
The results showed that Leptospirillum Group II CF-1 responds to oxidative stress and iron starvation by up-regulating the [Fe–S] cluster assembly isc genes and increasing the activity of IscS.

Acknowledgement: Fondecyt Grants 1120746, 1110203.
Background

The cyanobacterium *Arthrospira* sp. PCC 8005 has been selected by the European Space Agency (ESA) for producing oxygen and food during future long-duration manned space missions, as part of the bio-regenerative life support system 'MELiSSA'. PCC 8005 must continue to produce oxygen and conserves high nutritive value while exposed to cosmic radiation in space.

Objectives

The tolerance and the response of *Arthrospira* sp. PCC 8005 to ionizing radiation were investigated.

Methods

Metabolically active planktonic cells of were exposed to Cobalt-60-generated gamma radiation and Helium and Iron particle radiation. The molecular response to radiation was investigated via photosynthesis, pigment, antioxidant, proteome and transcriptome analysis.

Conclusions

The cells of PCC 8005 were able to survive doses of 6400 Gy of gamma, and 1000 and 2000 Gy of He and Fe particle radiation. During irradiation, cells switched quickly from an active growth state to a growth arrest mode, via a shut-down of photosynthesis and carbon fixation. Resources were rerouted to cellular protection and repair. Various antioxidant systems were activated, such as glutathione, to protect lipids, proteins and DNA. The cells activated also ssDNA repair systems and systems to remove damaged amino and nucleic acids from the cells. During recovery, the cells induced the *arh* genes, a new cluster of genes with unknown function. Finally, energy and metabolic pathways were restarted, and full recover of photosynthetic proliferation was obtained. These results show that *Arthrospira* sp.
PCC 8005 is a peculiar radiation resistant bacterium, useful for biotechnological applications, in space and on Earth.
Background

Carbon dioxide (CO$_2$), methane (CH$_4$) and nitrous oxide (N$_2$O) are three major greenhouse gases (GHG) which are naturally recycled mainly by microbiological mechanisms including respiration- CO$_2$ fixation, nitrification -desnitrification and methanogenesis-methanotrophy. Microbial groups involved in GHG recycling have been detected through molecular surveys in high altitude wetlands such as Salar de Huasco. This site is a high altitude wetland characterized by its extreme physical-chemical environmental conditions (high radiation, daily temperature shifts, salinity gradients) and by the presence of novel groups within Bacteria and Archaea domains and complex microbial structures (mats) in freshwater and salt-saturated sites.

Objectives

The aim of this study was to determine atmospheric and dissolved GHG concentration in Salar de Huasco wetland and dry Altiplanic adjacent areas (3,800 - 4,000 masl) and the potential communities involved in its recycling during the austral dry season (November 2014).

Methods

GHG were measured in discrete samples from air, water and gas bubbles below microbial mats inhabiting fresh water areas of Salar de Huasco wetland by standard chromatography. Also molecular analyses of the communities were studied using massive sequencing (metagenomic and 16S rRNA barcode).

Conclusions

This study indicates that Salar de Huasco microbial communities process GHG very fast since despite notorious accumulation of CH$_4$ bubbles (over N$_2$O) in cyanobacterial-microbial mats from fresh-water areas were found, the wetland was a net sink of GHG based on undersaturated values in the water and low ground air GHG levels compared with dry adjacent areas.
Acknowledgment: This work is part of the FONDECYT Project #1140356 and # 1140179.
HALOPHILIC FILAMENTOUS FUNGI FROM HYPERSALINE AND POLYHALINE ENVIRONMENTS

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Background

The existence of halophilic fungi in hypersaline environments has now been well established, negating the former belief that their presence was as simply ubiquitous fungi, or as being merely halotolerant. Several reports have indeed demonstrated the presence of these fungi in the Dead Sea and in solar salterns.

Objectives

Although hypersaline environments have been recorded as the primary econiche of halophilic fungi, the objective herein, was to ascertain the presence of this extremophilic group of fungi in polyhaline environments of the estuary and mangroves, in addition to solar salterns, and to characterise their nature of halophily.

Methods

Samples of water and sediment were obtained from these econiches and plated on to medium supplemented with high concentration of solar salt. The purified isolates so obtained were screened for their halotolerance levels, by growth on media containing increasing concentrations of salt.

Conclusions

Halophilic fungi were indeed isolated from polyhaline environments, as well as from hypersaline solar salterns. Most of the halophilic fungi screened were found to possess moderate halophily; some of the isolates were true halophiles, having an essential requirement of salt for growth. It is thus shown that this group of extremophilic fungi may be found in hypersaline as well as non-hypersaline marine environments.
Background

Background: By the end of 2008, and following a prolonged drought period, a mass death of shrubs was observed in a semi-arid region of the Northern Negev Desert.

Objectives

Objective: This study followed the development of biological soil crusts (BSCs) on the soil mounds in the sites of the dead shrubs, spanning over a three year period, from the disappearance of the shrub skeletons until the mounds were flattened.

Methods

Methods: In addition to on-site physical measurements, BSC samples were collected and analyzed for their physical and biochemical properties (15-17 parameters) and their microbial community structure and compared to those of mature crusts.

Conclusions

Conclusions: Statistical analyses showed significant differences in the biochemical properties and the microbial community structure between the developing BSCs and the mature ones during five sampling campaigns. Interestingly, significant differences were observed also between the north-facing and the south-facing slopes of the mounds. The extent of these observed differences was linked to the sampling dates, implying an effect of the soil moisture. Significant differences persisted even after the mounds were totally flattened. This slow development of BSCs on the bare soil mounds after shrub death and decomposition in semi-arid ecosystems, may allow the establishment of new shrubs in these sites and the recovery of the affected ecosystem.
GDGT LIPID COMPOSITION OF THE THERMOPHILIC ARCHAEA
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Background
The archaeal lipids differ significantly from the bacterial and eukaryotic analogs. The most studied lipids of thermophilic archaea are ether-bonded glycerides, resistant to usual lipid derivatization methods. Thus, characterization of archaeal lipid profiles is often not provided with the description of novel species.

Objectives
In our work, we have analyzed the composition of polar lipids, cellular fatty acids and the GDGT lipids of the several thermophilic archaea (Thermoplasma sp. DSM-1728ᵀ, Desulfurococcus sp. Z-1312ᵀ, and Fervidococcus sp. Kam-940ᵀ), among them are several recently described species.

Methods
Polar lipids were studied by two-dimensional thin-layer chromatography [1,2], GDGT lipids were analyzed by GC-MS after iodination with subsequent reduction by Zn/CH₃COOH [3]. Additionally, native GDGT lipids were analyzed by ESI-MS, and the polar groups of GDGT lipids were sylilated and analyzed by GC-MS.

Conclusions
The polar groups of GDGT lipids of DSM-1728ᵀ contained phosphate, mannose, arabinose and gulose. This is confirmed by glycolipids and glycosphingolipids revealed by TLC.

The GDGT lipids of Thermoplasma acidophilum DSM-1728ᵀ are dominated by GDGT4, which is confirmed by GC-MS analysis of core hydrocarbons. The polar lipid profiles of archaea studied are strikingly different, however, there are common glycosphingolipids for the DSM-1728ᵀ, Z-1312ᵀ, and Kam-940ᵀ strains. The further study of lipids of these archaea by LC-MS will clarify their structures.

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References
USING A FUNCTION-BASED SCREEN TO IDENTIFY ACTIVE RUBISCOS FROM HYDROTHERMAL VENT ENVIRONMENTS: UNRAVELING REGULATION AND ACTIVATION OF A RUBISCO ENCODED ON A METAGENOMIC FRAGMENT

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Background

Rubisco (Ribulose-1.5-bisphosphate Carboxylase/Oxygenase) is a key enzyme in the Calvin Benson Bassham cycle, which accounts for most of Earth’s primary production. Although extensive work on Rubisco enzymes has been conducted in the last decades, we still know relatively little about the regulation and activation of RubisCO enzymes.

Objectives

We have developed a new activity-based screen that enables the identification of recombinant Rubisco active enzymes from metagenomic fosmid libraries. Using this screen we successfully harvested Rubiscos from a metagenomic fosmid library that was constructed from a deep-sea hydrothermal vent. After verifying the Rubisco activity, sequencing of one of the metagenomic fragments revealed a 35 kb DNA insert which encoded a Rubisco form I and form II.

Methods

We investigated the role of adjacent DNA regions on the Rubisco form I and form II regulation and activation by individually deleting each of the flanking genes and measuring the mutant’s activity. Additional experiments with the genes’ transcripts and band shift assays, to assess binding properties of the respective enzymes and genes, have furthered our understanding of how the Rubisco is regulated and likely activated.

Conclusions

This screen and this approach to work with the metagenomic fragments opens the door to directly studying Rubisco from any environmental sample.
UV-RESISTANCE PROFILING OF A POLY-EXTREMOPHILIC GAMMAPROTEOBACTERIUM FROM HIGH-ALTITUDE ANDEAN LAKES

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Background

High-Altitude Andean Lakes (HAAL) at the South American Andes are exposed to high UV irradiation and high content of toxic elements. A wide variety of microorganisms named “extremophiles” were found to inhabit these environments as *Acinetobacter* sp. Ver3¹ and it was chosen as model poly-extremophilic HAAL’s microbe as it displayed high resistance to UV-B.

Objectives

The aim of this work is to integrate diverse experimental approaches to profile the UV resistance mechanisms of Ver3, herein called as “UV-resistome”.

Methods

DSMZ collection strains were used for comparison with Ver3. Strains were exposed to different doses of UV-B, the resistance was determined by colony forming unit counting². Survival, proteomic profiling and biofilm development after the exposure was evaluated by incubating cells under dark (DR) or light (PR) treatments. Genomic data were analyzed identifying components related with the “UV-resistome”.

Conclusions

Our results shown a superior resistance to UV-B radiation of Ver3 than control strains, in all cases the recovery was more efficient after PR, and the genome sequence supported the phenomenological observations. Ver3 highlighted a number of unique genes, such as a novel cryptochrome. An “UV-resistome” was defined, mainly genes related to UV-damage repair³,⁴,⁵ on DNA and genes conferring an enhanced capacity for scavenging the reactive molecular species responsible for oxidative damage. The proteomic profiling of UV-challenged cells identified up-regulated proteins such as a specific cytoplasmic catalase, a putative regulator of quorum sensing, biofilm development and down-regulated proteins related to several energy-generating pathways. This is the first report on a genome from a polyextremophilic *Acinetobacter* strain.
Background
The ability of microbes to thrive in high-temperature environments has prompted researchers to study these microorganisms to better understand their physiological and molecular adaptations and eventual utilization in various biotechnological applications. Natural geothermal springs, including terrestrial hot springs are one of the habitats of thermophilic microbes. On the territory of Nagorno-Karabakh many geothermal springs with different geotectonic origins and different physicochemical properties are found.

Objectives
This research is focused on investigation of the bacterial community structure of previously unexplored geothermal springs of Nagorno-Karabakh, Karvachar (70°C) and Zuar (52°C).

Methods
16S rRNA gene clone libraries were constructed from total community DNA using universal bacterial oligonucleotide primer sets. Sequences were used for phylogenetic assessment of the communities.

Conclusions
Sequence analysis of clones indicated that clones obtained from Karvachar samples originated from phyla *Proteobacteria* (48.6%), *Cyanobacteria* (29.7%), *Bacteroidetes* (5.4%), *Chloroflexi* (5.4%), *Verrucomicrobia* (2.7%) and *Planctomycetes* (2.7%) and clones obtained from Zuar samples originated from phyla *Proteobacteria* (42.3%), *Firmicutes* (19.2%), *Bacteroidetes* (15.4%), *Cyanobacteria* (3.8%), *Tenericutes* (3.8%) and yet unclassified phylotypes (15.4% for Zuar and 3% for Karvachar). The majority of the phylotypes detected in the gene libraries shared less than 95% sequence identity with their closest matches in GenBank, indicating a unique community structure of these geothermal springs. These thermal springs can represent a resource for novel thermophilic organisms and biotechnological tools.

The work was supported by the CPEA-2011/10081 grant from the Norwegian Cooperation Programme in Higher Education with Eurasia.
LIFE ON THE FROZEN CONTINENT: DIVERSITY OF RUBISCO, NIFH AND PUFLM GENES IN SOILS AROUND THE PRINCESS ELISABETH STATION, SØR RONDANE MOUNTAINS, ANTARCTICA.

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Background
In Antarctica, photosynthesis by Cyanobacteria is generally thought to be the main primary source of organic carbon for complex microbial communities. Many cyanobacterial species are also able to fix nitrogen. Therefore, they can survive and prosper in almost every habitat, including Antarctica, Earth's most extreme continent. However, several studies of Antarctic microbial communities have shown that Cyanobacteria are not always highly abundant. We explored the hypothesis that other bacteria must take over their role and produce organic matter as well as fix nitrogen, in order to sustain the microbial community. Light is an abundant energy source during the Antarctic summer and some bacteria can use rhodopsin-type pigments to exploit this, whereas aerobic anoxygenic photosynthetic bacteria can use bacteriochlorophyll for photosynthesis.

Objectives
The presence and diversity of non-cyanobacterial prokaryotes that possess one or several of these properties was studied in terrestrial samples gathered in the proximity of the Belgian Princess Elisabeth Station (Sør Rondane Mountains, Queen Maud Land, East-Antarctica).

Methods
RuBisCO, nifH and pufLM genes were investigated by construction of PCR clone libraries and Illumina MiSeq sequencing.

Conclusions
Preliminary results indicate an extensive diversity of the genes coding for these processes in terrestrial Antarctica.
Background
Soil water repellency is a common phenomenon affecting the hydrological responses of many soil and land use types in different climates. This newly recognised ‘extreme’ environment leads to decreased water infiltration, reduced vegetation cover, fertiliser run off and soil erosion. The fundamental (biological) causes of soil repellency and its dynamic behaviour remain poorly understood.

Objectives
This study aimed to apply metaproteomic and high-resolution imaging approaches to model and predict switches between hydrophilic and hydrophobic soil surface responses.

Methods
Extreme, moderate and sub-critical water-repellent UK grassland soils, including Park Grass at Rothamsted Research, were sampled under wettable and repellent conditions. Soils were subjected to new extraction methods for determining the specific hydrophobic and the general metaproteomes and to Atomic Force Microscopy for determination of topological and adhesion properties.

Conclusions
Using our ultrahydrophobic extraction protocol, we identified novel ultra-hydrophobic microbial proteins, which likely play an important role in the development of soil water repellency. Such proteins could be extracted from moderate and extremely hydrophobic soils with medium-low soil moisture levels, but were absent in the comparable wettable soils. In control extractions, hydrophobic proteins were only extracted from wettable soil containing a positive control, demonstrating the specificity of our novel extraction method. Our newly developed metaproteomic method required only up to 0.5g of soil. Initial comparisons revealed differences in protein profiles, suggesting altered microbial ecophysiology in response to development of water repellency. Atomic force microscopy of repellent soils showed the nanoscale coverage of repellency on particles and aggregates by determining surface adhesion properties from force curves.
Background
Metagenomic studies carried out in salterns located in Alicante and Huelva, Spain, revealed that in intermediate salinities ponds (13-21% of salts) inhabits a dominant bacterial group belonging to Gammaproteobacteria, related to Alkalilimnicola, Arhodomonas and Nitrococcus.

Objectives
On the basis of these metagenomic studies we designed different media and growth conditions in order to isolate this bacterium that have shown to be dominant at intermediate salinities. Subsequently, we studied the strategy of osmoregulation of this successful halophilic bacterium.

Methods
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Conclusions
We were able to isolate a microbe that has been cultured and described as Spiribacter salinus. Its complete genome has been sequenced and the recruitments against the available metagenomes of hypersaline aquatic habitats revealed its abundance in intermediate salinities, decreasing sharply at saturated and low salinities. S. salinus shows a simplified metabolic versatility, missing the chemolithotrophic and carbon fixation pathways.

We have combined physiological approaches and genomic analysis to derive a comprehensive picture of the molecular and cellular events that allow the adaptation of S. salinus to saline environments. A qualitative and quantitative assessment by natural abundance \(^{13}\)C-NMR spectroscopy and high-performance liquid chromatography (HPLC) analysis, respectively, have shown that S. salinus is able to synthesize ectoine when is exposed to high salinity, showing an essentially linear relationship between ectoine content of the cells and the external salinity. We have also tested representatives of multiple transporter families that have been proved to sense and respond to osmotic stress, from ABC transporter as OpuA, BCCT transporter as OpuD and from the TRAP- transporter family, TeaABC.
Background
Heshang Cave is a dissolution cave occurred in Cambrian dolomite. It is characterized by dark, oligotrophic, well-developed stalagmite and continuous dripping water throughout the year.

Objectives
Due to the great progress on the geochemical record and successful recover of microbial geolipids from stalagmite, it poses an urgent need to investigate microbial changes in terms of their relationship with environmental conditions which will help to decipher the microbial information in stalagmite.

Methods
Here we present a five year monitoring results about the variation of microbial communities in dripping waters in Heshang cave via 16S rRNA clone library construction and Biolog analysis of their carbon utilization. Meanwhile pH, temperature, conductivity, dripping rate, regional air temperature and precipitation were measured and collected.

Conclusions
Seasonal variations of bacterial communities were observed in composition, diversity and carbon utilization patterns. In autumn and winter bacterial community was dominated by Gammaproteobacteria, whereas in summer and spring Betaproteobacteria became dominant in water samples collected. Cluster and redundancy analysis indicated that regional air temperature strongly affect bacterial communities among the environmental factors investigated. Moreover the bacterial biodiversity was observed to increase with the temperature rising. These seasonal variation match well with the seasonal variation of microbial fatty acids in dripping waters in Heshang Cave which was also strongly affected by regional temperature. Our results show that microbial communities can response temperature change via multiple ways and the microbial variation recorded in stalagmite may be indicative of palaeo-temperature changes.
Background

The subsurface Boom Clay layer is investigated as a potential host rock for geological disposal of radioactive waste in Belgium. The HADES underground research facility (EIG Euridice c/o SCK•CEN), located at 230 m depth under the site of SCK•CEN (Mol, Belgium), provides access to this clay layer for \textit{in situ} geological, geochemical and geomicrobiological testing.

Objectives

In order to predict how microbiology will affect the biogeochemical processes in a disposal scenario, the resident microbial communities in the man-made structures within Boom Clay are being characterised.

Methods

In this study, water samples were collected from Boom Clay via various existing piezometers (diverse in depth, orientation, location, age, materials used). The aim was to assess differences or shared features of the microbial communities residing in piezometer boreholes, and to correlate variations to geochemical analyses.

Conclusions

Along the five piezometers, bacterial communities of the filters within one piezometer seem more similar to each other compared to those in other piezometers, despite a variety of filter materials or Boom Clay layers sampled within one piezometer. It shows that technical installations (such as piezometers) can introduce and promote local variations in the clay environment and the associated bioprocesses.

Further studies of other piezometers and of clay samples are needed, to pinpoint the source bacterial community underlying \textit{in situ} enrichment, to unravel the mechanism that shapes such microbial community in different repository conditions and to outline the relevance of the (dominant) microbial classes in defining borehole water (and gas) chemistry.
FEMS-3115
Host manipulation and bacterial survival

PHENOL-SOLUBLE MODULINS ALPHA ALTER THE CELL CYCLE OF EUKARYOTIC CELLS.

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Background

*Staphylococcus aureus* (SA) is a Gram-positive bacterium responsible for a wide range of infections in humans and animals. We previously demonstrated that SA USA400 MW2 strain induces a G2/M phase delay in human HeLa cells. This phenomenon was associated with the accumulation of the cyclin-dependent kinase Cdk1/cdc2 and with the accumulation of unphosphorylated histone H3. Additionally we showed that the G2 phase was preferential for staphylococcal internalization and intracellular replication (1).

Objectives

The objectives of the study were

-to identify the active substances, which are responsible for the cell cycle alteration,

-to understand, how this alteration hijacks defense functions of the host cells.

Methods

Using size exclusion chromatography of MW2 supernatant, followed by mass spectroscopy analysis and FACS, we identified phenol-soluble modulin alpha (PSMα) peptides as the likely candidates for cell cycle alteration.

Conclusions

Synthetic PSMα1 and PSMα3 caused a G2/M phase transition delay. The implication of PSMα in cell cycle alteration was confirmed by comparison of wild type LAC wt
strain with the isogenic mutant (LACΔpsmA), lacking the psmA operon that encodes PSMα1 to 4, for its internalization efficiency in HeLa cells. The decreased internalization rate of LACΔpsmA suggested a role of PSMα in host cell invasion.

Furthermore, PSMα-induced G2/M-transition delay correlated with a decrease in the defensin genes expression suggesting a diminution of antibacterial functions of epithelial cells (2). Our results open new perspectives for the investigation of the mechanisms of the SA infection.

o 1 Alekseeva et al. Plos One 2013

o 2 Deplanche et al. FASEB. J 2015
LACTOBACILLUS ACIDOPHILUS1 MAY PREVENT DIARRHEAL DISEASE BY VIBRIO CHOLERAE IN NEWBORN RABBITS
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Background
Acute diarrheal disease-ADD is the second cause of death in the world in children under five years old and particularly in Latin America a good proportion is caused by Vibrio cholerae. The high incidence of ADD produced by V. cholerae is mainly due to antimicrobial resistance, being mainly to get control of cholera with probiotics our scientific interest.

Objectives
To assess the in vitro and in vivo capacity of Lactobacillus acidophilus1 to prevent diarrheal disease caused by V. cholerae 01OGAWA, in newborn rabbits. Our working hypothesis is to evaluate the use of lactic acid bacteria-LAB in the prevention and control of ADD.

Methods
Isolated and identified by biochemical and molecular tests L. acidophilus1, from 35 Colombian children stools. The amylolytic capacity and biomass formation by L. acidophilus1 were standardized previously. With L.acidophilus1, in vitro and in vivo antagonistic activities against the enteropathogen were performed and prevention of diarrheal disease was evaluated by V. cholerae in newborn rabbits.

Conclusions
The isolate was identified as L. acidophilus1, who in an inoculum of 35x10^6 bacteria/ml was able to exert the most antagonistic in vitro effect on V. cholerae. After the Kaplan–Meier estimator, rabbits faced to the pathogen without receiving probiotic had lower survival probability of 0.25 compared to the group of animals challenged to the pathogen and simultaneously fed with probiotic whose probability of survival was 0.95. L. acidophilus1 is considered a probiotic microorganism, able to survive passage through gastrointestinal tract in an animal model and prevent intestinal colonization by V. cholerae in newborn rabbits.
FEMS-2672
Host manipulation and bacterial survival

HETEROLOGOUS EXPRESSION IN BUDDING YEAST OF DIVERSE BACTERIAL TRANSLOCATED EFFECTORS THAT TARGET COMMON CELLULAR COMPARTMENTS
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Background
Facultative and obligate intracellular pathogenic bacteria subvert host cell functions to create an intracellular environment for their replication and persistence. This is achieved by injection of bacterial proteins into the host cell cytoplasm via specialized type 3 or 4 secretion systems, thus reprogramming cellular pathways. Heterologous expression in the Saccharomyces cerevisiae yeast model is often used to understand the function of translocated effectors.

Objectives
We report the expression in S. cerevisiae of over 30 candidate and cognate effectors from Chlamydia trachomatis Coxiella burnetii and Salmonella enterica as N-terminal GFP fusions, aimed to study their subcellular localization as a means to gain insight in their function within eukaryotic cells.

Methods
We present data on co-localization with fluorescent markers and the use of a battery of yeast mutants defective at specific membrane traffic stages to address differential cues recognized by these effectors.

Conclusions
Most effectors showed a ubiquitous cytoplasmic distribution, either diffuse or concentrated in foci. Sometimes, as in Coxiella CaeA, these foci were consistent with accumulates of unfolded or aggregated protein. However, a few particular effectors displayed specific localizations. Notably, Coxiella AnkB was imported into the nucleus and associated to the nucleolus; C. trachomatis CT696, C. burnetii CBU-77 and Salmonella SteA specifically localized to cellular membranes. Particularly, C. trachomatis CT696 decorated the plasma membrane, C. burnetii CBU-77 localized at vacuolar membranes, and Salmonella SteA targeted simultaneously both
membranous systems. The utility of the yeast heterologous system to study the determinants for recognition by bacterial effectors of subcellular eukaryotic compartments will be discussed.
Host manipulation and bacterial survival

C. CANIMORSUS AFFECTS COAGULATION BY REDUCING THE ACTIVITY OF VITAMIN K DEPENDENT CLOTTING FACTORS

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Background

Capnocytophaga canimorsus is a Gram-negative bacterium belonging to the oral flora of dogs. The bacterium causes rare but severe infections in humans that have been bitten or licked. Patients frequently develop severe sepsis with disseminated intravascular coagulation (DIC).

Objectives

Considering that several bacteria interact with coagulation factors or platelets, as well as the frequency of bleeding abnormalities in C. canimorsus infection, we were interested if C. canimorsus interferes with coagulation.

Methods

We incubated normal pooled plasma (NPP) with C. canimorsus strain 5 (Cc5) and monitored thrombin generation by a calibrated automated thrombogram (CAT) assay. Additionally, clotting times of Cc5 treated NPP were measured. Factor deficient plasma was used to assess the activity of individual coagulation factors in Cc5 treated NPP.

Conclusions

Cc5 inhibited thrombin generation and clotting times of NPP incubated with Cc5 were significantly increased. However, pre-treatment of Cc5 with the irreversible serine protease inhibitor AEBSF completely abolished this increase.

We observed a specific impairment of the Vitamin K dependent (VKD) factors, FX, FIX, FII and FVII. We could also show that FX was cleaved by Cc5.

To conclude, our findings suggest a proteolytic mechanism by which Cc5 affects coagulation and which causes degradation of FX.

We are now interested in finding the cleavage site, to see if the other VKD clotting factors are equally degraded and to identify the bacterial protease which mediates the cleavage.

Inhibition of coagulation could promote bacterial dissemination and also aggravate DIC associated bleeding.
Vibrio vulnificus detected in the spleen leads to fatal outcome in a mouse oral infection model.

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Background
Vibrio vulnificus causes rapid disseminating septicemia by oral infection in infected individuals who have an underlying disease, especially chronic liver diseases. Although the elucidation of specific risk factors for V. vulnificus infection in patients with liver diseases is of urgent importance, no appropriate experimental animal model that mimics the liver diseases in this bacterial infection has been available so far.

Objectives
Discover the risk factors for V. vulnificus infection in liver diseases patients.

Methods
To discover these risk factors, we generated a liver disordered mouse by performing bile duct ligation (BDL). The BDL mice were infected with the V. vulnificus by orogastric route.

Conclusions
Hepatitis developed in the BDL mice, however this did not affect mortality in mice after orogastric administration of V. vulnificus, suggesting that the liver disorders caused by the BDL were not risk factors for V. vulnificus septicemia. When the dead and surviving mice were compared, V. vulnificus could be detected from the spleen only in the dead group. Furthermore, significantly higher numbers of V. vulnificus were detected from the intestines in the dead group than in the surviving group (p<0.001). These findings suggested that proliferation of the challenge inoculum in the intestine was needed for the oral infection with V. vulnificus, and that the elimination of V. vulnificus in the liver and/or spleen plays a critical role in survival of the host.
EFFECT OF HYPOBARIC HYPOXIA ON MICROBIAL HOMEOSTASIS AND PATHOLOGICAL CONSEQUENCES IN GUT

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Background
At high altitude (HA), hypobaric hypoxia is a hallmark of environmental stress that faced by millions of people like pilgrims, trekkers, scientists and military personnel and they generally suffer from a group of non-specific gastrointestinal complications like anorexia, dyspepsia, nausea, acidity, haematemesis, peptic ulcers, etc. These diseases are mostly related to the alteration of intestinal microbes and their related phenomenon.

Objectives
This experiment was conducted to explore the relationship between the altered atmospheric pressure with the microbial composition, which is the major functional counterpart of GI tract.

Methods
Albino mice were subjected to hypobaric atmospheric pressures (55kpa) in a chemostat for 15 days and after dissection intestinal luminal contents were collected. The population of indicator bacteria were monitored by selective culture based methods and DGGE. The expression of inflammatory markers of intestinal epithelium were evaluated by qPCR and western blot.

Conclusions
The quantity of facultative anaerobes like *Escherichia coli*, other strict (*Bacteroidetes sp.* and *Lactobacillus sp.*) and obligate (*Clostridium perfringens*, *Peptostreptococcus sp*) anaerobes were increased in many folds after exposure to hypoxic environment. The increased level of bacteria and their endotoxins activated p38 MAP kinase and TLR4 pathways which are related to the over expression of inflammatory cytokines and mediators like IL23, IL17F, TNF α, INOS, COX2 and HIF 1α. As a result mucosal layers of intestinal wall was severely inflamed and perforated which also documented by SEM studies. These consequences are greatly related to the prognosis of different gastrointestinal ailments developed during hypobaric hypoxic stress at high altitude.
IRON ACQUISITION MECHANISMS OF THE CYSTIC FIBROSIS (CF) PATHOGEN BURKHOLDERIA CENOCEPACIA AND THE CORRESPONDING HOST RESPONSE

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Background
B. cenocepacia, is a highly antibiotic resistant CF pathogen which produces low molecular weight iron chelators known as siderophores, predominantly ornibactin, to sequester iron in the host. In response to bacterial and fungal infection, mammals produce lipocalins, some of which bind siderophores.

Objectives
To characterise ornibactin production by B. cenocepacia under different conditions and the resulting host lipocalin response to ferrated ornibactin and to investigate alternative iron acquisition mechanisms by this pathogen.

Methods
Ornibactin gene expression detected using real-time PCR and siderophore levels measured using the CAS assay. Utilisation of iron sources assessed using growth assays. Lipocalin 1 (LCN1) was expressed in E. coli using the Champion™ pET 100 vector system.

Conclusions
This study demonstrated that in iron-depleted cultures siderophore production by B. cenocepacia is significantly upregulated by 4 h (P<0.001) with maximal production by 5 h. Furthermore, B. cenocepacia can acquire iron from exogenous sources such as host iron binding proteins including ferritin and hemin, and from the Aspergillus fumigatus xenosiderophores, fusaricine C and triacetylfusaricine C demonstrating a multifaceted iron acquisition strategy. Host response investigations have determined that recombinant LCN1 binds ferric-ornibactin with high affinity and not unferrated ornibactin. Ongoing studies are examining cellular lipocalin responses and the utilisation of additional xenosiderophores. Understanding the iron acquisition strategies and host responses to this pathogen is warranted given the potential to prevent colonisation by this pathogen if iron acquisition can be compromised.
Host manipulation and bacterial survival

INFECTION OF CAMPYLOBACTER JEUNI REDUCES CFTR MEDIATED CL- SECRETION IN T-84


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Background

Campylobacter jejuni is the common bacteria cause acute gastroenteritis in humans. It has been reported that the C. jejuni-infection was caused by very low infective dose, and it suggested that C. jejuni might have a resistance system against bacterial clearance system in intestinal tract. The mucus layer in the intestinal tract is the first defense system against bacterial infection, and the condition is well maintained by water movement. Cl- transport is closely associated with water efflux on intestine. Cystic fibrosis transmembrane conductance regulator (CFTR) is the major Cl- secretory pathway which is activated by cAMP. Dysfunction of CFTR causes dehydration of mucus and accumulation of bacteria in the intestine.

Objectives

CFTR is tightly related with infection of pathogenic bacteria. However, it is not clear how CFTR related with the C. jejuni-infection. To elucidate the role of CFTR on C. jejuni-infection, we investigated the Cl- secretion in T-84 on infection of C. jejuni.

Methods

Cl- secretion was measured by 125I- efflux on T-84 cells. C. jejuni-infection did not change 125I- effluxes in steady state. Next, we confirmed forskolin or prostaglandine E2, agonists of cAMP-dependent Cl- secretion, activated 125I- effluxes, which inhibited by CFTR inhibitor.

Conclusions

These indicated that C. jejuni-infection suppressed the activation of Cl- secretion dependent on CFTR. There were hypothesized that C. jejuni-infection effected on water movement and the mucus dehydration, which was related with bacterial clearance system in intestinal tract.
Host manipulation and bacterial survival

A STRATEGY OF PATHOGEN STREPTOCOCCUS ESCAPE MAMMALIAN PEPTIDOGLYCAN RECOGNITION PROTEINS KILLING

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Background
Our previous work showed that a new GI-type IVC secretion system location in the GI similar as previous described an 89K GI-like structure of S. suis named S4GIs is popular in the genus streptococcus.

Objectives
To reveal an unknown mechanism of a novel family of peptidyl-proly isomerases (PPIases) named SP1 from S4GIs interact with mammalian peptidoglycan recognition proteins (PGRPs).

Methods
In this study, we show that the function of killing bacteria of innate immunity proteins PGRPs could be blocked by protein SP1. We confirmed intracellular concentration of SP1 from streptococcus depends on secretion channel of S4G and increased expression of SP1 could be regulated by the concentration of PGRPs in environment using ELISA and qRT-PCR.

Conclusions
In conclusion, this study indicated a new escape way of pathogenicity Streptococcus protects against mammalian innate immunity proteins killing.
Background

Biofilms are highly diverse and complex multispecies microbial assemblages with characteristic spatial, i.e. three-dimensional organization. Elucidating function of a biofilm community would require a detailed characterization not only of the spatial distribution of the sessile organisms but also their respective metabolites.

Objectives

Mass spectrometry (MS) of homogenized biofilms (and other methods) have shown the importance of bacterial metabolites in the corrosion process. However, as seen in the profilometry map of a corroded coupon (Figure 1), the corrosion and the biofilm that causes it are not homogeneous. In order to gain a better understanding of the MIC process, we need to develop an instrument that can combine MS and spacial information.

Methods
An ambient mass spectrometry imaging (MSI) system was developed specifically for imaging living biofilms on corroding lab or field samples. The Laser Ablation and Solvent Capture by Aerosol (LASCA) instrument uses an IR laser to collect a 200 μm spot from the sample and analyzes it using a high resolution QToF. A single spot is analyzed in less than 10 seconds, allowing for thousands of spots to be analyzed and built into an ion image (Figure 2). The laser can be tuned to remove a specific

\[ \text{Image 1} \]

\[ \text{Image 2} \]

Conclusions

We present a system that can create ion images of thick, uneven biofilms in 3 dimensions. We demonstrate the use of LASCA with the correlation of ion images to corrosion damage. Further, method to probe the penetration depth of biocides into a biofilm is presented.
FLAVIN-BINDING FLUORESCENT PROTEINS - ADVANCED IN VIVO ANALYSIS OF BIOLOGICAL PROCESSES WITH HIGH SPATIO-TEMPORAL RESOLUTION
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Background
Genetically encoded fluorescent proteins (FPs), like GFP from the jellyfish Aequorea victoria, became one of the most variable and popular in vivo reporters in cell biology, microbiology and biomedicine. They can easily be detected by in vivo fluorescence techniques thus enabling the non-invasive analysis of complex cellular processes. However, beside the large size and relative slow maturation, a major drawback of GFP and its variants is their strict limitation to aerobic biological systems. This is primarily due to the fact that the autocatalytic synthesis of the chromophore depends on molecular oxygen.

Objectives
To conquer these limitations, we developed a new class of small cyan-green fluorescent proteins which can be used in microbes and mammalian cells under aerobic, oxygen-limited as well as anaerobic conditions. These FPs carry flavin mononucleotide (FMN) as chromophore and are thus termed FMN-binding fluorescent proteins (FbFPs).

Methods
To evaluate the applicability of FbFPs, the photophysical properties including the fluorescence spectra and brightness have been characterized and further improved. Furthermore, in vivo studies demonstrated their broad usability as reporter in (facultative) anaerobic bacteria and for bioprocess engineering. In addition, FbFPs were also be used to generate novel genetically encoded biosensors allowing the ratiometric intracellular monitoring of essential environmental parameters including molecular oxygen and pH.

Conclusions
The unique properties of FbFPs render them particularly valuable as in vivo tools for fluorescence imaging, quantitative in vivo analysis of gene regulation, protein
localization and bio-sensing.
Background

Structures of macromolecular complexes are traditionally solved by X-ray crystallography, requiring crystals and bright X-ray sources to obtain resolutions at which side-chain densities can be seen. Last year, a revolution has taken place in the field of single-particle cryo-electron microscopy (EM), resulting in numerous high-resolution un-crystallisable structures. These breakthroughs have been made possible by the conjunction of a new generation of microscopes, detectors, and software.

In single-particle EM, thousands of noisy images of individual biomolecules are computationally combined into one 3D model. Best results are obtained when the sample of interest has been extensively purified and stabilized. During these steps, the biological context disappears, interacting partners dissociate, and unforeseen conformations could be induced.

Objectives

Ideally, single-particle like methods could be applied to biomolecules within their native environment. Unfortunately, almost all eukaryotic cells are too thick to be imaged by EM.

Our lab has set the long term goal to derive structures from biomolecules within their cellular environment. We label complexes with fluorescent tags to localize regions of interest (ROI) within vitrified cells. The coordinates of these ROIs are, together with the sample, transferred under cryogenic conditions into a dual-beam FIB/SEM system to trim the ROIs into ca 150nm thin lamella. Finally, the trimmed sample is cryogenically transferred to a high-end transmission EM for subsequent imaging at different orientations.

Methods

In here, we will report on the first results obtained with our cryo-correlative workflow on mycobacteria.

Conclusions
We discuss challenges and opportunities, the latter including developments such as phase plates and super-resolution microscopy.
AUTOMATED SEGMENTATION OF INDIVIDUAL CELLS AT PHASE CONTRAST MICROSCOPY IMAGES

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Background

Nowadays, computer processing of microscopic images is becoming more common as a part of research in the field of cell science, replacing slow and time-consuming manual processing by human experts. A challenging task is for example to process images of live mammalian cancer cells from a phase contrast microscope.

Objectives

When processing the time-lapse images of live cells, the main goal is to describe the movement and behaviour of individual cells. There are many methods available in the cases when individual cells are separated from each other, However, separating individual cells from colonies or even from cell monolayers is much more difficult.
Methods

We developed a novel algorithm for segmentation of individual cells. First part separate the cells from the background and it is based on the differences in time between consecutive images and a combination of sophisticated thresholding, blurring, and morphological operations. It is fast and precise. The second part of our algorithm separates individual cells in the clusters. It uses the halos between cells (thresholding and modified skeletonization) and fills the missing parts by connecting the hanging branches of the skeleton via Dijkstra algorithm.

Conclusions

We tested the algorithm on images of four cell types acquired by two different microscopes, evaluated the precision of segmentation against manual segmentation performed by a human operator. We created the software which implements our segmentation method. We added the possibility to modify the resulting segmentation. User can modify the result by merging or splitting the cell regions that was found by out algorithm.
STRUCTURAL STUDIES OF LEGIONELLA EFFECTORS

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Background

Legionella pneumophila is a Gram-negative bacterium and is the causative agent of Legionnaires’ disease. The Dot/Icm type IV secretion system (T4SS) is one of the key virulence factors of L. Pneumophila. By transferring effector proteins into the host cell, the bacterium is able to replicate inside human alveolar macrophages by rapidly altering the normal host endocytic pathway after uptake. Currently, about 300 Icm/Dot dependent effectors have been identified in L. pneumophila; however, the biochemical functions for most of these proteins remain unknown.

Objectives

Lpg1496 is an effector protein containing a conserved sequence of the SidE family in its C-terminal region. The SidE family members are present early during intracellular replication and localize to the cytoplasmic face of the replicative phagosome upon translocation. No information on other regions of lpg1496 has been available.

Methods

We are exploring the three-dimensional structure of lpg1496 to probe for possible biochemical functions using structural similarity to proteins or domains of known function.

Conclusions

Here, we identified two novel regions of sequence similarity in lpg1496 and showed that they form independently folded domains. Moreover, we have determined high-resolution crystal structures of these domains. Each domain consists of two α-helices flanked by four β-strands on one side and four β-strands on the other side. Furthermore, we obtained the structure of the conserved C-terminal domain that shows structural similarity to HD domains and possesses phosphodiesterase activity. These studies will lead to a better understanding of the functional role of lpg1496 in Legionella pneumophila, and may contribute to the development of novel therapeutic treatments for Legionnaires’ disease.
NOVEL BACILLUS THURINGIENSIS ISOLATES WITH LARVICIDAL ACTIVITY AGAINST THE MOSQUITO VECTOR, CULEX PIPENS

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Background

The outbreak of mosquito vectors, the incidence of mosquito-borne diseases and the resistance of mosquitoes to conventional pesticides have recently caused a panic to the official authorities in the endemic countries. The bacterium Bacillus thuringiensis (Bt), a safe eco-friendly entomopathogenic biocontrol agent, is widely used to complement the chemical control in integrated mosquito control measures.

Objectives

This study was conducted to identify native mosquitocidal Bt isolates to be utilized in the battle against insecticides-resistant mosquito populations.

Methods

Bt isolates have been recovered from different environmental samples collected from different locations throughout Saudi Arabia. Their mosquitocidal activities were tested against Culex pipiens ³rd instar larvae compared to that of the reference B. t. israelensis (Bti-H14) strain. Three isolates were almost as active as Bti-H14, showing LC₅₀ of 4.2-4.8 µg/ml. Seven isolates showed 1.6-5.4 times more toxic than that of Bti-H14 (LC₅₀ of 0.91-3.03 v 4.88 µg/ml). Histopathological examination of the Bt-63 isolate-treated larvae showed cellular and subcellular alternations in midgut epithelia similar to that caused by Bti-H14.

Conclusions

Data of this study showed that spore-crystal mixtures of 7 native locally isolated Bt were higher mosquito larvicidal compared to that of Bti-H14. Molecular characterization of these potentially active isolates and their toxic parasporal protein crystals are currently being investigated. This study may lead to the identification of mosquitocidal Bt isolate(s) that could contribute to the battle against mosquito
vectors.
BACKGROUND
The evolution of resistance in agricultural pests to Cry proteins threatens the sustainable use of genetically modified Bt crops. The specific mechanisms of resistance are not well understood. According to the gut microbiota theory, extensive cell lysis caused by Cry proteins provide gut microbes access to the hemocoel where they germinate and reproduce, causing septicemia and death of the host.

OBJECTIVES
The main objective of this study was to determine whether microbes present in the mid-gut of Busseola fusca influence the efficacy of Cry 1Ab proteins.

METHODS
Larvae were collected from maize fields and dissected to excise the mid-gut. Mid-gut contents (intestinal bacteria) were enumerated on general media. Different morphological types were selected to test the antibiotic susceptibility of the bacteria. The most effective bacteriostatic and bactericidal antibiotics were used and the morphological types were exposed to different concentrations of these to visualise the effects of the antibiotics. This consisted of growth curve studies on all the selected bacteria. A mixture of ciprofloxacin, ampicillin and doxycycline (500 µg/ml) was incorporated into an artificial diet. Stemborer larvae were allowed to feed on this for 7 days. These larvae were then placed on Bt maize (MON810) plant material expressing Cry proteins. Larvae actively fed on the plant material.

CONCLUSIONS
Results suggest that by placing antibiotic reared larvae on a Bt plant, the absence of the mid-gut microbes contributed to larval survival on Bt maize.
Background
Genetically modified maize plants are engineered to express insecticidal toxins derived from the bacterium *Bacillus thuringiensis*. However, field-evolved resistance of African maize stem borers (*Busseola fusca*) against Bt-maize has developed and spread throughout South Africa. Studies suggested (1) that gut bacteria are required for *B. thuringiensis*-induced mortality in most Lepidoptera species and (2) that the toxicity of *B. thuringiensis* depends on microbial community interactions within the gut.

Objectives
This study aimed to identify the diversity of gut-associated bacteria by both culture-dependent and culture-independent approaches.

Methods
A total of 133 bacterial strains were isolated from the midgut of *B. fusca* larvae collected from 30 different sites. Molecular phylogenetic analyses of 16S rRNA gene sequences revealed bacteria affiliated to *Proteobacteria*, *Actinobacteria*, and *Firmicutes*. Taxonomic distribution of these sequences placed the isolates into 20 different genera. The majority of bacteria identified were belongs to the genera *Enterococcus*, *Klebsiella*, and *Bacillus*. Culture-independent methods involved the denaturing gradient gel electrophoresis fingerprinting (DGGE), and sequence analyses of a 16S rRNA gene from the excised band supported culture recovery results. However, additional bacterial taxa not determined via culture recovery were revealed using this methodology and included members of the genera *Chryseobacterium*, *Lactobacillus* *Lactococcus*, and *Spiroplasma*.

Conclusions
Some sequences represent hitherto uncharacterized novel organisms that may have not been characterised yet. The *Busseola fusca* gut represents an intriguing and unexplored niche for analyzing microbial ecology, which will provide opportunities for research involving the impact of diverse and dynamic microbial communities on developing resistance against Bt-maize.
Background

The cat flea, *Ctenocephalides felis*, is the most common flea species found on cats and dogs worldwide parasitizing a wide range of hosts. It has long been recognized that many arthropods carry passenger micro-organisms. *Wolbachia* alone infects in excess of 20% of insect species at any point in time. *Wolbachia*-infected species have been found to harbour lower levels of mtDNA diversity in closely related uninfected species.

Objectives

At the present work we carried out a molecular and phylogeographical study of *Ctenocephalides felis* and *C. canis* from different geographical regions (Spain, South Africa and Iran). Furthermore, the presence of *Wolbachia* sp. and its influence in these populations has been tested.

Methods

Molecular studies were based on the amplification and sequentiation of ribosomal DNA (Internal Transcribed Spacer 2, and 18S gene) and mitochondrial DNA (*cytochrome oxidase c-1* gene).

Conclusions

The presence of *Wolbachia* sp. was detected in the majority of fleas from different localities. The ribosomal data showed a great homology between all the populations of *C. felis* regardless the geographical origin but significative differences respect to *C. canis*. Nevertheless, *cytochrome oxidase c-1* sequences revealed significative differences in the population of *C. felis* from South Africa, appearing these sequences with high homology with that of *C. canis*. The endosymbiont *Wolbachia* could be responsible for selective sweeps on mtDNA variability within species. We suggest an introgression of mtDNA and *Wolbachia* between *C. felis* and *C. canis*.
Background

Tsetse flies are the sole cyclical vectors of the African trypanosomosis that causes sleeping sickness in humans and nagana in animals. *Glossina* species have established symbiotic relationships with three bacterial species: *Wigglesworthia glossinidia*, *Sodalis glossinidius*, and *Wolbachia pipientis*, mainly known as a reproductive symbiont. As salivary gland hypertrophy virus that affects reproduction of infected flies has been reported. Vector control remains the most effective approach for sustainable management of African trypanosomosis. The sterile insect technique (SIT) has proven to be such an effective method and it is based on the mass production of the targeted insect, the sterilization by irradiation, and the sequential release of sterile males.

Objectives

Such releases in endemic areas might increase the disease incidence before achieving eradication. Therefore, the development of symbiont-based strategies to produce tsetse strains refractory to trypanosome infection would be ideal for SIT programmes. One approach which is currently under consideration is to modify *Sodalis* to produce anti-trypanosome factor(s) in the released sterile males.

Methods

We investigated the impact of irradiation on the establishment of *Sodalis* in the tsetse fly *Glossina morsitans morsitans*.

Conclusions

The results indicate that irradiating 5-7 days old male flies with 110 Gy does not increase the mutation rate, as assessed in 15 genes, but it does have a negative impact on the replication rate of *Sodalis* and the salivary gland hypertrophy virus. In contrast, the irradiation treatment increased the prevalence of *Wolbachia*. These data are discussed in the frame of combining a symbiont-based and SIT-based control approaches.
FEMS-0933
Insect-microbes interactions

QUALITATIVE AND QUANTITATIVE ANALYSIS OF MICROBIAL DIVERSITY BY PYROSEQUENCING IN TETRANYCHUS URTICAE

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Background

Because of its extreme polyphagous nature, Tetranychus urticae is a good model organism to study the rapid adaptation mechanisms to various kinds of host plant toxins and acaricides. The symbiotic microorganism has been known as a pivotal factor in such chemical adaptation of many invertebrates. In T. urticae, several symbiotic bacteria (Wolbachia, Cardinium, etc) have been identified but research has been limited to the elucidation of their functional roles in cytoplasmic incompatibility.

Objectives

Analysis of overall symbiotic bacterial composition in T. urticae is necessary as a cornerstone for the integrative study on its host adaptation and acaricide resistance development.

Methods

16S rRNA pyrosequencing was conducted for nine T. urticae populations with different acaricide resistance properties (7 green types and 2 red types) with GS Junior Sequencing system (Roche, Branford, CT, USA) and data analysis was performed by CLcommunity program (Chunlab Inc., Seoul, Korea).

Conclusions

The operational taxonomic unit by CD-HIT and TBC methods were estimated as 85.3±22 and 737±220 in average, respectively. Most bacterial species (>99%) were categorized in the order Rickettsiales mainly composed of Wolbachia spp. and Rickettsia spp. except for the acaricide-susceptible UD strain. The UD strain was composed of Wolbachia spp. (43.4%) and Flavobacterium (53.6%). Green- and red-type mites were grouped into different clades in the cluster analysis using the quantitative and qualitative traits, suggesting that the microbial diversity might be related with the phenotypic characters of body color. Further analysis would be
necessary to elucidate the functional roles of endosymbiont bacteria in regulating the intrinsic physiology of *T. urticae*.
ANALYSIS OF YEAST-LIKE SYMBIOTE DIVERSITY IN THE BROWN PLANTHOPPER (BPH), NILAPARVATA LUGENS STÅL, USING A NOVEL NESTED PCR-DGGE PROTOCOL
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Background
Yeast-like symbiotes (YLS) are endosymbionts that are intimately associated with the growth, development, reproduction of their host, the brown planthopper, *Nilaparvata lugens* (Hemiptera: Delphacidae). However, it is unclear how many species of YLS are found within *N. lugens* and how they are related to each other.

Objectives
In order to overcome the difficulty in detecting low numbers of YLS in BPH or culturing the YLS *in vitro*, in this study, we developed a novel strategy, nested PCR–DGGE approach, which was used to analyze the diversity of YLS.

Methods
The nested PCR protocol was developed as follows: firstly, the 18S rDNA gene and 5.8S-ITS gene were amplified using fungal universal primers. Subsequently, these products were used as a template in a second PCR with primers ITS1GC-ITS2, ITS1FGC-ITS2 and NFGC-NR, which was suitable for DGGE. Using this highly specific molecular approach, we found several previously detected fungi: *Noda*, *Pichia guilliermondii*, *Candida* sp., and some previously undetected fungi, such as *Saccharomycetales* sp., *Debaryomyces Hansenii* and some uncultured fungi.

Conclusions
To the best of our knowledge, this is the first study to analyze the diversity of YLS in BPH by using the PCR-DGGE system. This methodological approach can be used to assess the relationship between variation in planthopper performance and YLS community.
GENETIC BASIS OF CYTOPLASMIC INCOMPATIBILITY CAUSED BY CARDINIUM

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Background

Endosymbionts of arthropods that cause cytoplasmic incompatibility (CI) manipulate host reproduction: bacteria in infected males sabotage the reproduction of uninfected mates, such that the relative fitness of uninfected females is depressed. Cardinium (phylum: Bacteroidetes) is the only bacterial lineage besides Wolbachia known to cause CI.

Objectives

Identify genes involved with CI and host interactions by transcriptional profiling of a CI-inducing Cardinium in male and female parasitoid wasps (Encarsia pergandiella) and genome sequencing of four Cardinium strains causing different phenotypes.

Methods

RNA was isolated from 1-3 day old male and female Encarsia pergandiella and bacterial mRNA was enriched using the Ribo-Zero™ Magnetic Gold Epidemiology Kit. Female and male samples were sequenced with Illumina Hi-Seq. The transcriptome was analyzed by mapping reads against the Cardinium hertigii genome. Cardinium genomes were also sequenced with Illumina HiSeq, assembled using reference-based and de novo assembly strategies and annotated using RAST.

Conclusions

First genome comparisons revealed evidence for distinct differences between Cardinium strains causing different phenotypes such as the absence of the biotin synthesis pathway in parthenogenesis-inducing Cardinium. Currently, the metatranscriptome is analyzed in more detail, focusing on sex-specific gene expression of candidate proteins for CI and eukaryotic cell cycle regulation. A ubiquitin-specific protease and a ubiquitin ligase, genes of the putative anti-feeding prophage, and other genes involved in host cell interaction were highly expressed. We expect that the results of the transcriptome sequencing and comparison of Cardinium genomes causing different phenotypes will reveal first deep insights into the mechanisms of reproductive manipulation in Cardinium.
Insect-microbes interactions

DIETARY AND PHYLOGENETIC DETERMINANTS OF GUT COMMUNITY STRUCTURE IN HIGHER TERMITES

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Background

The microbial symbionts of termites play critical roles in the digestion of lignocellulose. Unlike wood-feeding lower termites, which are associated with cellulolytic flagellates, higher termites harbor an entirely prokaryotic gut microbiota. Simultaneously, they have considerably extended the range of their diet to lignocellulosic plant litter in various stages of humification. Since the same dietary specializations have evolved in different evolutionary lineages, higher termites offer the unique opportunity to study potential drivers of microbial community structure in the intestinal environment.

Objectives

To assess the influence of host phylogeny and diet on the composition of the termite gut microbiota.

Methods

We analyzed the bacterial microbiota in the hindgut of 19 higher termite species from different feeding guilds using Illumina sequencing of amplified 16S rRNA genes. Sequence reads were taxonomically classified using a curated reference database and subjected to phylogenetic and statistical analysis.

Conclusions

The high similarity in the bacterial gut microbiota among the wood-feeding and humivorous members of different host lineages identified diet as a strong determinant of microbial community structure in higher termites. At higher taxonomic resolution, however, individual bacterial taxa showed a strong specificity for certain host groups, suggesting they are coevolving with their respective hosts. Nevertheless, evidence of co-cladogenesis is scarce and most bacterial lineages may not co-speciate with their respective hosts over a longer evolutionary time. Rather, the observed patterns of
host restriction seem to be enforced by a combined selection by microhabitat and ecological niche, enhanced by a vertical transmission of symbionts facilitated by the social lifestyle.
Δ-ENDOTOXINS OF BACILLUS THURINGIENSIS FORM AMYLOID FIBRILS THERE ARE INVOLVED IN THE FORMATION OF PARASPORAL CRYSTALS

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Background

The δ-endotoxins of the entomopathogenic bacterium Bacillus thuringiensis are multifunctional, so they are not only with highly specificity destroy invertebrate sensitive cells, but have an antimicrobial effect, leading to the destruction of cell walls and cytoplasmic membranes of some microorganisms like activities of others antimicrobial peptides and proteins (1, 2). We discovered for the first time the amyloid fibrils (AF) relating with parasporal crystals of B. thuringiensis.

Objectives

The crystals of B. thuringiensis subspecies: kurstaki, israelensis, amagiensis were the objects of our study.

Methods

Crystals and AF we studied using transmission electron microscopy. To confirm the formation of AF observed their interaction with the dye Congo red in the polarization interference microscope. Molecular mass of proteins was determined by PAGE electrophoresis.

Conclusions

δ-Endotoxins are capable to forming the AF having different thicknesses (like the previously studied AF). The degree of ordering of the AF increased near the crystals. Our research have given us reason to believe that the B. thuringiensis AF are involved in the formation of parasporal crystals. We discuss the ecological role and practical importance of these AF.

TARGETING GUT MICROBES: NOVEL APPROACH FOR INSECT CONTROL

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Background

Several insects are significantly affecting global economy through insect borne diseases in Agricultural and public health sector. Developing resistance towards frontline insecticides is the major challenge in insect control. Metabolic resistance is one of the major forms of resistance mechanisms. Role of insect gut microbes in supporting normal growth and development of insect is well documented. However, the role of insect gut microbes in developing resistance & favoring insect adaptation has not yet been deeply investigated. Targeting insect gut microbes could lead to unforeseen avenues for effective management of harmful insects.

Objectives

To development novel strategy of insect control by targeting insect gut microbes.

Methods

Two model insects are used in the present study malaria vector Anopheles stephensi and cotton pest mealybug Maconellicoccus hirsutus. Insect guts were cured with selected antibiotic treatment. These cured mosquito larvae’s and Mealybug were observed for pesticide susceptibility. M. hirsutus were observed for fecundity, crawler and adult development wax content. While An. stephensi were tested for altered susceptibility against Bacillus thuringiensis israelensis treatment

Conclusions

We observed that gut microbes cured mosquito larvae and Mealy bug became more susceptible to pesticide and environmental factors. Hence we put forward interesting effective vector or pest control strategy by targeting gut microbes.
MICROBIAL DIVERSITY IN GALL-INDUCED AND NON-INDUCED APICAL BRANCHES OF HAPLOPAPPUS FOLIOSUS (ASTERACEAE)

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Background

Plant galls are abnormal vegetative growths that involve the participation of insect, bacteria, fungi and nematodes to induce growth. The role of microbial associations in gall formation are however, unknown.

Objectives

Our objective was to describe and compare the culturable diversity of bacteria and fungi from galled and non-galled apical branches in *Haplopappus foliosus*, an endemic flowering plant of the Asteraceae from Chile.

Methods

Fungal and bacterial isolates were grown in Sabouraud and enriched nutrient broth respectively. Molecular identification of the isolates was performed by analysis of 16SrRNA, 18SrRNA and ITS DNA genes analysis. Bioactive fungal secretions were analysed by HPLC-MS.

Conclusions

Our results indicate that there was a significant difference in microbial communities associated with cecidia and non-galled tissue, comprising 72% and 28% respectively. According to the Margalef index (10.25 galls and 1.95 non-galled) there was a high presence of fungi in galls 52% (n=31), bacteria 43% (n=26) and yeast 5% (n=3). Interestingly, some bacterial isolates have been described as PGPR, such as *Massilia sp.* and *Bacillus simplex*, and cellulose degrading *Cellulomonas denverensis*. Fungal isolates, have been characterized by their capacity to secrete bioactive compounds and we are currently using analytical techniques to determine potential function of these secretions in the formation of the gall association.
Knowing the microbial composition in galled and non-galled tissue will allow us to identify potential key roles in the formation of cecidia structures and develop future biotechnological strategies to prevent gall formation in endemic plants of the region.
Background
There are organisms of different complexity, which can survive complete water loss conditions. Among them are bacterial and fungal spores, plant seeds, nematodes, rotifers, tardigrada. The most complex known organism with ability to withstand severe dehydration is larvae of African chironomid P. vanderplanki. During water depletion, larvae cells pump out water and accumulate trehalose and a number of protective proteins – Lea, Hsp, thioredoxins, etc.

Objectives
Here we attempted to investigate the bacterial community inhabiting the gut of sleeping chironomid Polypedilum vanderplanki, an insect able to survive complete dehydration by induction of special metabolic state anhydrobiosis.

Methods
To assess the variability of gut microbiota in P. vanderplanki larvae, we performed 454 pyrosequencing of 16S rRNA gene.

Conclusions
Comparison of larvae maintained under controlled laboratory conditions for more than 10 years and freshly collected from wild allowed to identify the core microbiota. Interestingly, representatives of larvae core microbiota were absent in soil samples suggesting true associations with host. By production of germ free larvae we showed that the absence of bacteria does not affect the rate of successful anhydrobiosys. However, sterile animals are suffering from the fungal infestation. We revealed that some isolated bacterial strains have antagonistic activity against fungi. Moreover, we found that during rehydration millions of virus-like particles are accumulating in the interspace between gut epithelium and perithrofic membrane. These viruses could promote the development of anhydrobiosys phenomenon in P. vanderplanki insect.

The work is performed according to the Russian Government Program of Competitive Growth of Kazan Federal University.
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Insect-microbes interactions

METABOLIC ACTIVITIES OF STRICTLY AND FACULTATIVELY ANAEROBIC GUT BACTERIA AND THEIR INTERACTIONS IN A GERM-FREE COCKROACH MODEL

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Background

The diverse microbial communities in the guts of termites and cockroaches represent a complex metabolic network of individual populations. In view of the special microenvironmental conditions within the gut and possible interactions among the microbiota, it is essential to investigate in how far the metabolic properties of pure cultures reflect their activities in their natural environment.

Objectives

To develop a protocol for the inoculation of germ-free cockroaches with defined cultures of autochthonous gut bacteria and to compare the metabolic profiles of the isolates in pure culture (in vitro) with their activities and interactions in the gut environment (in situ).

Methods

Pure cultures of gut bacteria were isolated from the cockroach Shelfordella lateralis. After inoculation of germ-free cockroaches, the strains were quantitated and localized in situ using qPCR, GFP-fluorescence and FISH. Gut conditions and metabolites were analyzed using microsensors, HPLC and GC.

Conclusions

The germ-free cockroach model provides first insights into the factors affecting the metabolism of the gut microbiota in their native environment. The strictly anaerobic Fusobacterium sp. (strain FuSL) and the facultatively anaerobic enterobacterium (strain EbSL) exclusively colonized the hindgut. Both strains showed high cell densities in mono-association, but the abundance of strain FuSL was much lower when co-inoculated with strain EbSL. Oxygen strongly influenced the metabolic products both under in vitro and in situ conditions. The availability of oxygen in the gut would also explain why the anaerobic strain FuSL is outcompeted by the
facultatively anaerobic strain EbSL, which should achieve higher growth yields under microoxic conditions at the gut wall.
Insect-microbes interactions

EVALUATION OF ANTIBACTERIAL EFFECT OF AMERICAN COCKROACH HEMOLYPH ON SOME NOSOCOMIAL PATHOGENIC BACTERIA

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Background

Insects due to their long evolutionary history are able to recognize many pathogenic microorganisms and defend against them. Today, due to the development of resistance to synthetic antibiotics, researchers are trying to apply insect immune-derived products.

Objectives

This study was planned to investigate the antibacterial effect of the American cockroach (Periplaneta Americana) hemolymph on susceptible and resistant strains of nosocomial bacteria.

Methods

Adult cockroaches were reared in insectarium (25 ± 2 \textdegree C and 60\% humidity). To stimulate their immune system, 20\textsuperscript{th} of 10\textsuperscript{6} Escherichia coli cells were injected into coelom. After 6 hours, induced hemolymph were collected. The antibacterial effect of extracted hemolymph were assayed in the agar diffusion method on susceptible and resistant bacteria to antibiotics of ceftazidime, imipenem and, methicillin including, susceptibleE. coli strain "ATCC 25922"& "PTCCIBRC-M 10708", Staphylococcus aureus "ATCC25923", Pseudomonas aeruginosa "ATCC 27853" and, resistant strains of E.coli, Pseudomonas aeruginosa and, Staphylococcus aureus that were isolated from the hospital environment and stored in bank of bacteria. Also, non-induced hemolymph effect was evaluated on the same bacteria.

Conclusions

Evaluation of induced hemolymph effect on types of strains showed that induced hemolymph affected about 75\% of susceptible bacteria strains (P-Value< 0.001), whereas it had no effect on resistant strains. Among bacterial tested, ceftazidime-sensitiveE. coli ) PTCC ( ,ceftazidime-sensitiveE. coli (ATCC 25922), and methicillin-sensitive S. aureus (ATCC 25923) showed sensitivity to induced hemolymph (P-Value< 0.001).
These results showed that stimulation of immunity system American cockroach leading to production of antibacterial proteins and peptides which have inhibitory effect on bacterial depending on the bacterial strains and their sensitivity.
Intracellular survival

THE ACTIVATION OF THE AUTOPHAGIC PROCESS BY EIEC PROVIDES A SMALLER SPREAD IN THE HOST CELLS.

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Background

Autophagy has been described as an intrinsic host defense system for recognizing and eliminating intracellular-invading bacterial pathogens. However, invading pathogens have evolved mechanisms to avoid autophagic detection. Enteroinvasive Escherichia coli (EIEC), an important diarrheagenic Escherichia coli, are closely related to Shigella, showing remarkable phenotypic and genotypic similarities. However, the disease induced by EIEC is generally less severe than that induced by Shigella spp.

Objectives

EIEC express much less icsB than S. flexneri, our hypothesis is that EIEC are being efficiently recognized and eliminated by the host cell autophagic process. Moreover, we examined the role of icsB in this process.

Methods

We generate an icsB EIEC mutant by pGEM-T easy Vector System, pJP5603 plasmid and DH5-αλpir system. The EIECΔicsB was confirmed by RT-PCR. HeLa cells were challenged with wild type EIEC and EIECΔicsB for different time points. The delipidation of LC3B was analyzed by Western Blot. Bafilomycin was used as a positive autophagy control.

Conclusions

Differently from Shigella, EIEC induce autophagy in HeLa cells and this process seems to be independently of IcsB. Our results suggest that other virulence factors than IcsB are involved in the autophagic process induced by EIEC and that IcsB seems to not be a mechanism of EIEC camouflage against autophagic recognition. Additionally, the activated autophagic process could be involved with the slightest spread of EIEC in the host cell, providing a better control of infection.
SECRETORY PROSTATE APOPTOSIS RESPONSE-4 MIGHT REGULATE INTRACELLULAR SURVIVAL OF MYCOBACTERIA
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Background

Regulation of apoptosis has become one of the hot topics in mycobacterial infection. Recently, prostate apoptosis response-4 (Par-4), a tumor suppressor protein, is known to cause apoptosis in prostate cancer cells. Par-4 also participates in apoptotic pathway via interaction with glucose regulated protein 78 (GRP 78), which is activated by endoplasmic reticulum (ER) stress under stressful situation. Previously, we reported that ER stress-induced apoptosis plays a critical role as a host defense mechanism against Mycobacterium tuberculosis (Mtb). In this study, we investigated the role of Par-4 during mycobacterial infection.

Objectives

The aim of this study was to investigate the effect of Par-4 expression on mycobacterial apoptosis in macrophages.

Methods

We showed that mycobacterial infection induced expression of Par-4 and GRP78. Additionally in this study, strong interaction between Par-4 and GRP78 was shown at Mtb-infected macrophage surface through quantitative colocalization analysis. To determine if apoptosis could be initiated by GRP78 and Par-4 complex, we used siRNA for Par-4 before Mtb infection. Annexin V staining was used to identify apoptotic cell death. As expected, siPar-4 decreased apoptotic cell death induced by mycobacterial infection.

Conclusions

We found that Mtb infection activated Par-4 expression as well as ER stress induction. The interaction of extracellular Par-4 and cell surface GRP78 led to apoptosis via activation of caspase-8/caspase-3 pathway. The intracellular survival of
Mtb H37Ra was increased in Par-4 siRNA treated macrophages. These data suggested that Par-4 plays a crucial role in the intracellular survival of mycobacteria.
Background

Short and long term dietary interventions influences the structure and activity of gut bacterial communities. Recent literature suggests that the impact of dietary changes depends on microbial community structure at the time dietary changes are initiated.

Objectives

Investigate the effects of long-term dietary fibre intervention on the gut microbiota of rats from the same and different litters.

Methods

Forty-five, 21-day old female rats from six different litters were fed one of three diets: 5% fibre, 26% fibre (insoluble-cellulose) and a diet consisting of 50% of a basal diet and 50% cooked red kidney beans (rich in soluble-fibre). Each animal in a litter was randomly assigned to one of the three diets. Gut microbiota composition was determined before and after 14-weeks of treatment. Microbial communities were characterized using 16s amplicon sequencing. Taxonomic affiliations were determined using Mothur and SILVA bacteria database.

At the start of the experiment the microbial communities present in a rat varied with a rat’s litter membership. After 14 weeks on the experimental diets an animal’s microbial community composition significantly varied with diet, but significant litter membership effects remained. After diets treatments fibre, short-chain fatty acid, nitrogen and carbon composition of the faeces depended on an animals litter membership and diet.

Conclusions

The results suggest that efforts to enhance the health of humans and other animals through the use of prebiotics may have limited success in general due to among individual differences in the composition of their microbiotas and differences in how these microbiotas respond to dietary manipulation.
ENTEROCYTE-ASSOCIATED MICROBIOME OF THE HADZA HUNTER-GATHERERS

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Background

In a recently paper (Schnorr et al., 2014), we provided the first characterization of the fecal microbiota for a community of human hunter-gatherers, the Hadza of Tanzania. Our data suggested a new mutualistic layout for the gut microbiome (GM), with an enrichment in opportunistic bacteria and a depletion in health-promoting \textit{Bifidobacterium}.

Objectives

To obtain a deeper understanding of the GM-host mutualism in the Hadza, we explored the enterocyte-associated microbiome (EAM), whose layout of the EAM can inform about the functional influence on the host epithelium and other cell types in the mucosa, with critical implications for the maintenance of immune homeostasis. Microorganisms that directly interact with the enterocyte surface have a primary role in the microbiota-host cross-talk.

Methods

By means of a previously developed non-invasive \textit{ex-vivo} minimal model, based on mucus-secreting HT29 cells (Centanni et al., 2013; 2014), we characterized the EAM of 21 Hadza compared to 9 urban living Italians by 16S rRNA barcoded sequencing, and inferred the functional profiles via PICRUST.

Conclusions

Compared to Italians, the Hadza EAM were characterized by a greater amount of adhesive and opportunistic microorganisms, such as Enterobacteriaceae and Pseudomonadaceae, resulting in a functional enrichment in cell motility, signal transduction, interaction and biofilm formation. Our results depict an interesting mutualistic configuration of the intestinal mucosal microbiome in Hadza that is
capable of enhanced microbiota-host cross-talk and interaction at the mucosal surface. These findings stress the importance of a deep microbe-host interaction at the intestinal mucosal interface along the course of human evolution.
EFFECTS OF LINSEED AND HEMP SEEDS DIET SUPPLEMENTATION ON CAPRINE RUMEN BACTERIAL DIVERSITY.

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Background

The rumen, an heterogeneous microbial community including bacteria, archaea, protozoa and fungi, is a natural habitat that has evolved into an efficient system for lignocellulose degradation. Thanks to this microbiota, ruminants convert the plant materials into digestible compounds, such as volatile fatty acids, their main energetic fuels, and bacterial proteins. The equilibrium of ruminal microbiota is dependent on the diet, which carries fermentation substrates, and the efficiency of ruminal microbiota can be strongly affected by dietary changes.

Objectives

The objective of this study was to assess the effects of linseed or hemp seeds diet supplementation on bacterial diversity in the rumen content of goats using the Next Generation Sequencing.

Methods

In nine pluriparous Alpine goats fed the same pre-treatment diet for 40 days, ruminal fluid samples were collected, before feeding, using an aesophageal polyethylene probe. After this pre-treatment period the goats were arranged to three dietary treatment groups consisting of control diet (C), control diet supplemented with linseed (L) or hemp (H) seeds. Ninety days later, the same ruminal sample collection procedure was performed. The bacterial DNA was extracted using a protocol described in literature and 16S rRNA gene amplicons on V3-V4 region analyzed by Miseq (Illumina).
Conclusions

In the three dietary treatment groups, bacterial community was dominated by Bacteroidetes and Firmicutes with a high abundance of Prevotellaceae, Porphyromonadaceae and Veillonellaceae and a low presence of Ruminococcaceae and Lachnospiraceae. However, L treatment seemed to affect the bacterial population, reducing the microbial diversity.
Background

Many microbial infections involve formation of biofilms containing multiple species. Besides the difficult treatment, the taxonomic and functional characterization of the microbial communities is a major challenge and classic culture-dependent methods often fail to fully characterize the infection.

Objectives

Our aim is the development of Next-Generation Sequencing based methods combining analysis of 16S-amplicons with metagenomics to reveal both the taxonomic and functional diversity of infection related microbial communities with a focus on virulence and presence of resistance determinants.

Methods

We analyzed samples from chronic lung infections and acute infections of dental implants (peri-implantitis). Taxonomical composition was determined by sequencing barcoded amplicons of the 16S rRNA V1-V2 variable regions using Illumina technology. Sequences were compared with the Ribosome Database Project for the characterization of taxa. Selected samples were also used to isolate whole genomic DNA for metagenomic sequencing using Illumina technology. Sequences were assembled and annotated by bioinformatic gene prediction and alignment to the NCBI non-redundant nucleotide database.

Conclusions
The taxonomic composition of the samples both from chronic lung infections and peri-implantitis revealed complex communities in both settings. The dominant taxa included pathogenic taxa like *Pseudomonas, Staphylococcus* and *Streptococcus* in the cystic fibrosis lung infections and several Bacteroidetes like *Tannerella* and *Porphyromonas*, which are commonly described in such infections. Analysis of Metagenomic sequences is ongoing to reveal the functional content of the communities with focus on virulence factors and resistance determinants. Employing metagenomics and amplicon sequencing combines taxonomic accuracy and cost-efficiency with a deep insight in the functional genomic content of microbial communities.
Background

The nose is rich in bacterial species that produce outer membrane vesicles (OMV) known to participate in inter- and intra-species signalling. These structures, however, are highly reminiscent of eukaryotic exosomes and synthetic liposomes: all of these nanostructures involve lipid bilayers containing biomolecules such as DNA, RNA and/or protein. The blood-brain barrier has long scuppered efforts of drug delivery to the brain. Encouragingly, studies have revealed that the brain can be accessed across the olfactory nasal epithelium with biotherapeutics and nanoparticles.

Objectives

The main aim was to investigate the hypothesis that 1) the bacterial material is present in the brain of healthy rodents without causing disease and that 2) the possible entry route is via the nasal cavity across the olfactory epithelium. In addition, we analysed the microbial DNA signatures at the phylum and class level.

Methods

We first attempted PCR amplification of the V3 hypervariable region of bacterial rRNA genes from the mice and rats brain DNA. Produced amplicons were the subjects of 16S phylogenetic analysis by Ion Torrent Next-Generation Sequencing and RDP analysis.

Conclusions

The study proved existence of microbiomes in healthy mammalian brains. The comparison of the microbial signatures indicated that at least some of the bacterial DNA is common at both tissue loci, in support of the precept that nasal bacteria or their products might enter the brain through the olfactory epithelium. In addition, there is some similarity of the bacterial species between mice and rats, though some differences were down to host-species intricacies.
Background
Protein energy malnutrition is a potentially fatal body depletion disorder. The catastrophic effects of malnutrition include diarrhoea, malabsorption, increased intestinal permeability and alleviated immune response, thereby aggravating other pathological conditions and contribute to the global disease burden. Since effective treatment for malnutrition is lacking, therefore, metagenomic procedures allow to access the complex cross-talk between the gut and its microbial flora and understand how a different community composition affects various states of human health.

Objectives
The aim of the study is to develop protein energy malnourished (PEM) mice model and to evaluate the various changes in the body and organ weight, histological changes and the total protein content as compared to control. Moreover, a metagenomic approach has been employed for analysing the differences between gut microbial communities obtained from malnourished and apparently healthy mice.

Methods
Twelve mice were assigned to two groups (6 each/group) and fed either a malnourished (protein=2%, energy=295.2 calories) or a normal (protein=16%, energy=397.2 calories) diet. Body weight was taken till four weeks after which they were sacrificed. Weight of the visceral organs weight and their histological parameters were analysed. Faecal samples were further analysed for the differences in microbial communities between the two groups through RT-PCR. Serum samples stored under refrigerated conditions (-20°C) were also analysed for total protein content between both the groups.

Conclusions
The present study reveals the development of PEM model by analysing various morphological, anatomical and histological parameters. Moreover, it characterizes the microbial community resident in the gut of malnourished mice as compared to control.
MUCOSA-ASSOCIATED MICROBIOTA IN THE SNOW TROUT INTESTINE (SCHIZOTHORAX ZARUDNYI, NIKOLSKII, 1897) ARE DIFFER FROM LUMEN-ASSOCIATED MICROBIOTA, AS REVEALED BY 454 PYROSEQUENCING

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Background
As in mammals, studies on the intestinal microbiota of fish have revealed that the bacterial community may influence growth, feed conversion, epithelial development, and immunity of the host.

Objectives
This study aims at applying 454 pyrosequencing approach to study the autochthonous and allochthonous-associated microbiota from snow trout.

Methods
In total, 32 genomic DNA samples were obtained and then Roche 454 pyrosequencing was applied to the pooled set of 16S rRNA gene amplicons. Sequence data were prefiltered and analyzed with the Quantitative Insights Into Microbial Ecology (QIIME) v 1.8.0 pipeline using default parameters.

Conclusions
From our analyses, distinct microbial communities were detected between the snow trout gut-associated and intestinal content-associated microbiota, as has also been observed in human, pig, mice and alligators. According to the results Firmicutes phylum members composed the prominent phyla of the mucosa-associated microbiota regardless of the fish gender. However, in luminal-associated microbiota Fusobacteria phylum group made up the most dominant group in intestinal content of male and female fish. This suggests that the abundance and diversity of bacterial populations in gut mucus is, in general, quite different from the microbiota in gut contents, indicating that some microbial species poorly colonise gut mucosa layer. Many of these bacteria might be of high physiological relevance for snow trout as these groups have been implicated in vitamin production, nitrogen cycling and carbohydrate fermentation.
DETECTION OF SAPOVIRUS GV.2 BY THE NEXT GENERATION SEQUENCER IN THE STOOL SPECIMENS OF PATIENTS OF GASTROENTERITIS OUTBREAK FROM WHICH PATHOGEN HAD NOT BEEN IDENTIFIED

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Background

Causative pathogens have not been identified by ordinal examinations in some sporadic and outbreak cases of gastroenteritis received at Toyama Institute of Health. In contrast, some pathogens have been identified by metagenomic analysis using the next-generation sequencer (NGS).

Objectives

In this study, pathogens were searched by NGS in one outbreak of gastroenteritis from which pathogen has not been identified by conventional PCR analysis.

Methods

RNA-seq libraries were constructed from six specimens collected in the outbreak occurred in 3 facilities of health center for the elderly in April 2013, and were analyzed by MiSeq sequencer (Illumina).

Conclusions

About 20,000-50,000 reads were obtained in each specimen by NGS analysis, and 2 to 6 reads of nucleotide sequence of sapovirus (SaV) were identified from 3 specimens. These reads agreed with the part of the sequence of sapovirus Hu/Nagoya/NGY-1/2012/JPN (SaV GV.2) reported firstly in the food-borne gastroenteritis (Nagoya City in 2012) with 93-99% identity. Since multiple sequences of SaV were detected from the same specimen, the virus was suspected to be causative agent. Specific primers for SaV GV.2 were generated and PCR was performed. Products were obtained in all six samples by PCR, and their sequences
were revealed to be 99.8 % identity with that of SaV GV.2. Therefore, SaV GV.2 was concluded to be the causative agent of the present case.
Pathogens that are not able to be detected by conventional primes because of low homology may be identified by metagenomics analysis that is independent on specific primers as in this case.
Background

Celiac disease (CD) is a chronic inflammatory disorder of the small intestine that presents only in a fraction of genetically predisposed individuals following gluten consumption. Recently, several studies have suggested that abnormalities in the small intestinal microbiota might be involved in the development or the pathogenesis of CD.

Objectives

The objective of this study was to characterize the specific composition of the duodenal microbiota of untreated CD patients and non-CD controls without mucosa damage in order to identify possible differences in bacterial communities in the upper small intestine.

Methods

Intestinal bacterial communities were identified by pyrosequencing of 16S ribosomal DNA extracted from duodenal biopsies. The study group included 9 untreated CD patients and 9 non-CD controls without duodenal mucosal atrophy.

Conclusions

Analysis of a total of 180,825 reads of the 16S rDNA showed that the majority of them were classified within two phyla: Firmicutes and Proteobacteria. Bacterial richness and diversity was higher in non-CD controls than in untreated CD patients. The principal coordinates analysis revealed that bacterial communities of non-CD controls and untreated CD patients were dispersed without forming a clear group according to the presence or absence of the CD. There is no characteristic pattern of bacterial communities associated to untreated CD patients. There are not differences in any bacterial group community frequencies in the upper small intestine between untreated CD patients and non-CD controls.
FEMS-1665
(Human) microbiome

METAGENOMIC ANALYSIS OF MICROBIOTA IN THE SEMEN OF PROSTATITIS PATIENTS: MOLECULAR APPROACHES TO ASSESS THE DIVERSITY OF MICROBIAL COMMUNITIES
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Background

Prostatitis is one of the most common urological diseases in men and accompanied by bacterial infection in the prostate gland which occurs in 5-10% of prostatitis patients and also, frequently causes recurrent urinary tract infection. Urogenital health and diseases rely on the interaction between the host and the urogenital microbial community.

Pyrosequencing a next generation sequencing (NGS) technologies allow us to characterize microbial communities using bacterial 16S rRNA sequences with high magnitude faster than previous Sanger dideoxy chain-termination method. Therefore, the aim of this study was to identify specific microbial descents that may play crucial roles in the development of prostatitis and to examine whether the presence or abundance of these bacterial changes in semen of prostatitis patients.

Material and Methods

Semen samples were collected from prostatitis patients and extracted genomic DNA for pyrosequencing by using DNA extraction kit.

Results and Discussion

In this study, some abundant pathogenic genus, such as Anoxybacillus, Staphylococcus, Corynebacterium, Pseudogluconobacter and Enterococcus in the prostatitis sample were found by using the pyrosequencing techniques.

In this study, in case of normal control, Streptococcus and Corynebacterium were abundance to 33.5% and 21.2%. On the other hand, the most abundant genera in semen of prostatitis patient were Enterococcus (20.7%), Pseudogluconobacter (16.2%) and Anoxybacillus (14.3%). These data provided the comprehensive report on bacterial communities between normal and prostatitis patients and also, it is concluded that prostatitis is related to substantial alteration in the composition and representation of the urogenital bacterial biota.
This identified variability in community membership and structure has important implications for understanding the etiology of prostatitis, for developing diagnostic tools and for treatment.
Background
Dandruff is a global common problem, up to 50% of both genders suffer from
dandruff. However, although bacteria and fungi associated with dandruff are known,
understanding the etiology of dandruff is incomplete.

Objectives
Correlation of the bacterial and fungal communities with dandruff is needed because
the human skin is associated with many microorganisms with potential for both
beneficial and detrimental interactions.

Methods
For all cases, samples from normal scalps and severe dandruff patients were
collected and analyzed under protocols approved by Chung-Ang University College
of Medicine IRB (Protocol #2012-02-01). From the collected samples, we amplified
variable regions V1-V3 of the 16S rRNA gene (bacteria) and the D1/D2 region of the
26S rRNA gene (fungi) using universal primers and GS-FLX pyrosequencing.

Conclusions
Firmicutes were the most dominant in dandruff patients and showed an increase from
5.2% in cases defined as normal scalps to 91.0% in severe dandruff patients.
Whereas, Proteobacteria decreased from 60.1% in the normal scalp to 6.2% in
dandruff patients. Similarly fungi of the phylum Basidiomycota were associated with
the normal scalp, decreasing from 66.6% to 24.7% in severe dandruff. In the
Firmicutes Staphylococcus spp. were dominant in the dandruff scalp. In contrast,
Malassezia spp. in the Basidiomycota were the most abundant fungus in the healthy
scalp. This switch from Malassezia to Staphylococcus provides new comparative
information on the microbiomes of the normal scalp and patients with exacerbated
dandruff. Consequently, our results can be expected to lead to improved diagnosis
and treatment.
GENOMIC ANALYSIS OF NOVEL CHITINOLYTIC SUBSPECIES OF CLOSTRIDIUM SARTAGOFORME FROM BUFFALO RUMEN REVEALS ITS IMPORTANT ROLE IN VOLATILE FATTY ACID PRODUCTION
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Background
The rumen of herbivores is an open fermentation chamber for digestion of complex plant biomasses by highly efficient bacteria.

Objectives
Isolation of anaerobic rumen microbes from buffalo and its phenotypic-genotypic analysis. Correlate the genomic analysis with metagenome data for implication of the isolate’s role in rumen.

Methods
A strain of Clostridium sartagoforme species was isolated from buffalo rumen, involved in cellulose and chitin degradation. The isolate was cultured between 37°C and 42°C temperature with an optimum pH of 7.5 in Hungate’s medium supplemented with cellulose. Whole genome sequencing was performed using ion torrent PGM. Taxonomy study using local BLAST, showed highest phylogenetic relation with Clostridium sartagoforme DSM 1292 strain based on comparison of the 16S rDNA sequences. Comparison of sequences coding for housekeeping genes phosphoglycerate kinase and gyrase B confirmed its taxonomic novelty within the Clostridium genus. Biochemical assessment of strain’s fermentation ability using AN-Biolog plate revealed that the strain utilized more than 30 different metabolite precursors. Pathway analysis made known the organism to be highly active in the production of volatile fatty acids (VFAs).

Conclusions
The isolate represents a new subspecies, for which the name is proposed Clostridium sartagoforme AAU1 (GenBank Accession:ASRV00000000). Genomic analysis gives an insight about the role of bacteria in determining ruminant host physiology. VFAs produced by the isolate play a prominent role in animal health maintenance by contributing a major proportion of the ruminants’ daily energy requirement. A deeper understanding of genes involved and enzymes encoded allow further industrial applications and modulation of the rumen for enhanced farming applications.
FEMS-2593
(Human) microbiome

METAGENOMIC ANALYSES REVEAL A CELLULOYTIC AND FERMENTATIVE LIFESTYLE IN THE GUTS OF EPULOPISCIUM-REPLETE SURGEONFISHES

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Background
Epulopiscium fishelsoni, is a giant bacterium found exclusively in the guts of surgeonfishes. Three years after the publication of an incomplete draft genome of Epulopiscium sp. type B from Naso tonganus, we still have no clue into the metabolic potential of this hitherto uncultivated symbiont, and more so, the functions of the gut microbiota in reef fishes.

Objectives
Hence we were prompted to fill this knowledge gap, and thus provide a foundation for understanding the physiology and ecological roles of their microbiota.

Methods
We therefore employed a metagenome-sequencing approach to explore the phylogenetic diversity of Epulopiscium, and through binning of assembled metagenomic contigs, also shed insights into their metabolism.

Conclusions
Our data shows that distinctive feeding guilds of surgeonfishes harbour different Epulopiscium-like phylotypes, and that the low-to-high intestinal abundances of these bacteria correlate with their algal diet types. Metabolic reconstruction of the draft Epulopiscium-like genomes from these fishes revealed a capacity to catalyze hemicellulolysis and an anaerobic fermentation lifestyle that produces acetate and lactate. Our data thus provides the first in-depth analysis into their diversity and putative symbiotic role, which in turn illuminates the potential metabolic links shaping the symbiosis between the host and the giant bacteria. We further present more insights from the draft genomes, focusing on the nature of this symbiosis, and its interconnection to the trophic ecology of the fishes.
Background

Aromatic compounds comprise one-quarter of the Earth’s biomass and are the second most widely distributed class of organic-compounds in nature, next to carbohydrates. In tropical-countries, the buffalo are fed on lignocellulosic agricultural by-products like cereal-straws and tree-foliage. Ruminants digest such plant-materials by microbial-processes. The role of microbiota as reservoir of its unique functional capacity related to the metabolism of aromatic-compound needs to be explored in buffalo rumen.

Objectives

In the view of above concern, objective of the present study was to account the comparatives profiling of phylogenetic and functional-potential of genes related with the metabolism of aromatic-compound in Bubalus bubalis rumen fed with different diets.

Methods

The metagenomic analysis of liquid and solid-fraction of the rumen-biomaterial collected from Bubalus bubalis was carried out using high-throughput sequencing. The experimental animals received M1 diet for 6weeks followed by M2 for 6weeks and
then M3 for subsequent 6 weeks. On the last-day of each experimental feeding period, rumen-samples were collected 3h post feeding using stomach tube.

**Conclusions**

The work presented here describes composition of overall functional-capacity related with the genes for metabolism of aromatic-compound and responsible taxonomic-communities of buffalo rumen-ecosystem. The higher % abundance was found to be related with genes involve in peripheral-pathway for catabolism of aromatic-compound in all treatment-groups. The taxonomic analysis of metagenomic reads indicated that rumen-microbiomes were dominated by Bacteroidetes and Firmicutes phyla in all treatments-groups. The information obtained from this research will open new-horizons towards a full understanding of functional-genes related with metabolism of aromatic-compound and metabolic-capabilities of complex-compound degrading rumen-microorganisms.
INTAKE OF A LICHEN-BASED DIET ALTERS THE POPULATION OF METHANOCENIC ARCHAEAE IN THE RUMEN AND HINDGUT OF REINDEER

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Background
Reindeer are large Holarctic herbivores, which rely on a consortium of anaerobic microorganisms in the rumen and hindgut to digest plant cell wall carbohydrates. Reindeer may include a large fraction of lichens in their winter diet. Lichens are high in phenolic secondary compounds, which may affect the reindeer gut microbiome, and possibly alter the output of methane. Methane is produced by methanogenic Archaea mainly from CO₂ and hydrogen.

Objectives
In this project we examined the effect of lichens on the reindeer rumen and hindgut methanogenic Archaea.

Methods

Rumen and cecum samples were collected from two groups of Norwegian reindeer (Rangifer tarandus tarandus), one group (n=4) fed lichens and one fed a standard pelleted reindeer feed (n=3). The diversity of methanogens was assessed using 454 pyrosequencing. Real-time PCR experiments were performed to estimate the population densities. All animals were sacrificed in accordance with Norwegian regulations, Sections 9 and 10 in the Norwegian Animal Welfare Act.

Conclusions

Densities of methanogens and protozoa were higher in the cecum of lichen-fed reindeers but no differences were found in rumen samples. Methanobrevibacter (≥ 97% similarity) was the major genus of Archaea in all the samples, with M. thaueri as the dominant species in all reindeers fed lichens (>60% on average). Remarkably, M. ruminantium suffered a significant increase (up to 23,8%) with a lichen-based diet in both sampling sites. In conclusion, the intake of lichens affected to some extent the microbial diversity and density of the microbiome housed in the rumen and cecum of reindeer.
Background

Drinking water quality is a public health concern worldwide. Growing evidences depict drinking water as a complex matrix, in which a wide diversity of microorganisms interact in a dynamic network. Recent studies reveal that drinking water treatment process can affect the microbiome structure. Moreover the occurrence of antibiotic resistance genes in water is becoming an issue of great interest as the mobile resistome can easily spread among species. Molecular techniques can give a deeper knowledge, going beyond the limit of culture-dependent methods.

Objectives

In this study we evaluated and standardized a new pipeline for microorganisms concentration, DNA extraction and amplification, suitable for molecular analysis and optimized for High-Throughput Sequencing (HTS) approaches.

Methods

We collected samples in a water treatment plant in Milan (Italy), at different steps of the potabilization processes, from the aquifer to the tap. We analyzed the presence and the relative abundance of bacteria and eukaryotic microorganisms across the water treatment plant. Furthermore the presence of specific antibiotic resistance genes was detected and quantified with Real Time PCR, at each step of water treatment process. Since molecular techniques are unable to differentiate between viable and nonviable microorganisms, live/dead ratio was estimated using SYTO9/propidium iodide staining coupled with microscopy visualization. These analyses are integrated in a broader study characterizing microbiome structure variability using HTS techniques, in order to better understand this complex ecosystem.

Conclusions

The results agree with those obtained in the few recent studies published till now and can help to unravel the dynamics underlying water microbiome changes.
Background
Phototrophic and heterotrophic bacteria have been found directly inside halite evaporites that formed as bottom-growth crusts in a hyperarid core and inside gypsum minerals. The role of microorganisms in the precipitation of authigenic minerals like carbonates, sulphides and silicates, has been documented by some authors in several natural systems.

Objectives
In this study the interface between biological and geochemical components in the surface crust of a saline soil was investigated. The bacterial community structure and diversity as well as the possible role of the photosynthetic microbial community in the formation of the soil crust was evaluated.

Methods
A variable pressure scanning electron microscope equipped with energy dispersive X-Ray diffraction analysis was used for geochemical analysis, while pyrosequencing of the V2-V3 16S rRNA gene region was used for characterizing microbial diversity and community structure.

Conclusions
The organization and diversity of the microbial taxa was analyzed according to the physical and chemical heterogeneity of that peculiar habitat. Mineral structures made of gypsum and halite were documented as crystallized around the filaments of cyanobacteria. A univocal causal relationship could not been established between the crystallization of gypsum or halite and cyanobacterial or algal metabolism but their compartmentalization respect to the biological mat suggested that the organisms
could have at least a physical control over the distinctive structures produced. The results provided information on the type of distribution of different bacterial and cyanobacterial groups as a function of the biogeochemical interfaces, suggesting also possible forms of microbial interaction.
Background
Bacteriophages are the most abundant biological entities in the biosphere. They influence the evolution and dynamics of microbial populations. Although phage populations were studied extensively for the marine environment only few studies were dedicated to freshwater ecosystems.

Objectives
The main objective of this project is to study the phage population in Lough Neagh, the largest freshwater lake in the British Isles and to analyse interaction of these phages with bacterial hosts.

Methods
Both total microbial and viral subpopulations were investigated using standard protocols. Isolation of viral fractions was performed by common procedures. Pyrosequencing was used to identify bacterial communities and the dynamics of the bacterial population’s changes in Lough Neagh are compared with seasonal variation. Illumina sequencing was used to characterise viral communities in the same environment.

Conclusions
16S rRNA analysis detected a diverse range of bacterial phylum in both water and sediment. Proteobacteria, Acidobacteria, Bacteroidetes and Planctomycetes are found in the freshwater samples which are comparable to the sediment samples. The water samples taken from Lough Neagh have bacteria phylum which are not present in the sediment samples such as Chlamydiae. Analysis of viral metagenomes detected the presence of 16S rRNA sequences probably originated from transducing particles. For example Pelagibacteraceae was identified and. this bacterial family is more commonly found in the oceans rather than a freshwater lake. Possibly this may indicate interactions between bacterial species from two different environments. Illumina analysis of viral metagenomes provided a diverse range of information and can be compared to other published metagenomes.
OPTIMIZED AND STANDARDIZED SINGLE INDEXING SOLUTION FOR HIGHLY MULTIPLEXED MICROBIAL COMMUNITY PROFILING ON ILLUMINA MISEQ PLATFORM

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Background
The growing need to survey the tremendous microbial diversity in a culture independent manner, has led to the development of molecular methods through sequence profiling of conserved genes, in scientific field like ecology, agronomy, biotechnology, plant, animals or human health. Recently, the improvement of the Illumina MiSeq platform to a 2×300 bases paired-end version made it much more attractive for amplicons sequencing in metagenomics. Nevertheless, the lack of nucleotide diversity at initial sequencing positions due to sequence conservation is problematic on Illumina platforms. Moreover, validated Illumina amplicon sequencing protocols do not support more than 96 samples per run, which underutilized the overall capacity of a sequencing run.

Objectives
We therefore worked on the development of a standardized and optimized high-multiplexed metagenomic solution for the exploration of complex environments.

Methods
We developed an integrated solution combining (i) a custom and standardized amplification protocol for library preparation; (ii) a 192 custom single index validation strategy within a single run; (iii) the inclusion of a nucleotide sequence variability at the first sequencing cycles; (iv) an accurate paired-end reads assembly process and (v) a dedicated pipeline for taxonomic affiliation and microbial communities analysis.

Conclusions
Our high-multiplexing sequencing solution validated within complex metagenomics samples, provided high sequencing accuracy and technical reproducibility with no index biases based on taxonomic distribution analysis. The association of technical and bioinformatical improvements yields substantial cost reductions and provides greater target flexibility to investigate structure as well as functions of microbial communities through 16S rDNA, 18S rDNA, ITS and functional genes profiling.
Background

In marine sediments, a novel group of filamentous sulfide-oxidizing bacteria has recently been discovered, which induce electrical currents over centimetre-scale distances. These so-called cable bacteria grow from the oxic zone into the sulfidic part of the sediment up to a depth of several centimetres. Cable bacteria use a radically novel microbial metabolism, in which electron donor and electron acceptor reactions are spatially separated: the oxidation of sulfide in anoxic sediment layers is coupled to the reduction of oxygen at the sediment surface. This mechanism is only possible when electrons liberated from sulfide oxidation are transported from cell to cell along the length of the filament to the oxic sediment surface.

Objectives

To identify a marine sediment where cable bacteria are active in situ and to study the sediment microbial community using shotgun metagenomic sequencing.

Methods

Depth profiles of oxygen, sulfide and pH were taken with microsensors and cable bacteria distribution with depth was evaluated using fluorescent in situ hybridisation. Metagenomic analysis was performed for different depths of the sediment: the oxic surface sediment, the suboxic zone where neither oxygen nor sulfide was present, and the deeper sulfidic zone without cable bacteria. Extracted sediment DNA was sequenced using Illumina MiSeq with 300 bp paired end reads.

Conclusions

At the examined North Sea site, microsensor profiles indicated that metabolic activity of cable bacteria dominated the sediment geochemistry. The obtained metagenomic
data provide insight into the microbial community composition and functioning, and possible interactions between cable bacteria and other taxonomic groups.
Granulation is important for successful start-up of high-rate anaerobic reactors and usually takes long time. The influence of inoculum’s Sludge Volume Index (SVI) on granulation in high-rate upflow Hybrid Anaerobic Reactor (HAR) was studied.

Objectives
Understanding of microbial community changes due to variations in SVI will help in providing novel reactor setting up conditions to obtain faster granulation rate in high rate anaerobic reactor.

Methods
Ten HARs were operated with inoculum having different values of SVI (100 to 325), SVI were varied by adding different amount of inoculum (10% to 50% of the reactor volume). The methanogenic and bacterial community structures of anaerobic sludge obtained from these ten HARs were examined using culture-independent techniques like DGGE and qPCR.

Conclusions
Results showed that granulation was achieved in HARs with SVI values ranging from 150-210 or 18% to 27% inoculum of the reactor volume. DGGE identified 17 methanogenic and 22 bacterial operational taxonomic units in the ten reactors’ samples. DGGE analyses showed dominance of *Methanosaeta* and *Methanosarcina-*like species in reactors where granules were formed. qPCR analysis also confirmed that granulating reactors were rich in aceticlastic methanogens especially *Methanosaetaceae*. *Bacterial* rRNA genes obtained from DGGE results, were affiliated to four Orders -*Clostridiales*, *Lactobacillales*, *Actinomycetales* and *Bacteroidales*. But, no significant correlation was observed between granulation and bacterial abundance. The concentration of bacterial 16s rRNA genes was found to be less in granulating than non-granulating systems unlike methanogenic genes. Thus, this study suggests to make conditions favorable for growth of aceticlastic methanogens to decrease granulation time.
Background
Environmental DNA derived from uncultured bacteria has huge potential as a source of novel bioactive molecules as shown by recent screening campaigns of metagenome libraries which revealed a number of novel protein families, extremozymes and secondary metabolites. Before constructing a metagenomic library, the choice of the habitat should be carefully considered because it predefines the types and properties of biocatalysts to be discovered.

Objectives
In this study, we have constructed a metagenomic library in Escherichia coli DH10b with DNA isolated from the microbial population of a slaughterhouse drain biofilm. By comparative screening for lipolytic, proteolytic and detergent activities we have identified number of clones producing bioactive molecules.

Methods
By applying a functional screening assay for biosurfactant activity, two haemolytic and surface-active clones (SA343, SA354) were identified. Sequence and structural homology analyses revealed that they contained genes encoding N-acyl amino acid synthases (NAS) located on environmental DNA fragments.

Conclusions
The respective NAS proteins (Nas343 and Nas354) were shown to be responsible for the haemolytic phenotype and the biosurfactant synthesis in vivo. The molecular organisation of the NAS domain linked here to a c-di-GMP binding domain has so far not been identified in any other known protein. The produced biosurfactant was purified and identified by NMR spectroscopy as N-myristoyl-tyrosine. Its biophysical properties including the critical micelle concentration and the ability to reduce surface tension were compared with chemically synthesised N-myristoyl-tyrosine and shown to be in a similar range. Interestingly, N-acyltyrosine also showed antibiotic activity against various Gram-negative and Gram-positive bacteria.
Background
Oxygen minimum zones (OMZs) play a major role in marine biogeochemical cycling. They are characterized by a high microbial diversity adapted to life along an oxic-anoxic gradient. In particular, anammox and/or denitrification are dominant processes in these zones accounting for significant loss of nitrogen from the ocean. However, other processes within the nitrogen and also carbon cycling have not been explored well.

Objectives
To get insights into the organisms involved in carbon and nitrogen cycling in the Arabian Sea OMZ, we studied the meta-genomes of filtered ocean water recovered at two different depths, the upper zone (170m deep, PA2) and the central zone (600m, PA5).

Methods
We systematically screened for marker genes indicative for key nitrogen and carbon cycling processes by blasting the meta-genomes data against manually curated functional gene databases.

Conclusions
Both zones are characterized by a large fraction of alpha, gamma and delta-proteobacteria. In PA5, anammox contributed a significant amount to the overall diversity (about 8%) whereas Thaumarchaea were present in higher numbers in the upper zone (about 9%). The predominance of nitrifying archaea over bacteria could also be confirmed by amoA diversity. No bacterial amoA was detected in the entire dataset, and only very few sequences related nitrogen fixation were recovered. Furthermore, no canonical methane oxidizers could be found. However, pmoA sequences only distantly related to known diversity are present and could be linked to a published single cell genome, indicating the possibility of a cryptic methane cycle in the Arabian sea OMZ.
MICROBIOMES OF THE BUILT ENVIRONMENT ARE ALTERED BY DIFFERENT ROOM MAINTENANCE

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Background
The excessive removal of microbes from controlled built environments like intensive care units, operation theatres and especially cleanrooms is daily routine. However effects of these stringent maintenance procedures on our surrounding microbiomes in indoor environments are unknown.

Objectives
Since recent studies showed the enormous potential of the human microbiome towards our health, this study aims to resolve the interactive interference of all microbiomes in the built environment and determines its potential for human well-being indoors\textsuperscript{1}.

Methods
High throughput deep sequencing technologies were applied for shotgun metagenomes and 16S/18S rRNA and ITS gene amplicons of Archaea, Bacteria, Eukaryota and fungi. Viability assays using ATP (adenosine tri-phosphate) and PMA (propidium monoazide) were deployed to identify potential viable microbiomes and supported by qPCR to investigate microbial abundance\textsuperscript{2,3}. The biotechnological potential was further characterized by VOC’s (volatile organic compounds) assays of culturable indoor bacteria\textsuperscript{4}.

Conclusions
These assays revealed high reduction of viable microbial abundance in cleanrooms\textsuperscript{5}, higher similarities of viable microbiomes between controlled and uncontrolled areas compared to the total microbial fraction, distinct profiles of microbial communities from floors, medical devices and workplaces in an intensive care unit, and for all controlled built environments not only an overlap with the human, but also the plant microbiome. Hence plant leaves could be identified as a main source for microbial distribution on the surrounding indoor environment.

The knowledge of certain key species and their ecological key functions in controlled as well as uncontrolled built environments will help to control indoor environments in a more sophisticated way for our health inside buildings in the future.
Background

Presence of heavy metals impedes use of sewage sludge as a fertilizer. Simultaneous sludge digestion and metal leaching (SSDML) combines conventionally distinct stages of metal leaching and sludge stabilisation into one, making the process economically attractive. Insights into the microbial community involved could enable further optimization of the process.

Objectives

To evaluate the performance of SSDML and understand corresponding microbial (bacterial) community shifts.
Figure 1. Bacterial DGGE profile during the process (0, 2, 4, 6, 8, 10, 12 and 15 are the n\textsuperscript{th} day of operation of experiment)
Figure 2. Unweighted pair group method with arithmetic mean (UPGMA) analysis of DGGE profile in Figure 1
Methods
Batch study was carried out at 37°C for 15 days after adding sulfur to heavy metal contaminated sludge, with aeration rate of 0.6 vvm. Samples for heavy metals, volatile suspended solids (VSS) analyses and DNA extraction were collected regularly.

Conclusions
Bio-oxidation of sulfur to $\text{H}_2\text{SO}_4$ lowered the pH aiding leaching of metals. Zinc, chromium, cadmium and lead reduced by 86.75%, 64.26%, 79.17% and 69.58% in the dry sludge respectively. Concomitantly, VSS reduction of 49.40 % was obtained making the sludge stable.

*Acidithiobacillus ferrooxidans* and *Acidiphilium acidophilum* were found to be the main bioleaching microorganisms through 16S rDNA PCR-Denaturing Gradient Gel Electrophoresis (DGGE) analysis, in contrast to the commonly reported sulfur-oxidising *Acidithiobacillus thiooxidans*. *Enterobacter*, *Alicyclobacillus cellulosilyticus* and *Pseudomonas* were the dominant heterotrophic organisms present across all samples. UPGMA analysis showed a significant microbial shift during the course of operation which could be mainly attributed to the change in pH and solids content.
Thus, after the single stage treatment of SSDML, sludge obtained was stabilised with reduced heavy metal load. PCR-DGGE analysis showed that both heterotrophs and bioleaching microorganisms were present and adapting well according to the dynamic pH.
QUALITATIVE AND QUANTITATIVE BIAS ESTIMATION OF MICROBIAL COMMUNITY ANALYSIS USING BARCODED 454 FLEX+ METHODOLOGY FOR CLAY CONTAINING GEOLOGICAL SAMPLES

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Background
High throughput sequencing methodology can be used to determine the composition of environmental microbial communities. Generally, a barcoded PCR amplicon of the omnipresent 16S rDNA gene is used to unravel complex microbial communities. However, several biases and technical difficulties linked to this methodology, may jeopardize the outcome of the community analysis. For instance, it is often difficult to extract DNA out of environmental samples due to interferences caused by the geological matrix. This is especially true for clay containing geological samples. Beside, often containing highly reactive dissolved organic materials, clayey soils are also known for their high sorption capacity of (biological) macromolecules. Therefore, the presence of clay minerals may be responsible for additional biases when analyzing microbial communities of soil or geological samples.

Objectives
To quantify the biases, qualitatively and quantitatively, caused by the presence of clay minerals, during analysis of the composition of microbial communities in geological samples, with high throughput barcoded 454 Flex+ DNA sequencing methodology.

Methods
Four identical bacterial mockup samples were made, by mixing 18 different soil bacteria. Boom clay was only added to two samples. DNA was extracted, with an identical DNA extraction procedure. The 16S rDNA gene was PCR amplified with four different barcoded 16S\textsubscript{341}-forward primers and one 16S\textsubscript{1492}-reverse primer. The amplicons were sequenced using the 454 Flex+ DNA sequencing methodology. The data were treated with our in house developed bioinformatics pipeline, including the tools, NoDe and CATCh.

Conclusions
At the start of the FEMS-symposium 2015 the results and conclusions will be ready to be presented.
Background
The revolution in new sequencing technologies has led to an explosion of possible applications, including microbial biodiversity studies via bacterial 16S rRNA amplicon sequencing.

Objectives
All 16S rRNA amplicon sequencing data suffer from the presence of erroneous sequences: (i) chimeric sequences introduced by the PCR reaction, and (ii) sequencing errors produced by the sequencing platform itself. As such, there is a need for efficient algorithms to remove those erroneous sequences in order to be able to accurately assess the microbial diversity.

Methods
First, a machine learning method called CATCh (Combining Algorithms to Track Chimeras) is able to integrate the output of existing chimera detection tools into a new more powerful method, outperforming all existing tools. Second, NoDe (Noise Detector) was introduced as an algorithm to correct 454 pyrosequencing errors, showing a significant improvement in reducing the error rate (reduction of 67 to 75%) and dramatic decrease in computational cost compared to state-of-the-art tools. Similarly, IPED (Illumina Paired End Denoiser) was introduced as first tool in the field for the denoising of MiSeq sequencing data.

Conclusions
Integrating both CATCh and NoDe/IPED into the preprocessing pipeline of 16S rRNA amplicon sequencing data significantly increases the reliability of the data, demonstrated by the positive effect on the clustering of the sequencing data in operational taxonomic units (OTUs). When tested on mock communities (MiSeq as well as 454 pyrosequencing platform), the number of OTUs closely approaches the actual number of species present in the mock samples. Both tools are freely available at http://science.sckcen.be/en/Institutes/EHS/MCB/MIC/Bioinformatics/.
MYCOSPHERE EFFECT OF THE PINE MUSHROOM (TRICHOLOMA MATSUTAKE) ON THE DIVERSITY, COMMUNITY STRUCTURE, AND FUNCTIONAL PROFILES OF SOIL MICROBES

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Background
The mycosphere is a distinct soil environment affected by fungal hyphal activities. Because mycosphere affects microbial activity, it is a valuable source of understanding microbial dynamics in soil ecosystem.

Objectives
The main objective of this study is to explore the mycosphere effect of T. matsutake by comparing bacterial and fungal diversity, community structure, and functional profiles in different zones of the fairy ring. We expanded on the design of past studies by comparing multiple fairy rings across two geographical locations.

Methods
We extracted soil DNA from mycosphere samples in fairy ring of T. matsutake and adjacent soil samples. After processing pyrosequencing data, we conducted metagenomic analysis for microbial diversity, community structure, and functional profiles.

Conclusions
Across samples, community was similar within but different between mycosphere and non-mycosphere samples—microbial diversity was lower in the mycosphere compared to non-mycosphere zones. Many ectomycorrhizal fungi were negatively correlated with the presence of T. matsutake. Analysis of bacterial functional profiles predicted distinct bacterial activity between zones; in mycosphere samples, xenobiotic biodegradation and metabolism, and amino acid metabolism were high, but carbohydrate metabolism, and glycansynthesis and metabolism were low compared to non-mycosphere samples. Overall, similar functional activity of mycosphere zones across fairy rings and geographic locations imply a mycosphere effect of T. matsutake, by recruiting mutualistic taxa and excluding antagonistic ones based on the functional trait.
COMPARATIVE ANALYSIS OF METAGENOMES FROM FILTER UNITS OF FULL-SCALE HYBRID TREATMENT WETLAND


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Background

Treatment wetlands (TW) have become widely used for wastewater treatment over the recent decade for their cost-effective building and maintenance. While physical and chemical pollutant removal mechanisms in TWs are well known, understanding of microbial processes related to pollutant removal in TWs is still low. Metagenomics offers the ability to examine directly the genomic content of microbial communities, complementing taxonomic information with functional capability.

Objectives

(1) to characterize microbial communities in vertical and horizontal sub-surface flow (VSSF and HSSF respectively) filter units of a full-scale TW treating wastewater from a schoolhouse for over ten years; (2) evaluate the functional potential of bacterial and archaeal communities.

Methods

Water samples, including influent, effluent and intermediate well, together with filter media biofilm from both units were used for shotgun sequencing with Illumina MiSeq system. Metagenomes were assembled into contigs and used for microbial profiling, identifying functional genes and relating them to nitrogen, phosphorus and carbon cycle.

Conclusions

Domain distributions showed the dominance of Bacteria (>95%), a small fraction of Eukaryotes (>2%), Archaea (>1%) and Viruses (0.2%). Samples of filter material had similar bacterial dominant phyla, but different distribution of lower taxonomic ranks. Wastewater samples had diverse bacterial community distribution between samples. Over one-third of archaeal sequences belonged to methanogenic species. Nitrogen metabolism in VSSF and HSSF filter units was dominated by ammonia assimilation related genes followed by nitrate and nitrite ammonification, denitrification and nitrogen fixation related genes. Phosphorous metabolism was dominated by phosphate metabolism genes.
Background

Metagenomics aims for sequencing and identification of DNA from communities of microorganisms in their natural environment. By estimating and comparing gene abundances between samples we can get a picture of the functional role of the metagenome, i.e. which genes and pathways are abundant at different conditions. Methods for functional metagenomics typically rely on identification of functional domains such as PFAMs, TIGRFAMs and COGs. However, this results in a broad classification of gene products since many proteins can be classified into the same functional domain. The human gut gene catalog, for instance, is predicted to contain more than 5 million genes, but the number of PFAM domains is only around 10,000.

Objectives

We present HierBin, a new method for functional annotation and quantification in metagenomes, giving a more detailed functional description of the metagenome and performing better in identification of changes between conditions.

Methods

First a supervised classification step is used to identify known functional domains in the data. Secondly, each domain is divided into subdomains using unsupervised clustering based on sequence similarity. Finally the abundance of each subdomain is quantified by mapping the sequence reads to the subdomain sequence and differentially abundant subdomains are identified.

Conclusions

We show, by evaluating on resampled data, that our method performs better in predicting differential abundant genes than methods using only functional domains for gene prediction and detects differences at higher resolution that would be invisible at lower resolution.

Finally, we apply our method both to metagenomics data from the human gut as well as environmental samples.
Background
Rapid sand filtration is a drinking water production technology widely used in Europe and other countries around the world. This technique allows the removal of ammonia, manganese, ferrous iron, methane, sulfides and other compounds from groundwater by a combination of physical, chemical and biological processes. During many years, it has been recognized that microbial activity plays an important role in these processes.

Objectives
Therefore, it is essential to study the microbial composition in these rapid sand filters and how these communities interact and facilitate the removal of pollutants.

Methods
In this study, six samples from a rapid sand filter were analyzed through a metagenomic approach. By building a non-redundant gene catalog we identify the microbial diversity and the metabolic pathways occurring in this system.

Conclusions
Nitrospirae and Proteobacteria were the dominant phyla. The functional genetic potential included most of genes related to nitrogen oxidation (specially abundant in the nitrification pathway) and methane oxidation. Genes involved in different CO2 fixation pathways were also abundant. Genera typically implicated in manganese oxidation as Hyphomicrobium and in iron oxidation as Gallionella were also abundant in the catalog. This investigation has so far revealed the main biological processes occurring in these ecosystems. In the next step, we will try to assemble genomes of the most abundant genotypes of the rapid sand filter community.
INTRODUCING A COMPLETE METAGENOMICS ANALYSIS PIPELINE, BASED ON AN OPTIMAL COMBINATION OF ILLUMINA SEQUENCING TECHNOLOGY AND AN EASY TO USE ONLINE PLATFORM

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Background
Direct sequencing of metagenomes is commonly implied for bacterial community analysis related to food research, biotechnological processes and clinical diagnostics.

Objectives
Commonly the taxonomic classification of sequences is based on unique target regions such as the 16S ribosomal gene which includes a number of unique variable regions. In this work we present an overview of a new and comprehensive Metagenomics analysis pipeline that we are using to prepare, sequence and analyze metagenomics samples from a range of environments.

Methods
In this holistic approach we start with laboratory techniques that take into account and reduce as much as possible the sources of possible bias that can be introduced into metagenomics datasets by laboratory steps like sampling procedures and DNA isolation. We further include both biological and technical controls to continually validate the process.

Conclusions
The bioinformatic analysis is performed using overlapping paired-end reads which are annotated through the Greengenes database (De Santis et al., 2006). Finally we introduce the ‘BaseClear metaGenome Browser’ (BmGB)™, https://metagenomics.baseclear.com, an online platform in which researchers (with or without any bioinformatics training) can easily interpret the taxonomic composition of hundreds of metagenomes using intuitive interfaces and efficient analyses. The workflow is tested on a number datasets and results are discussed.
LIMITS TO ROBUSTNESS, REPRODUCIBILITY AND ECOLOGICAL CONSISTENCY IN THE DEMARCATION OF OPERATIONAL TAXONOMIC UNITS
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Background

The demarcation of Operational Taxonomic Units (OTUs) from complex sequencing datasets is a key step in large-scale characterizations of microbial communities. However, it remains unclear how well the various proposed OTU clustering algorithms approximate ‘true’ microbial taxa, and many conceptually distinct approaches continue to be used.

Objectives

We explored different aspects of OTU clustering, in particular the robustness, reproducibility and ecological consistency of OTU demarcation.

Methods

In a global and comprehensive survey of roughly one million microbial SSU sequences, we objectively quantified biases introduced by several widely employed algorithms.

Conclusions

We observed surprising trends in the robustness of sequence clustering to changing parameters, and with regard to the reproducibility of OTU-based findings. Moreover, we explored the ecological consistency of OTUs – based on the assumption that, like true microbial taxa, they should show measurable habitat preferences (niche conservatism). We systematically parsed sequence annotations to obtain broad ecological descriptions of sampling sites. Based on these, we observed that sequence-based microbial OTUs generally showed high levels of ecological consistency. However, different OTU clustering methods resulted in marked differences in the strength of this signal. We found that hierarchical average and complete linkage clustering provided the most robust and reproducible partitions with regard to a wide range of parameters. Moreover, complete linkage clustering provided the ecologically most consistent clusters. Assuming that ecological consistency can serve as an objective external benchmark for cluster quality, we
concluded that hierarchical complete linkage clustering, should be the default choice for OTU clustering.
Background: Turf algae holobionts (host macroalgae plus microbiome) are widespread in coral reefs worldwide. They are also the most abundant benthic structures in the Abrolhos reef Bank (Brazil), with more than 50% benthic coverage. Abrolhos reefs turfs are morphologically diverse regarding color and texture, but their microbial matrix is not well known. Objectives: Our aim was to perform a metagenomic characterization of the prokaryotic microbiome of the different morphologic types of turf algae holobionts from Abrolhos reefs. Methods: We used metagenomic approaches to obtain the whole communities associated with turfs assemblages, collected at different reefs during March and October 2013, totaling 22 samples. Results: Filamentous, non-heterocystous cyanobacteria were the dominant microbial phototrophs of the holobionts. Main macroalgal in the holobiont were red, green and brown seaweeds. 15,27 million reads were generated using Illumina sequencing. Proteobacteria (36.8%, N = 1.564,678), Cyanobacteria (28.8%, N = 1,222,446), and Bacteroidetes (10.2%, N = 434,897) were dominant among the turf algae prokaryotic microbiome. Major microbial components of the turf algae holobiont are Roseobacter sp., Silicibacter sp., Dinoroseobacter sp., Congregibacter sp., Maribacter sp., and Cyanobacteria. Genes belonging to aerobic anoxygenic photosynthesis (AAnP), sulfur metabolism (e.g. S oxidation, and DMSP consumption) and virulence potential (adhesins, fibronectin/fibrinogen-binding protein, type IV pili, superoxide dismutase) were found in the turf algae microbiomes. Conclusion: The complex assemblies of microbial guilds render turf holobionts important competitive advantages allowing them to outcompete other benthic organisms and dominate vast reef areas.
EXPLORING MICROBIAL DIVERSITY PATTERNS IN THE SØR RONDANE MOUNTAINS (EAST ANTARCTICA) USING NEXT GENERATION SEQUENCING AND ARISA

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Background
Antarctica is a continent of extremes. Low availability of liquid water and nutrients, extreme low temperatures and high levels of radiation exert high selective pressures on organisms. Consequently, most life forms are microbial. Estimations indicate that as little as 1-3 % of the continental surface represents ice-free regions. Relatively few biological studies focus on terrestrial samples which most often originate from the McMurdo dry valleys that make out the largest contiguous ice-free area. Mountain tops protruding through the ice sheets (nunataks) are less frequently studied although they might function as havens and reservoirs for terrestrial organisms. Geological data shows the presence of different kinds of bedrock in the Sør Rondane mountains (Queen Maud Land, East Antarctica). Patches of macroscopic organisms (lichens, mosses and arthropods) are scattered throughout these ice-free islands, indicating a high amount of variability in the presence of organic matter, and hence nutrients for microbial life. Here we present the results of a large scale sampling effort in the eastern Sør Rondane Mountains of such ice-free regions near the Belgian Princess Elisabeth station.

Objectives
We examined the composition and distribution of bacterial communities in these refugia, and investigated the possible impact of environmental parameters on these community compositions.

Methods
Samples were subjected to both a genetic fingerprinting technique (ARISA) and second generation sequencing (Illumina MiSeq 300PE).

Conclusions
Preliminary results indicate an effect of bedrock type and presence of macrobiotic organisms.
Background

Salmonella 4,[5],12:i:- has become a new epidemic serotype in Europe associated with human infections, being the identification/tracking of its clones crucial to contain their spread.

Objectives

We assessed the trends in S. 4,[5],12:i:- clones distribution and their association with antibiotic and metal resistance/tolerance genes in Portuguese isolates. Results were also compared with previous data from the last decade (2002-2010).

Methods

S. 4,[5],12:i:- isolates (n=158), confirmed by PCR (fliB-fliA/fljB), from different sources (clinical/food) and regions of Portugal (2010-2014) were analyzed. They were screened for sulfamethoxazole resistance genes (sul1-sul2-sul3) and other class 1 integrons genes (intI1, qac and gene cassettes) by PCR. Detection of metal tolerance genes [copper (pcoD), silver/copper (silA) and mercury (merA)] and other antibiotic resistance genes by PCR, susceptibility to 10 antibiotics [ampicillin (A), chloramphenicol (C), gentamicin (G), kanamycin, nalidixic acid, ciprofloxacin, streptomycin (S), sulfamethoxazole (Su), tetracycline (T) and trimethoprim (Tr)] (CLSI/EUCAST) and clonality by PFGE were performed in representative isolates.
Conclusions

We detected the presence of the 3 clones currently circulating in Europe: i) "European clone" (75%; sul2 and absence of intI1/sul1/qacEdelta1; mostly ASSuT-bla\textsubscript{TEM}-strA-strB-sul2-tetB and carrying pcoD+silA+merA), which has expanded throughout this study period; ii) "Spanish clone" (6%; intI1; qacEdelta1+qacH; mostly AC(G)SSuTTTr-bla\textsubscript{TEM}+cmlA-floR-(aac(3)-IV)-aadA-sul1-sul2-sul3-tetA-dfrA12 and carrying merA-silA] and iii) "Southern-European clone" (1%; intI1; qacH; CSSuTTTr-cmlA-aadA-strA-strB-sul3-tetB-dfrA12) mostly with similar MDR and/or PFGE-types described since 2002. A marked decreased frequency of Spanish and Southern-European clones was observed contrasting with the expansion of the European clone characterized by ASSuT-phenotype and copper/silver/mercury tolerance genes, which might facilitate adaptation and success of these strains.
BIOAUGMENTATION WITH METAL-RESISTANT PLANT GROWTH-PROMOTING RHIZOBACTERIA IMPROVE THE METAL RHIZOACCUMULATION POTENTIAL OF SPARTINA MARITIMA

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Background
As an adjunct to phytoremediation strategies and as part of an effort to make this technology more efficacious, a number of scientists have begun to explore the possibility of using plants and the microbial populations colonizing the rhizosphere.

Objectives
A glasshouse experiment using soil from a metal-contaminated estuary was designed to investigate the effect of a native bacterial consortium, isolated from Spartina maritima rhizosphere and selected owing to their plant growth promoting (PGP) properties and multiresistance to heavy metals, in plant growth and metal accumulation.

Methods
Plants of S. maritima were randomly assigned to three soil bioaugmentation conditions (without inoculation, one inoculation and repeated inoculations during experimental period) for 30 days. Growth parameters and photosynthetic traits, together with total concentrations of several metals, including As, Cu, Pd and Zn were determined in roots and/or leaves.

Conclusions
Bacterial inoculation improved S. maritima root growth, through a beneficial effect on its photosynthetic rate ($A_n$) due to its positive impact on functionality of PSII and chlorophyll concentration. Also, bacterial inoculation favoured water use efficiency (WUE) of S. maritima, through the increment in $A_n$, stomatal conductance and in root-to-shoot ratio. Moreover, this consortium was able to stimulate plant metal uptake specifically in roots, with increases of up to 19% for As, 65% for Cu, 40% for Pb and 29% for Zn. On the basis of these results, bioaugmentation of S. maritima with the selected bacterial consortium can be claimed to enhance plant adaptation and metal rhizoaccumulation during marsh restoration programs.
ANALYSIS OF ADAPTATION TO HIGH CONCENTRATIONS OF NICKEL IONS OF A NOVEL SPHINGOBIUM STRAIN BY RNA-SEQ

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Background
While bacteria belonging to the Sphingobium genus are generally known for their capacity to degrade poly-aromatic hydrocarbons, their response to high concentrations of metal ions has been largely disregarded. Only S. cupriresistens, a species isolated from a copper mine, has been reported to tolerate high concentrations (0.9 mM) of Cu²⁺ [1]. We have recently reported the whole genome sequence of a novel Sphingobium strain able to tolerate Ni ions up to 20 mM [2].

Objectives
In order to understand the molecular mechanisms by which this strain is able to adapt to high concentrations of Ni ions we are currently investigating its transcriptome profiles by RNA-seq approach.

Methods
Sequencing of rRNA-depleted RNA was carried out for independent triplicate cultures in the presence or absence of 10 mM NiCl₂, using the SureSelect strand-specific RNA library prep kit (Agilent) for Illumina MiSeq multiplex sequencing.

Conclusions
Transcriptomic data show the differential expression (fold change ≥ |2|, p-adjusted < 0.05) of about one-hundred genes. Most of them are up-regulated in the presence of Ni²⁺ and include genes for membrane proteins and metal efflux systems. Gene annotation was further enriched with description of operons and UTRs as identified by the EuGene integrative gene finder tool [3]. The observed metal resistance of this Sphingobium strain highlights its possible use for biodegradation of xenobiotic compounds in metal-rich environments.

ADAPTABILITY OF TRICHODERMA ASPERELLUM TO HIGH CONCENTRATIONS OF VARIOUS METALS
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Background
*Trichoderma asperellum* is a biological agent capable of removing heavy metals from the environment via biosorption and/or bioaccumulation. Most studies investigated the use of dead fungal biomass for metal biosorption. The potentials of using live cells to transform toxic metals into less harmful forms (bioaccumulation) is lesser known.

Objectives
In this study, we explored the adaptability of *T. asperellum* to increasing concentrations (100-6000 ppm) of various heavy metals (Al, Cd, Cr, Cu, Pb and Zn).

Methods
Adaptive tolerance index (TI) of fungus in Potato Dextrose Agar was measured.

Conclusions
The results conveyed bioaccumulation potential of *T. asperellum*. Results revealed that *T. asperellum* has adaptability towards increasing concentrations of Cd (TI: 0.01-0.04), Cr (TI: 0.22-0.28) and Cu (TI: 0.24-0.31) at the beginning of the experiment. Decreasing TI values (from 1.08 to 0.01) with increasing Al, Pb and Zn concentrations indicated poor adaptability. The most tolerable concentration across all metals, except Cu, was 1000 ppm. In fact, *T. asperellum* tolerated up to 6000 ppm Al. Concentrations of 1500-2000 ppm generally exerted inhibitory effects with Cu being most toxic (tolerable up to 300 ppm). The adaptability of *T. asperellum* to high metal concentrations was evident with SEM micrographs showing hyphae growth, in spite of some structural deformities, metal precipitates and particles deposited on the hyphal surface. Nevertheless, in cases where growth was inhibited, the severely deformed hyphae displayed irregular, grooves and rough texture. Our study highlights the potential of live *T. asperellum* cells in removing heavy metals from the environment in a cheap and environmental-friendly manner.
Background
Salar de Atacama (Chile) is the largest basin in Atacama Desert (2900 Km²) and is located at 2300 m.a.s.l. This hypersaline system possess an average concentration of lithium as brine of 1500 ppm. Lithium is obtained industrially through the process of solar evaporation ponds, reflecting the conditions present in this Salar, including high UV radiation, high evaporation and low rainfall; however, this process is non-selective and is pollutant. We propose the recovery of lithium from brines based on biosorption using Gram-positive bacteria selectively accumulate lithium.

Objectives
The aim of this study was to describe microbial diversity present in brines of Salar de Atacama and to isolate native bacteria with lithium adsorbent potential.

Methods
Natural brines and concentrated lithium brines of Salar de Atacama were characterized. Such brines present hostile conditions for life, including high salt concentrations (0-100 g/L of Na⁺; 190- 450 g/L of Cl⁻; 0.2 -8 % of Li) and pH between 0.9 and 7. Simultaneously, microorganisms from the Salar were grown in a range of mineral mediums with or without organic carbon, and different concentrations of LiCl (0- 300 uM) through which the ability to absorb lithium was determined.

Conclusions
Bacteria and Archaea were detected in both brines. These microorganisms are likely naturally adapted to the differences in concentration of the elements present, especially lithium. Furthermore, bacteria capable of adsorbing lithium after 12 hours were obtained. Analyses of microbial diversity of the brines and development of a process for obtaining lithium by native microorganisms are currently underway.
MODELING ARSENITE OXIDATION BY SINORHIZOBIUM SP. M14 IN CHANGING ENVIRONMENTAL CONDITIONS

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Background
A complement to the chemical methods of treatment of water contaminated with arsenic are the biological methods i.e. biooxidation. Among the biological methods, technologies using bacteria, which oxidize arsenites, appear to have significant potential. In the literature, many bacterial strains able to oxidize arsenates are described, but most of them are not able to survive in new conditions. These disadvantages can be overcome if we use strains or microflora enriched by the gene pool localized on the plasmid pSinA Sinorhizobium sp. M14, carrying genes responsible for arsenic metabolism.

Objectives
The aims of this study were to (i) investigate the impact of biotic and abiotic factors, simulating the natural environment, on the growth kinetics and (ii) develop a kinetic model of arsenite oxidation by Sinorhizobium sp. M14.

Methods
The effect of different factors on arsenite biooxidation (i.e. start OD, substrate concentration, mixture of arsenic compounds, temperature and pH) on the efficiency of oxidation of arsenites and the changes of physiological condition of the studied strains was investigated. Cultures were grown on MSM supplemented with 0,04% yeast extract. As speciation samples were analyzed by HPLC/ESI MS, while the amount of biomass was monitored by OD/CFU determination. Based on the collected data, a kinetic model of the process was developed.

Conclusions
Culture of microorganisms in different concentrations of As (1;2,5;5 ppm), in mixture of arsenic compounds -As(III)/As(V) (2,5ppm/2,5ppm; 1ppm/4ppm), in different start OD (0,01;0,025;0,06;0,08;0,1;0,15), in various pH (4;6;7,5;10) and in different temperatures (10;16;22;30°C) allowed to develop a model of kinetics arsenite oxidation by Sinorhizobium sp. M14.
INVESTIGATIONS ON MULTIMETAL UPTAKE IN THE PRESENCE OF PESTICIDE BY FUNGAL ISOLATE

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Background
Wastewaters are often contaminated with complex cocktail of pollutants, out of which heavy metals and pesticides pose significant health risks. However, majority of the researches focus on bioremediation of individual metals or pesticides in isolation.

Objectives
Therefore, efforts are needed to obtain microbial strains which can simultaneously handle multiple pollutants.

Methods
Here a newly isolated fungal strain was assessed for its multimetal and pesticide uptake potential in the composite salts medium using glucose and lindane pesticide as the carbon and energy sources. This strain was able to grow in the presence of 1% glucose, 30 mg/L lindane and 30 mg/L multimetal (5 mg/L each of Cd²⁺, Cr⁶⁺, Cu²⁺, Ni²⁺, Pb²⁺ and Zn²⁺) and could degrade maximum amount of lindane to 1.92 ± 0.01 mg/L in about 72 h. The cube root growth kinetic constant (k) of the strain was obtained as 0.0211 g¹/³ l⁻¹/³ h⁻¹ in batch study. However, the presence of lindane caused an inhibition effect in the uptake of heavy metals and thus only Pb and Zn were brought down below the permissible mandates for irrigation. The results indicate that the growth kinetics and pollutant uptake is affected by the nature of contaminant matrix. More studies towards the elucidation of mechanisms of metal removal by the fungus in presence and absence of pesticide by various spectroscopic and microscopic techniques such as SEM, TEM-EDX, FTIR, XRD and XPS have been conducted.

Conclusions
These results reveal the inherent potential of this fungal strain to remove multimetals in the presence of pesticide from contaminated wastewater.
SEARCH FOR BACTERIA IN A MEDIEVAL ARCHEOLOGICAL SITE IN VERDUN (FRANCE) AND ISOLATION OF CUPRIAVIDUS NECATOR B9, A NEW METAL RESISTANT STRAIN

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Background
Remains of a well-preserved medieval foundry were excavated by archaeologists in 2013 in Verdun (France). Three to four ancient workshops specialized in brass and copper alloys were found with an activity between 13th to 16th c. Levels of Cu, Zn and Pb reached 20 000, 7000 and 6000 mg kg⁻¹ (dw), respectively, in several soil horizons.

Objectives
The objective was to examine the microbial community in this contaminated site.

Methods
A total of 8–22 10⁶ reads were obtained by shotgun metagenomics in each horizon (length : ± 220 pb). Bioinformatic analyses suggest the presence of very complex bacterial communities dominated by Proteobacteria. Using selective media three Cupriavidus necator/eutrophus strains were isolated. One of those strains, strain B9, differed from C. necator N1 and C.eutrophus H16 on several aspects. For instance, stain B9 was not able of autotrophic growth but was copper resistant (up to 1.2 mM). The strain features a plasmid that differs in size in comparison to the one observed in R. eutropha H16. Stain B9 was therefore characterized through genomic sequencing and a total of 516 contigs were obtained (Illumina sequencing, N50=25246 pb).

Conclusions
Preliminary analyses of the contigs confirm the absence of cbb genes necessary for autotrophy and show the presence of a ~70kb region containing metal detoxication genes (mainly copper but also a mercury resistance merRTPCA operon) also found in environmental Ralstonia pickettii strains and mainly an extended cluster of cop genes (cop KH3SRABCDIH2JGFOL1L2QH) largerly syntenic with the cop region of C. metallidurans plasmid pMOL30.
METAGENOMIC PROFILING OF MICROBIAL METAL INTERACTION IN RED SEA DEEP-ANOXIC BRINE POOLS’ WATER

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Background
Different geochemical studies reported high metal abundance in Red Sea deep-anoxic brine pools, especially in Atlantis II Deep, which has the highest metals content. Brine pools showed wide diversity of biologically essential and non-essential metals. Several metals known for their toxicity to biological life were detected in these pools. Yet, our previous microbiome analyses of the pools demonstrated vast microbiological diversity.

Objectives
In this study, we compare metal-resistant prokaryotic microbiomes in different metal-rich brine water samples from; Atlantis II lower convective layer (ATII-LCL), Atlantis II upper convective layer (ATII-UCL), Discovery Deep (DD) and Kebrit Deep (KD). Moreover, we investigate genome evolution of microbial communities in response to such excessive metal abundance.

Methods
Using 16S rRNA pyrotags and shot-gun 454-pyrosequencing, we perform a comparative analyses of a-taxonomic assignment of Operational Taxonomic Units to major bacterial and archaeal groups and b-metal resistant protein-coding genes, of the microbial communities and metagenomes. The ATII-LCL, ATII-UCL, DD and KD brine pools metagenomes protein-coding genes involved in microbial-metal interaction and resistances were assessed for abundance, diversity and novelty.

Conclusions
We report specific microbial diversity of these three brine pools. Functional analyses of the metagenomes revealed different metal resistance mechanisms. This was supported by the strong correlation between specific high metal/s concentration in selected brine water, where; metal resistance, enrichment of metals metabolism and transport were revealed. As expected, ATII-LCL showed the highest relative abundance of genes involved in microbial-metal interaction. Additionally, we report significant abundance of peroxidases-encoding genes, mainly in ATII-LCL, and we hypothesize that generation of H₂O₂ occurs through interaction of pyrite deposits.
Background
The brown rot fungi are important components of the coniferous northern hemisphere forest ecosystem and are characterized by their ability to colonize and degrade lignocellulosic materials; preferentially metabolizing hemicellulose and cellulose components and leaving a residue of modified lignin. Multiple aspects of the metabolism and degradative capabilities of these organisms are dependent upon cations, including but not limited to iron, manganese, and calcium.

Objectives
As wood degrades, it is characterized by decreasing pH and increasing concentrations of selected cations. The decay fungi, as they colonize wood, differentially translocate cations and actively modulate their ionic environment. The base cations are needed for many metabolic functions including membrane stabilization and they also function as enzyme cofactors and electrolytes. Transition metals are directly involved in lignocellulose degradation. We will examine the influence of cation concentrations on fungal sheath formation, and the activity of a biologically unique iron-based non-enzymatic degradative system in the brown rots.

Methods
The role of iron in the chelator-mediated Fenton (CMF) system will be examined as it is particularly crucial to the ability of the brown rot fungi to depolymerize lignocellulose. Methods used include but are not limited to ICP and XFM. Organisms used in this work include: *Serpula lacrymans, Postia placenta, Fomitopsis pinicola* and *Gloeophyllum trabeum*.

Conclusions
The role played by transition metals, pH, oxalate production and calcium oxalate crystal formation in brown rot physiology and cell wall breakdown mechanisms is discussed along with the potential ecological significance of cation mobilization and redistribution within the forest floor ecosystem.
ADHESIN COMPETENCE REPRESSOR (ADCR) FROM STREPTOCOCCUS PYOGENES CONTROLS ADAPTIVE RESPONSES TO ZINC LIMITATION AND CONTRIBUTES TO VIRULENCE
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Background
Altering zinc bioavailability to bacterial pathogens is a key component of host innate immunity. Thus, the ability to sense and adapt to the alterations in zinc concentrations is critical for bacterial survival and pathogenesis.

Objectives
To understand the adaptive responses of group A Streptococcus (GAS) to zinc limitation
To elucidate the regulation of adaptive responses by metalloregulator AdcR

Methods
RNA sequencing
Gel mobility shift assay
qRT-PCR
animal infection studies
X-ray crystallography

Conclusions
Genes involved in zinc mobilization and conservation are derepressed during mild zinc deficiency, whereas the energy-dependent zinc importers are upregulated during severe zinc deficiency.
We also demonstrated that transcription activation by AdcR occurs by direct binding to the promoter. However, the repression and activation by AdcR is mediated by its interactions with two distinct operator sequences.
Mutational analysis of the metal ligands of AdcR caused impaired DNA binding and attenuated virulence, indicating that zinc sensing by AdcR is critical for GAS pathogenesis. Together, we demonstrate that AdcR regulates GAS adaptive responses to zinc limitation and identify molecular components required for GAS survival during zinc deficiency.
MECHANISMS OF CHROMATE RESISTANCE AND REDUCTION BY KLEBSIELLA PNEUMONIAE CHROAQ1 ISOLATED FROM A CHROMIUM CONTAMINATED AQUIFER

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Background

Chromium-reducing microorganisms as well as their enzymes chromate-reductases have taken great interest due to their potential application in bioremediation processes. They are phylogenetic and metabolically diverse; in some cases Cr(VI) reduction is result of the metal respiration as in Geobacter. However in other microorganisms like genus Klebsiella has little knowledge about the metal reduction mechanisms.

Objectives

The present study was aimed at the characterization the mechanisms of Cr(VI) resistance and reduction in Klebsiella pneumoniae ChroAq1, isolated from long-term Cr(VI)-contaminated aquifer located in Guanajuato, México.

Methods

It was determined the ability to reduce Cr(VI) to Cr(III) by growing cells and cell-free extracts of K. pneumoniae ChroAq1. The cellular fractionation was performed by ultracentrifugation. Moreover the complete genome of ChroAq1 was sequenced with illumina technology, automatically annotated and analyzed. Some putative genes involved in resistance and reduction were deleted by double recombination.

Conclusions

The isolated strain ChroAq1 was identified as Klebsiella pneumoniae, its resistance observed was 1.8mM of Cr(VI) in anaerobic conditions and 22mM in aerobic conditions and was able to reduce Cr(VI) only anaerobically. The cell-free extract from this strain showed a NAD(P)H-dependent chromate-reductase activity associated to the soluble cell fraction. The gene chrA is present in the genome of
ChroAq1 however its deletion did not abolish the chromate resistance. In other hand, we deleted four genes presumptively involved in Cr(VI) reduction, one encodes a protein 84.57% identical to YieF (ABJ74146), other encodes one 86.58% identical to NemA (P77258) from *E. coli*, however they had no effect on the chromate reduction capability, the other two are being studied.
PRODUCTION OF Selenium AND Zinc ENRICHED BIOMASS AND NANOPARTICLES USING LACTOBACILLI
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Background
Selenium (Se) and Zinc (Zn), are the trace elements which play a pivotal role in humans well-being. Symptoms resulting from deficiency of these essential metals are diverse but chronic and severe and if untreated, can prove fatal. Bioproduction of Se and Zn enriched biomass and nanoparticles by lactobacilli can be explored and exploited for development of dietary organic sources of these minerals to overcome the mineral deficiencies and to improve the human health.

Objectives
To produce Se and Zn enriched biomass and Se⁰ and zinc oxide nanoparticles using lactobacillus spp.

Methods
Fifty human origin lactobacilli were screened for their ability to accumulate Se and Zn by growing them on medium added with different concentration of sodium selenite and zinc sulfate respectively. Estimation of Se and Zn accumulated by cultures was carried out by ICPES and AAS, respectively. Selected cultures were used for biogenic production of Se and ZnO nanoparticles and their size were determined by Scanning Electron Microscope.

Conclusions
Among all the isolates, L. reuteri NCDC77 was found to have greater ability to accumulate Se (820 μg/g dry weight) coupled with potential probiotic properties while L. plantarum YM2C was able to accumulate large amount of Zn (1.32mg/g dry weight). In addition, nanoparticles of Se and Zn produced by the selected strains, were found to be in the range of 100-200nm. Harnessing these potential cultures can serve as a novel application of these cell factories for the production of Se and Zn enriched functional foods and as dietary supplement.
Background
The inhibitory effect of metal(-ion)s on the growth of microorganisms is mainly determined by their chemical binding properties to essential biochemical molecules (e.g. thiols, proteins, enzymes, RNAs, DNA) and the effective intracellular concentration. Active control of the intracellular metal-ion concentration is one of the successful survival strategies microorganisms use against toxic metal-ion concentrations and they can harbor a variety of resistance systems to control the intracellular concentration of different metal-ions. Cupriavidus metallidurans CH34 is one of the model bacteria used to study survival in the presence of elevated metal-ion concentrations. Numerous studies have been performed using C. metallidurans CH34 to elucidate its various metal-ion defense mechanisms. As a consequence, a substantial amount of physical, chemical and biological data is available on the impact of several metal-ions on the behavior of C. metallidurans CH34.

Objectives
To test whether it is possible to establish a metal-ion toxicity model that is able to predict the Minimal Inhibitory Concentration for C. metallidurans CH34.

Methods
A two-variable metal-ion characteristics toxicity model, based on $\log K_{OH}$ ($K_{OH}$ = first hydrolysis constant) and $\chi^{2}_{m,r}$ (= covalent index), that efficiently correlates ($r^2=0.90$) with the measured MIC of 20 different metal ions, has been developed for C. metallidurans CH34 cultivated in chelating-free mineral medium. To test this chemical model for its applicability, the MIC of Pd$^{2+}$ was first calculated and afterwards experimentally determined.

Conclusions
The model prediction, 0.032 mM, corresponded very well to the measured MIC of 0.029 mM, indicating that the two metal-ion characteristics, $K_{OH}$ and $\chi^{2}_{m,r}$, can be used to reliably predict MIC values.
SEVEN MERA ALLELES ARE SPREAD AMONG ENTEROCOCCUS FROM DIFFERENT SPECIES, ENVIRONMENTS AND CLONAL BACKGROUNDS (1926-2012)

Background
Successful bacteria accumulated different genetic features shaping their evolution and fitness to diverse environments/hosts. Mercury (Hg) is widespread in Nature and possibly contributed for selection of particular strains.

Objectives
To evaluate the dispersion of diverse merA alleles among enterococci from several origins and clonal backgrounds.

Methods
merA alleles was searched in Enterococcus available genomes, used to construct a maximum-likelihood phylogenetic tree. A PCR scheme+RFLP+sequencing was developed to detect the six merA alleles identified, namely I-(GenBank-AECE01000068), IIA-(AIUL01000023.1), IIB-(ASDU0100008.1), III-(NZ_KB947199.1), IV-(AECE01000068.1) and VI-(NZ_KB030055.1), among 918 Enterococcus (Portugal; human/animal/environment/food; 1996-2012). Clonality was evaluated by PFGE/MLST, merA-transfer by conjugation and Hg-phenotype in m_Enterococcus+128mg/L HgCl2.

Conclusions
merA were found in 4% (n=37/918) of Enterococcus studied. PCR+RFLP+sequencing distinguished the six alleles previously found at Genbank and a new one (type V). They were distributed among diverse sources and clones, in our and GenBank isolates: types I and IV-human E. faecalis (Efls; n=5; USA; 1987-88; ST9/ST206); IIA-human/pig/trout/feed/hospital-sewage E. faecium-
Efm/E. hirae/Enterococcus_sp (n=17/1/1; Portugal/France/Denmark/Germany; 1989-2012; 12ST-Efm including from ST78-ST17-ST18-lineages-CC17); IIB-human Efls/E. dispar (n=2/1; Portugal/USA; 1926-2001; ST206/ST105/ST107); III-human/animal/hospital-sewage Efls/E. casseliflavus (n=15/1; Portugal/USA/Canada/Japan; 1961-2012; ST64/ST9/ST30/ST159/ST245); V-human/trout/pig-manure Efm (n=4; Portugal; 2001-2006; ST94/ST890); VI-human/hospital-sewage/piggery-soil/piggery-manure/trout Efm (n=23; Portugal, Italy France, Germany, Denmark, Hungary, Switzerland, Ireland, Norway, Brazil, USA; 1961-2012; 14ST including ST78-ST17-ST18-lineages-CC17). The presence of merA among major Efm/ST9-Efls lineages associated with human infections, suggests that mercurial-compounds potentially contributed for their selection/maintenance. merA⁺-Enterococcus grown in >128mg/L-HgCl₂. Transfer occurred for IIA and VI alleles. All but VI-merA alleles, were also identified in genomes of different species of Firmicutes (data not shown), suggesting genetic exchange of enterococci with bacteria sharing the same communities/environmental challenges.
THE SENSORY PROTEIN HBPS FROM THE SOIL BACTERIUM STREPTOMYCES RETICULI SPECIFICALLY INTERACTS WITH IRON, HEME AND AQUO-COBALAMIN

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Background
A novel type of redox-signaling system has been recently characterised in bacteria, the HbpS-SenS-SenR sytem from streptomycetes. The extracellular HbpS protein binds ferrous ions, heme and aquo-cobalamin.

Objectives
Elucidation of molecular mechanisms that modulate the function of HbpS-SenS-SenR, and analyses of binding sites as well as binding kinetics in HbpS-ligand interactions.

Methods
Disruption and site-directed mutagenesis; growth assays; crystallization; FRET, UV/Vis and CD spectroscopy; bioinformatics

Conclusions
HbpS-SenS-SenR is involved in the protection of Streptomyces against oxidative stress and is conserved in many other actinobacteria. HbpS acts as an accessory module of the two-component system SenS-SenR. Analysis of the HbpS crystal structure and biochemical studies revealed that HbpS assembles as an octamer that is crucial for interaction with the sensor kinase SenS. Under conditions of oxidative stress this leads to the autophosphorylation of SenS that, in turn, phosphorylates the response regulator SenR. This activates the transcription of anti-oxidative genes.

HbpS sequesters large quantities of ferrous iron ions which might protect Streptomyces from the effects of iron-based oxidative stress. HbpS is also an unusual heme-binding protein in which a specific threonine apparently binds to the tetrapyrrole macrocycle. In vitro and in vivo studies have also shown that HbpS can degrade heme. This activity may be responsible for HbpS-mediated protection against toxic concentrations of heme. Moreover, HbpS binds aquo-cobalamin. The calculated \( K_d \) of 34 \( \mu \text{M} \) suggests that HbpS might bind aquo-cobalamin in both bacterial cultures and in the Streptomyces natural environment the soil. The physiological relevance of this interaction remains, however, to be elucidated.
Background

Calcium carbonate precipitation is a widespread phenomenon among bacteria, with relevant implications in natural processes and great potentiality in numerous applications. Nevertheless, the molecular aspects of the process are still unknown.

Objectives

We have been studying CaCO₃ precipitation in the model bacterium *Bacillus subtilis* by different multidisciplinary approaches to identify: a) bacterial genes, and b) cell structure(s), involved in biomineralization; c) analytical markers able to distinguish the "biosignature" of natural calcite.

Methods

For objective a), we produced *B. subtilis* mutants by insertional mutagenesis and checked their precipitation phenotype. For objective b), we tested bacterial dead cells and cell fractions in a suitable precipitation test in solution. For objective c), we analyzed calcite crystals produced by *B. subtilis* by X-ray powder diffraction (XRPD) and Electron Paramagnetic Resonance (EPR) spectroscopy.

Conclusions

By analysis of mutants impaired in calcite formation, we identified a gene cluster as involved in the process, and two genes of the cluster, *etfA* and *etfB*, as the minimal gene set necessary for precipitation. The *B. subtilis* cell wall fraction, called BCF, was able to induce calcite formation in solution. BCF was applied on stone specimens and on a monumental site as an eco-friendly biotreatment for stone consolidation. New calcite formation and a little cohesion increase were observed in the treated stones. Both XRPD and EPR revealed unusual spectral parameters, attributed to the effects of the bioprecipitation of the mineral. The resultant spectroscopic fingerprint of bacterial calcite would be useful for identifying traces of bacterial activity in fossil carbonate deposits.
ALUMINUM TOXICITY AND TOLERANCE IN MICROORGANISMS
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Background

In acid soils below pH 4.5, aluminum becomes more soluble and toxic to plants, a few micronutrients, such as manganese and iron, become more soluble and toxic; and most plant nutrients, such as phosphorus, become more limited. This situation can cause serious destruction of the environment and significant economic problems. To overcome these problems, understanding of the toxicity of acid soils and the tolerance mechanisms in plants and microorganisms, especially the Al-toxicity and tolerance mechanisms, is essential.

Objectives

We have isolated many plants and microorganisms well adapted to highly acidic soils. Although there have been numerous reports on molecular mechanisms of Al tolerance in plants and microorganisms, such as organic acid secretion, alteration of cell walls, and Al-sequestration, our isolates suggested the complexity of the mechanisms of Al toxicity and tolerance. In this study we studied the Al-toxicity and tolerant mechanisms.

Methods

Microorganisms: Acidocella aluminiiidurans NBRC 104303(T), Burkholderia bannensis NBRC 103871(T), B. acidipaludis NBRC 101816(T) and 103872, B. heleia NBRC 101817(T), Pullulanibacillus sp. CA42, Rhodotorula taiwanensis CGMCC 2.4753, and Saccharomyces cerevisiae BY4741 and their derivatives.

Al tolerance test: Low phosphate synthetic minimal medium containing 1% glucose was buffered with 5 M succinic acid at pH 4.5. Cells were cultured in the presence of different concentrations Al at 28°C.

Conclusions

The results suggest that in addition to the aforementioned mechanisms of Al tolerance, an extracellular polysaccharide, cellular functions, and alteration of
membrane lipids, are relevant with the Al toxicity and tolerance. The knowledge will greatly help to overcome the acid soil problems.
Background

Metal nanoparticles have been produced using chemical and physical methods for many years. However, the exploitation of strong reducing agents may lead to undesired toxicity issues. It is, therefore, important to develop alternative and ecofriendly methods. Recently biosynthetic methods employing microorganisms have emerged as simple and viable alternative to produce metal/metalloid nanoparticles. In particular, the chalcogens selenium and tellurium in their elemental forms exhibited interesting antimicrobial activity. Moreover, nanoparticles produced with these elements show intriguing optoelectronic and semiconducting properties.

Objectives

A microbial strain characterized as *Ochrobactrum* sp. MPV1, isolated from the highly metal-polluted site of Scarlino(GR), was studied for its ability of effectively reducing both the oxyanions selenite and tellurite to their elemental forms in aerobic conditions and consequently producing elemental selenium and tellurium nanoparticles.

Methods

The isolate is capable of reducing 2 mM SeO$_3^{2-}$ in 48 hours and 0.3 mM TeO$_3^{2-}$ in 96 hours. The intracellular accumulation of nanoprecipitates was demonstrated through SEM-EDX and TEM analysis. Moreover, several analysis were performed in order to shed light on the mechanisms involved in selenite and tellurite reduction to the elemental state. The results obtained suggest that selenite and tellurite are reduced through two different mechanisms in *Ochrobactrum* sp. MPV1. Glutathione seems to play a major role in selenite reduction, while tellurite reduction could be ascribed to the catalytic activity of intracellular NADH-dependent oxidoreductases.

Conclusions

In conclusion, *Ochrobactrum* sp. MPV1 is an ideal candidate for the biogenesis of Se0 and Te0 nanoparticles, with possible biotechnological and industrial applications.
INVESTIGATION OF FACTORS DETERMINING STRESS RESISTANCE OF SOLVENTOGENIC BACTERIAE STRAIN CLOSTRIDIUM ACETOBUTYLICUM DERIVED BY ADAPTIVE SELECTION METHOD

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Background
Biobutanol is a promising type of motor fuel and feedstock for chemical industry.

Objectives
To elaborate efficient technology of biobutanol manufacturing from renewable materials adaptive selection technique was applied to derive solventogenic bacterial strain Clostridium acetobutylicum producing butanol in concentration up to 18.7 g/l. It was shown that the selected bacterial strain when compared to the parent strain was capable to withstand 1.3 times higher butanol level and was distinguished by osmotolerance, resistance to temperature impact and antibiotic chloramphenicol.

Methods
To define the factors governing enhanced resistance of selected strain C. acetobutylicum to stress exposure, lipid fatty acid composition of cellular membranes in the parent and butanol-adapted clostridial strains was analyzed. Using real-time PCR technique, we carried out comparative examination of expression of heat shock gene groEL and key gene adhE responsible for butanol biosynthesis in parent and adapted clostridial strains grown on butanol and solvent-free media.

Conclusions
It was found that the ratio of saturated to unsaturated acids is 1.84 times higher in butanol adapted strain C. acetobutylicum than in parent strain, which leads to stabilization of structure, promotes membrane fluidity and induces resistance to stress factors.

It was established that expression of groEL gene in the parent strain declined 100-fold on butanol-containing media, while expression of gene adhE was not recorded, in contrast to adapted strain C. acetobutylicum showing only minor decrease of gene expression under similar conditions. The obtained results indicate that cell metabolism of adapted bacterial strain was less sensitive to adverse butanol effects as compared to wild-type bacteria.
Background
The start-up of trial operation of biogas plant usually consists of adding leachate from other biogas plants or inoculation by cattle slurry. An alternative to the traditional start-up operation is the use of the freeze-dried microbial consortia that were previously selected in anaerobic digestion process.

Objectives
The aims of this work were investigate the effects of freeze-drying of methanogenic consortium selected from agriculture anaerobic digestion plant on: (i) biogas production efficiency and (ii) stability of microbial community structure.

Methods
Lab-scale anaerobic digestion process. Freeze-drying. Biogas yield: daily biogas production, methane content were analyzed by gas chromatography GC-MS. Physical and chemical analysis: pH, volatile fatty acids (VFA), chemical oxygen demand (COD), ammonium. Determination of total cell counts by fluorescence microscope (Japan) using the filter set WU for DAPI detection. DNA and RNA isolation. PCR amplification and 16S rRNA gene amplicons libraries.

Conclusions
The conducted analyses revealed that the consortium selected from anaerobic digester plant is active and able to effectively produce biogas after long-term storage. 16S rRNA gene amplicon libraries analyses showed that the microbial consortia have the same methanogenic community composition after long-term storage. These results demonstrated that described method for long-term storage and revitalization of methanogenic consortia could be used as a starter to quick start-up industrial process of biogas production.
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Microbes and alternative energy sources

GENETIC ENGINEERING OF CYANOBACTERIA FOR HYDROGEN PRODUCTION

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Background

Hydrogen can be naturally produced by several groups of organisms, including cyanobacteria. As a biofuel, hydrogen has substantial advantages over carbon-based compounds as it does not generate carbon dioxide and other atmospheric pollutants, and has a higher mass energy density than any other fuel. However, the current cost of its industrial production and the low yield of hydrogen by living organisms are not conducive to increasing its use as an environmentally friendly alternative to the traditional fuels.

Objectives

Our goal was genetic engineering of cyanobacteria for the purpose of increasing the yield of hydrogen that was generated by these strains.

Methods

For this purpose, we employed homologous and heterologous expression of hydrogenases in cyanobacteria.

Conclusions

Genetic engineering of cyanobacteria allowed us to significantly increase their hydrogen-producing capacity without compromising the stability of the mutant strains. As the details of molecular regulation of hydrogen production in the cell become increasingly available, new steps are being taken to improve the level of hydrogen production by cyanobacteria. Due to a photoautotrophic nature and low maintenance cost of these microorganisms, they represent the most economical system for biological hydrogen production with a potential for commercial application.
NEW GENETIC APPROACH TO ENGINEERING OF MUTANT STRAINS OF CYANOBACTERIUM ANABAENA VARIABILIS ATCC 29413 WITH ENHANCED LEVELS OF BIOHYDROGEN PHOTOPRODUCTION

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Background
Heterocystous cyanobacteria are capable to efficient nitrogenase-dependent H₂-photoproduction in the case of mutational elimination of uptake hydrogenase activity. In this study we used previously isolated chemically induced H₂-producing mutants of A.variabilis PK84 and PK17 with impaired activity of uptake hydrogenase and also ammonium producing ethylenediamine-resistant (ER⁶) mutants ED21 and ED93 characterized by derepressed levels of nitrogenase activity and low levels of glutamine synthetase activity. Our sequencing data revealed the point mutations in conservative sites of genes hypF (PK84: Asp374Asn), hupL (PK17: Cys446Tyr), glnA (ED21: Ala382Thr, ED93: Pro465Leu).

Objectives
The construction of A.variabilis strains with mutations in both hypF (or hupL) and glnA genes and analysis of their H₂-producing capacities in comparison with reference strains PK84 and PK17.

Methods
The mobilizable plasmid vector pRL498 was used for cloning and transfer of DNA fragments with mutations into Anabaena cells via triparental mating with E.coli. Mutant strains PK84 and PK17 were used as recipients for cloned fragments with glnA-mutations (with selection of ED⁶ recombinants) whereas mutants ED21 and ED93 were recipients for hypF or hupL genes inactivated by insertion of spectinomycine resistance cassette (with selection of Sp⁶ recombinants)

Conclusions
In both variants of gene transfer we have selected some biotechnologically perspective double mutants with lower growth rate and higher level of H₂-production compared with strains PK84 and PK17 (up to 130 % per biomass unit) that might be explained by enhanced levels of nitrogenase activity accompanying by a partial block in biosynthetic or assimilatory processes caused by additional alterations in glnA gene.
IMMOLIZED BIOCATALYSTS FOR BIODIESEL FUEL PRODUCTION

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Background

Biodiesel comprising of fatty acid alkyl esters is a promising alternative fuel. The biodiesel fuel can be produced under milder conditions and with fewer steps using biocatalysts instead of conventional alkali catalyst. In order to increase the biocatalyst stability and to ensure its easy separation from the biodiesel fuel, it is required to immobilize the biocatalyst on a suitable carrier. The comparison of biodiesel fuel production with different immobilized biocatalysts will be helpful for the process optimization and for further industrial implementation.

Objectives

In our work, we studied the immobilization of lipases on silica, diatomite, and ion exchange resins, and the immobilization of yeast cells and mycelium on both soft (polyurethane foam) and rigid (haydite) carriers to perform the comparison of different immobilized biocatalysts in stirred-tank and packed bed bioreactors.

Methods

_Pseudomonas fluorescens_ lipase, _Rhizopus oryzae_ lipase, Lipase _Candida antarctica_, glutaraldehyde, tributyrine, and triglycerides standards were from Sigma-Aldrich. _Aspergillus niger_ DSM823 and _Yarrowia lipolytica_ DSM8218 strains were from DSMZ. Activity of biocatalysts was assayed by the hydrolysis of tributyrine with analysis of butyric acid by GC. Methanolysis of triglycerides was controlled by HPLC. The immobilization of microorganisms was controlled by SEM (see Fig.1, DSM8218 on haydite, bar 10 µm).
Conclusions
The highest yield of biodiesel fuel (fatty acid methyl esters) was observed for the DSM823 mycelium immobilized on polyurethane foam in packed bed bioreactor. This suggests the whole-cell biocatalysis in continuous mode bioreactors is the most promising approach in biodiesel fuel production.

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THE USE OF MIXED HYDROLYTIC BACTERIA IN THE PRODUCTION OF SUPPLEMENTS ENHANCING BIOGAS PRODUCTION PROCESS

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Background
During the degradation of the lignocellulosic biomass by hydrolytic bacteria many organic compounds such as: volatile fatty acids, simple carbohydrates and many intermediate metabolites are released. These compounds are used by successive groups of microorganisms involved in the subsequent stages of anaerobic digestion process.

Objectives
The main aim of this study was (i) degradation of lignocellulosic biomass (maize silage) by mixed hydrolytic bacteria and (ii) production of supplements dedicated for enhanced biogas production.

Methods
(I) Preparation of the supplements: aerobic degradation of lignocellulosic biomass (3% d.m. of corn silage) by mixed hydrolytic bacteria for 72 hours at 30°C at pH 7. Centrifugation and extraction of the supernatant from the culture. (II) Analysis of influence of produced supplements on anaerobic digestion. Lab-scale anaerobic digestion process. Biogas yield: daily biogas production, methane content were analyzed by gas chromatography GC-MS. Physical and chemical analysis: pH, volatile fatty acids (VFA), chemical oxygen demand (COD), total solids (TS), volatile solids (VS).

Conclusions
In the presented work it was showed that the mixed hydrolytic bacteria can be used for preparation of supplements dedicated for the methane fermentation process. The results demonstrate that supplements produced by hydrolysis mediated by mixed hydrolytic bacteria, can increase the efficiency of biogas production up to 20-30%. Such supplements can be used in both well-working biogas plants in order to increase the efficiency of the biogas production process.
ANALOBIC CO-DIGESTION OF HARDLY BIODEGRADABLE ORGANIC MATERIALS WITH THE USE OF MAIZE SILAGE
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Background
Many organic residues are often used as a substrate in anaerobic digestion, but the efficiency of the process is determined by the level of biodegradability of waste materials. To increase digester gas production from hard-degradable compounds various co-digestion processes are applied. If co-substrates are used in anaerobic digestion system it improves the biogas yields due to positive synergisms established in the digestion medium and the supply of missing nutrients.

Objectives
The main aim of this study was to verify if maize silage, which is the most popular substrate used in production of biogas in agriculture plants, can be also a valuable co-substrate used for utilization of hardly biodegradable substrates such as sewage sludge waste (SSW) or residues from coal mines (RCM).

Methods
Laboratory anaerobic digestion process in various ratios of corn silage and utilization of waste (3\%, 2+1\%, 1+2\%, 0\%). Biogas yield: daily biogas production, methane content were analyzed by gas chromatography GC-MS. Physical and chemical analysis: pH, volatile fatty acids (VFA), chemical oxygen demand (COD), total solids (TS), volatile solids (VS). Determination of total cell counts by fluorescence microscope, using the filter set WU for DAPI detection.

Conclusions
Obtained results showed that maize silage can be used as valuable co-substrates during utilization of various industrial waste materials. The addition of only 1\% of the dry matter of maize silage to sewage sludge waste and residues from coal mine increased the biogas yield (even up to 300\%).
CHARACTERIZATION OF AN ANAEROBIC THERMOPHILIC GLYCEROL-DEGRADING ENRICHMENT CULTURE
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Background
The glycerol market was totally changed by the biodiesel industry, which resulted in the production of an excess of this compound as an industrial by-product. As a consequence, the price of glycerol dropped and a huge interest in alternatives for its valorisation emerged since then. In the field of Biotechnology research, glycerol is an attractive compound for the microbial production of chemical building blocks.

Objectives
The aim of this work was to investigate thermophilic anaerobic communities capable of conversion of glycerol.

Methods
Thermophilic sludge from a lab-scale anaerobic reactor fed with skim milk and sodium oleate (50:50% chemical oxygen demand) was incubated at 55°C in closed bottles containing bicarbonate-buffered medium supplemented with 10mM glycerol. Periodic successive transfers of the glycerol-converting enrichment culture, combined with serial dilutions were performed. After eight generations a highly enriched, low diversity (microscopic observations and 16s rRNA DGGE profiling) microbial culture was obtained.

Conclusions
The enriched culture converted glycerol mainly to methane (6mM) and acetate (7mM) within 6 days of incubation. A yet unknown organic compound was also produced. Sequencing results obtained on the Illumina platform showed the bacterial predominance of an uncultured Thermotoga species (75 % of the retrieved sequences), an uncultured Anaerobaculum species (13 %) and a close relative to Thermoanaerobacter pseudethanolicus (5 %). Isolation of the new uncultured Thermotoga and Anaerobaculum species is ongoing and their role in glycerol degradation will be assessed.
Background
Bioethanol fermentation can use the high cellulose substrate such as banana pseudo-stem that has 35.96% of cellulose content. The research about simultaneous and separated saccharification of banana pseudo-stem waste (Musa x paradisiaca var. sapientum L.) and fermentation of the hydrolysate for bioethanol production to get an effective enzyme dosage of hydrolysis, consortium of microbes, method of fermentation has been carried out.

Objectives
The purpose of the research are to get the most effective consortium and method in bioethanol fermentation from banana pseudo-stem waste (Musa x paradisiaca var. sapientum L.) using Simultaneous Saccharification and Fermentation (SFS) and Separated Hydrolysis and Fermentation (SHF) methods.

Methods
The experimental method was used in fermentation process of banana pseudo-stem waste with Complete Randomize Design (CRD) consist of 2 factors and 3 replications. First factor were the combination of method and consortium (K) and second factor were sampling time (T).

Conclusions
The result showed that the most effective dosage during dosage optimization of α-amylase enzyme was dosage 1 with Dextrose Equivalent (DE) about 23.1; hemicellulase enzyme was dosage 2/3 with DE about 34.9; cellulase enzyme and glucoamylase enzyme were dosage 2/3 with DE about 57.4. The result showed that consortium of Pichia stipitis and Saccharomyces cerevisiae and Simultaneous Saccharification and Fermentation (SFS) method were the best consortium and method in bioethanol fermentation of banana pseudo-stem waste that produce the highest ethanol concentration about 6.376% in 72 hours fermentation.
Background

Vinasse is a liquid by-product of the ethanol production. Its high organic content (COD of 20-40g/L) means that its disposal into the environment is hazardous and has a considerable pollution potential. However, vinasse can be treated by fermentation process in bioreactors. In this case, thermophilic anaerobic digestion can be used to treat this wastewater from sugar cane industry.

Objectives

The aim of this work was to isolate thermophilic fermentative bacteria present in vinasse.

Methods

Thermophilic bacteria was isolated by anaerobic serial dilution technique on plates containing fermented vinasse with pH of 6.5 and at 50°C. Colonies were identified by MALDI-TOF mass spectrometry technique.

Conclusions

Bacillus thermoamylovorans, facultatively anaerobic and amylolytic bacterium was isolated from fermented vinasse. This strain ferments carbohydrate producing lactate, acetate, ethanol, and formate but not hydrogen (Combet-Blanc et al, 1995). The presence of this bacterium shows that vinasse is a source of microorganisms that can be used as inocula in anaerobic treatment of vinasse.
ENDO-BETA-1,4-MANNANASES FROM THERMOTOLERANT BACILLUS SP.

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Background

Endo-β-1,4-mannanases are important catalytic agent in several industries. The enzymes randomly cleave the β-1,4-linkage in mannan backbone and release short β-1,4-mannooligosaccharides and mannose. They are classified as glycoside hydrolases.

Objectives

The present study, the endo-β-1,4 mannanase was isolated from thermotolerant bacteria and physicochemistry properties of purified enzyme were characterized.

Methods

Microorganisms (320 isolates) were screened on minimum medium containing locust bean gum at 45°C. The species of selected strain was identified by 16S rDNA sequence analysis. An endo-β-1,4-mannanase was purified to homogeneity by using anion-exchange, hydrophobic, and size-exclusive column chromatographies. The molecular mass was analyzed by SDS-PAGE.

Conclusions

The 16S rDNA sequence revealed that the highest mannanase producing microorganism was Bacillus sp. The specific activity of purified enzyme was 14.5 U/mg. The apparent molecular mass was 38 kDa. The optimal pH and temperature for enzyme activity were pH 6.0 and 60°C, respectively. The enzyme was stable in a pH ranges from 5 to 9, after 16 h of incubation at 4°C and stable up to 60°C for 1 h, and more than 80 % of initial activity remained. This endo-β-1,4-mannanase can be applied in several industries such as animal feed, pharmaceutical, paper and pulp, coffee, and bioethanol.
ESCHERICHIA COLI [NI-Fe]-HYDROGENASES ACTIVITY DURING GLYCEROL FERMENTATION UPON FORMATE SUPPLEMENTATION

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Background

*Escherichia coli* encodes four [Ni-Fe]-hydrogenases (Hyd).

Objectives

H₂ producing Hyd activity was studied during glycerol fermentation at different pHs upon external formate supplemented (10 mM).

Methods

H₂ evolution was measured by using pair of Pt and Ti-Si redox electrodes.

Conclusions

At pH 7.5 and 6.5 wild type cells showed similar H₂ production rate (V₉₂) when in the assays glycerol was added, whereas in formate supplemented assays it was decreased ~1.5 fold at pH 6.5. V₉₂ was lowered ~2 fold in selC (coding for formate dehydrogenases) single mutant at pH 7.5. At both pHs when formate was added in the assays, V₉₂ decreased in the strains where Hyd-3 or formate hydrogen lyase 1 related enzymes were disturbed. When glycerol was supplemented in the assays, V₉₂ was significantly lowered in triple hyaB hybC (coding for large subunits of Hyd-1 and Hyd-2, respectively) selC or hyaB hybC hycE (coding for large subunit of Hyd-3) mutants at both pHs.

Taken together it might be suggested that Hyd-3 becomes mainly responsible for H₂ production during glycerol fermentation when external formate is added. Besides, in the glycerol supplemented assays, three hydrogenases can work in H₂ producing mode and only deletion of three of them decreases the production of H₂. This effect might be due to disturbance of H₂ cycling. Therefore, responsible Hyd enzymes have been revealed under the conditions above.

All this is of significance in application of different carbon sources, especially mixed carbons, in H₂ production technology using bacteria.
PURIFICATION OF FERULIC ACID ESTERASE FROM BACILLUS MEGATERIUM

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Background
Ferulic acid esterase (FAE) are a subclass of carboxylic acid esterases (EC 3.1.1.73) that play role in complete enzymatic hydrolysis of hemicelluloses by hydrolyzing the ferulate ester groups in the cross-linking between hemicelluloses and between hemicellulose and lignin.

Objectives
To screen and purify ferulic acid esterase from thermotolerant bacteria

Methods
A total of 250 microorganisms collected from soil and rotten wood were isolated on minimum medium containing 1% rice straw at 45°C for strains that produced high FAE activity. The selected isolate was identified by 16S rDNA sequence analysis. Enzyme was purified by using anion-exchange, hydrophobic, and size-exclusive column chromatographies. The purified enzyme was characterized for its temperature and pH optima and stability.

Conclusions
The 16S rDNA analysis of selected isolate revealed that it belong to Bacillus megaterium. The optimum temperature and pH of purified FAE were 60°C and pH 7.0. The enzyme was stable in a wide pH ranges (pH 1–8); more than 80 % of initial activity remained after 16 h of incubation at 4 °C and stable up to 80°C for 1 h. The specific activity was 298.01 U/mg. This ferulic acid esterase have potential applications for several industries that high temperature and wide pH range are needed.
EFFECT OF PHOSPHOGYPSUM AND SEWAGE SLUDGE FERTILIZATION ON
HEMP (CANNABIS SATIVA) YIELD AND MYCORRHIZA AND SOIL
RESPIRATION, MICROBIAL BIOMASS AND DEHYDROGENASE ACTIVITY
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Background
Phosphogypsum and sewage sludge are formed as a by-product during production of fertilizers from phosphate rock and industrial or municipal waste-water treatment, respectively.

Objectives
The aim of present study was to investigate the hemp yield and arbuscular mycorrhizal fungi (AMF) root colonization and spore formation in response to phosphogypsum and municipal sewage sludge fertilization. Additionally the effect of phosphogypsum and sewage sludge on soil respiration, microbial biomass and dehydrogenase activity were also examined.

Methods
The field spots with three hemp varieties Beniko, Bialobrzeskie and Tygra were studied. An equivalent of 170 kg per ha pure nitrogen was used as fertilization in treatments with sewage sludge. The phosphogypsum at the level 100, 500 and 1000 kg per ha was applied.
AMF root colonization by microscopic and molecular techniques were studied. For determination of soil microbial biomass fumigation-extraction method (PN-ISO 14240-2) was used. Soil respiration by determination of CO₂ release by titration (PN-ISO 16072) and dehydrogenase activity by TTC method (PN-ISO 23753-1) were measured.

Conclusions
Application in agriculture seems to be one of the most reasonable possibility for utilization of phosphogypsum and sewage sludge, especially as fertilizer in energetic plant production. Additionally, phosphogypsum in combination with sewage sludge significantly increased soil biological activity.

Financial support was provided by EU Project at Warsaw Life Sciences University 'A program to improve the level of the didactic approach to the question of how to obtain raw plant materials for the purposes of energy production in the context of the Europe 2020 Strategy objectives'.
PALLERONIA ABYSSALIS SP. NOV., ISOLATED FROM THE DEEP MEDITERRANEAN SEA AND THE EMENDED DESCRIPTION OF THE GENUS PALLERONIA AND OF THE SPECIES PALLERONIA MARISMINORIS

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Background
Three strains designated 221-F¹, 221-F² and 3030-F¹ were isolated from the Matapan Vavilov Deep canyon, also known as Calypso Deep in the Eastern Mediterranean Sea, at a depth of 4,908 meters.

Objectives
To describe a new species based on 16S rRNA gene sequence analysis and phenotypic characteristics. These strains were found to be most closely related to Palleronia marisminoris and Hwanghaeicola aestuarii.

Methods
The description is based on a polyphasic approach with extensive phenotypic, quimiotaxonomic, genotypic and phylogenetic analysis.

Conclusions
The strains were observed to be red-pigmented and to form non-motile cocci or pleomorphic cells. The cells were found to stain Gram-negative, to be strictly aerobic, oxidase and catalase positive. Strains 221-F¹, 221-F² and 3030-F¹ were found to be mesophilic and to grow in medium containing up to 13 % NaCl. The major polar lipids of the three strains were identified as diphosphatidylglycerol, phosphatidylglycerol, phosphatidylcholine, an unidentified glycolipid and an unidentified aminolipid. Ubiquinone 10 (U-10) was found to be the major respiratory quinone. The DNA G+C content of strain 221-F¹ was determined to be 64.7 mol %. The new isolates were unambiguously more closely related to the type strain of Palleronia marisminoris (95.3% of similarity) than to Hwanghaeicola aestuarii (94.7% of similarity). Based on phylogenetic, physiological and biochemical characteristics we describe a new species represented by strain 221-F¹ (=CECT 8504T =LMG 27977T) for which we propose the name Palleronia abyssalis sp. nov. We also propose to emend the description of the genus Palleronia and the species Palleronia marisminoris to reflect new results obtained in this study.
Background
The Black Sea is a world’s largest meromictic basin, where deep sulfide-rich waters do not mix with the upper oxic waters. Interestingly, sulfate-reducing bacteria (SRB) were detected there not only in the anoxic zone but also in the oxic water layer. A pure culture of a new SRB species, possessing different systems of antioxidative defense, was isolated for the first time from the Black Sea oxygenated water column.

Objectives
We analyzed the phylogenetic composition of SRB community in the Black Sea oxic waters where representatives of Desulfovibrio-Desulfomicrobium, Desulfococcus-Desulfonema-Desulfosarcina and Desulfotomaculum subgroups were revealed. The pure culture of psychrophilic Gram-negative SRB was isolated from the subsurface oxic waters and described as Desulfofrigus euxinos. This new species possesses different systems of antioxidative defense including O$_2$ reduction and ROS detoxification.

Methods
FISH, nested and qRT-PCR with primers to 16S rRNA and dsrB genes, DGGE and nucleotide sequencing were used to investigate the SRB community. DNA-DNA hybridization, fatty acids analysis and growth tests with different electron donors/acceptors were performed to characterize the SRB species. Oxygen consumption rates were measured with a Clark-type electrode. Key genes of antioxidative defense were identified by DOP-PCR, and inverse PCR was carried out for the sequence analysis of the entire genes.

Conclusions
While some SRB are resistant to aerobic conditions because of multi-component antioxidative mechanisms, their presence in the sea oxygenated waters is likely to depend also on inhabiting anoxic microniches within suspended organic particles. The work was supported by RFBR projects nos. 10-04-00220-a and 12-04-91052-CNRS-a (PICS #6041).
Background

Bacteria play an essential role in food webs and biogeochemical cycles in aquatic ecosystems. Investigating ecological relationships among bacterial taxa and between abiotic and biotic ecosystem components is critical to environmental control and functional traits of individual bacterial group. However, such information is scarce. Here we present the results of a study on the spatio-temporal dynamics of bacterial communities in a stratified lake.

Objectives

The main objectives of the present study were to: (a) investigate the bacterial diversity and dynamics through time and space; (b) identify relations between specific bacterial groups and environmental parameters.

Methods

We collected over a year monthly samples throughout the water column and from the sediment of a holomictic lake in the Netherlands. Subsequently, we sequenced the 16S rRNA genes of bacteria on an Illumina MiSeq platform to determine the diversity and relative abundance. Simultaneously different physical and chemical parameters were measured. Statistical analysis was performed to detect possible interactions among different populations and to determine relations between specific bacterial groups and environmental parameters.

Conclusions

All sequences were affiliated to members of phyla commonly found in freshwater systems. Bacterial community changed dramatically over time and space in the water column, but clear seasonal successions could be observed. Contrastingly, the bacterial community in the sediment was quite stable, and mainly composed of Proteobacteria and Bacteroidetes. Statistical analysis showed Actinobacteria were positively correlated with temperature, while Cyanobacteria and Planctomycetes co-varied with nitrate. Betaproteobacteria, Deltaproteobacteria, Gammaproteobacteria,
Epsilonproteobacteria and Firmicutes were positively correlated with ammonium and negatively correlated with dissolved oxygen and pH.
Background
The high-altitude wetlands of the Chilean Altiplano (Atacama Desert) are unique and remote aquatic ecosystems, and are considered part of the cold biosphere. These ecosystems characteristically contain organisms with a high level of endemism that can prosper under extreme environmental conditions. Furthermore, microbial diversity is typically high, and includes a large contribution of previously undescribed groups of Bacteria and Archaea. Microbial mats are common, but are typically small in area and depth relative to those described from other environments.

Objectives
To describe members of the rare biosphere from a micro-profile taken from Salar de Huasco microbial mat.

Methods
Bacterial diversity was described by pyrosequencing of 16S rRNA genes of each layer (5) of a microbial mat (<5 mm depth) as well as metagenomic analysis (454 Roche) of microbial mat samples. Oxygen microprofiles were performed in situ using a microprofiling system (Unisense). Microbial diversity was characterized by the presence of groups of low relative abundance (rare biosphere) and also low sequence identity with sequences of available databases. At the phyla level, rare bacteria from the microbial mats (1.6-0.05% of relative abundance) were affiliated with Planctomycetes, Verrucomicrobia, Chlorobi, Acidobacteria, SR1, Armatimonadetes (ex-OP10), OD1, BRC1, Saccharibacteria (ex-TM7), OP11, Lentisphaerae, Spirochaetes, Synergistetes, and others.

Conclusions
Different members of the rare biosphere were present across the microbial mat micro-profile. Particular rare groups could be associated with the specific conditions in each layer of the microbial mat, providing clues to their likely metabolic function.

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MICROBIAL COMMUNITIES IN OPERATIONAL DRINKING WATER DISTRIBUTION SYSTEMS: IMPLICATIONS FOR DRINKING WATER QUALITY

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Background

Microorganisms inhabiting drinking water distribution systems (DWDS), particularly those attached to pipes forming biofilms, can affect the safety and quality of the water. The presence of unfavourable microorganisms in DWDS is influenced by a number of factors ranging from physico-chemical properties of the source water to the characteristics of the pipe infrastructure itself. The difficulty of accessing the internal surface of pipes has limited the study of the microbial ecology of DWDS. Most previous research in this area has been based on laboratory models and/or assessing only few selected microorganisms under controlled conditions which do not represent real systems.

Objectives

To better understand the microbial ecology of DWDS, microbial communities were monitored in two chlorinated networks supplied with different source waters.

Methods

Biofilm sampling devices fitted with coupons designed to maintain boundary layer conditions at the pipe water interface were installed in the networks. Over a one year-period, water physico-chemical parameters were measured and the dynamics of plankton and biofilm communities were monitored using Illumina MySeq sequencing.

Conclusions

Clear differences in the composition of the microbial communities supplied with different source waters were detected. Microbial diversity in the groundwater supplied network was higher than previously thought in chlorinated systems. Source water and the hydraulic regime in the system were critical factors shifting microbial communities and influencing the quality of the water. This new understanding of the microbial ecology of DWDS is vital to improve control and management strategies to help safeguard drinking water quality and ultimately public health.
CYTOTOXIC AND ANTIMICROBIAL ACTIVITIES OF SECONDARY METABOLITES PRODUCED BY MARINE ACTINOMYCETE STRAINS ISOLATED FROM SAUDI COASTAL HABITATS

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Background

Cancer and infectious diseases are still the main two health problems facing the humankind. Actinomycetes from the coastal habitats of Saudi Arabia can represent a good source for drug discovery.

Objectives

The main objectives of the present study were screening the marine actinomycete strains isolated from the coastal habitats of Saudi Arabia for production of anticancer and antimicrobial compounds and identifying them to the species level by the polyphasic approach.

Methods

Eight samples were collected from the Arabian Gulf habitats of Saudi Arabia. The selective isolation of actinomycetes was carried out on three different recommended media. Twenty six strains were selected to study their diversity by examining their morphology and determining their cell wall diaminopimelic acid type and whole-cell sugar pattern. Sixteen representative strains were screened for their cytotoxic and antimicrobial activities and their taxonomic positions were confirmed by the phylogenetic analysis of the 16S rRNA gene sequence.

Conclusions

Based on dissimilarities in their appearance on two media, twenty six strains were selected and primarily assigned to 8 actinomycete genera; *Micromonospora*, *Streptomyces*, *Nocardiopsis*, *Amycolatopsis*, *Microtetraspora*, *Nonomuraea*, *Actinopolyspora* and *Saccharomonospora*. These results showed that there is good actinomycete genus diversity in the Saudi coastal habitats. Out of the selected sixteen strains, seven strains showed promising activity against the tested Gram-positive, Gram-negative bacteria and yeast strains. In addition, the actinomycete extracts showed potent anticancer activities against human T cell leukemia (Jurkat) and human laryngeal carcinoma (Hep-2) cell lines. The results are encouraging as novel strains have been isolated and identified and the active secondary metabolites are promising.
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MICROBIAL IRON CYCLING IN COASTAL MARINE SEDIMENTS – COMPEITION AND INTERRELATION OF FE(II)-OXIDIZERS AND FE(III)-REDUCERS
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Background
Iron is an abundant redox-active element in many environments and iron(III)-reducing and iron(II)-oxidizing bacteria are largely responsible for microbial iron redox cycling. Three metabolic types of neutrophilic Fe(II)-oxidizing bacteria are known, i.e. microaerophilic, nitrate-reducing, and phototrophic Fe(II)-oxidizers. Based on the geochemical conditions in redox-stratified marine sediments, all three can potentially co-exist with overlapping niches.

Objectives
Consequently, there is potential for competition for their electron donor, Fe(II), that is formed by Fe(III)-reducing microorganisms which can interact with the iron-oxidizing microbial community. However, most studies of iron cycling have so far focused only on a single metabolic type of iron(II) oxidation coupled to iron(III) reduction.

Methods
In our study, we therefore determined the spatial distribution of the different metabolic types of iron-oxidizing and iron-reducing bacteria along the steep redox gradients within a coastal marine sediment from Aarhus Bay, Denmark, by MPN studies, isolations and qPCR. Furthermore, the iron(II)-oxidizing and iron(III)-reducing activity of the different metabolic types and their potential contribution to iron mineral formation within the sediment were quantified by microcosm studies.

Conclusions
From our results we conclude that microbial iron cycling occurs in these sediments and involves microaerophilic, phototrophic and nitrate-reducing Fe(II)-oxidizers as well as Fe(III)-reducers. The quantification of rates of iron oxidation and reduction under dynamic environmental conditions, including day-night cycles, input of nitrate or O₂, reveals the competition and ecological network of iron-oxidizing and -reducing bacteria and is an essential step in order to evaluate the importance of microbial iron cycling in coastal marine sediments.
DIVERSITY AND BIOSYNTHETIC POTENTIAL OF CULTURABLE YELLOW-PIGMENTED BACTERIA ASSOCIATED WITH THE SURFACE OF ANTARCTIC MACROALGAE
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Background

Algal surfaces are an untapped source of microbial diversity and could represent a promising source of novel therapeutic agents. Very little is known about the diversity and bioactive potential of Gram-positive bacteria associated with the surface of Antarctic macroalgae. Furthermore, it remains unclear the natural distribution, diversity and ecological contributions of Antarctic pigmented heterotrophic bacteria. To our best knowledge, this study is the first research carried out on the diversity and biosynthetic potential of pigmented bacteria associated with Antarctic macroalgae.

Objectives

This study aims to explore the phylogenetic diversity and potential to produce secondary metabolites of Gram-positive, yellow-pigmented bacteria isolated from the surface of intertidal and subtidal Antarctic macroalgae.

Methods

Representative species of green, red and brown algae were collected from the intertidal and subtidal zone (5 and 30 m) of King George Island, Antarctica, in January 2014. Surface-associated bacteria were investigated by cultivation-based methods and 16S rRNA gene sequencing. Yellow-pigmented isolates were screened for the presence of genes encoding polyketide synthases (PKS) by PCR amplification with degenerate primers.

Conclusions

The phylogenetic analysis showed that the yellow-pigmented epibionts belonged to the genera Arthrobacter, Citricoccus, Kocuria, Labedella, Microbacterium, Salinibacterium and Staphylococcus. PKS sequences were detected in Kocuria and Staphylococcus isolates. It highlights that Antarctic macroalgae are a unique source of microbial diversity for natural product research.

Acknowledgments
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Background
Bacteria associated with algae differ markedly from those living freely in seawater and represent great potential for the production of diverse bioactive compounds as they interact in multiple complex ways with their host.

Objectives
This work aims to study cultivable bacteria associated with the brown alga *Ascophyllum nodosum*, showing polysaccharolytic activities.

Methods
To isolate cultivable microorganisms, algal thalli of *Ascophyllum nodosum* were swabbed with sterile cotton tips and marine agar plates were inoculated. Three-hundred isolated bacteria were screened for agarase, kappa- or iota-carrageenase activities on specific marine media. Thirty-two bacteria with polysaccharolytic activities were isolated and a part of their 16S rDNA (8F-1492R) were amplified and sequenced. Twenty-seven were classified as *Flavobacteria* and five as *Gammaproteobacteria*. Putative new strains and species of *Zobellia, Maribacter, Cellulophaga, Shewanella, Glaciecola, Pseudoalteromonas* and *Colwellia* were identified by phylogenetic analysis. Genomics libraries with their DNA were constructed in *Escherichia coli* and *Bacillus subtilis* and are currently screened for diverse enzymatic activities (agarases, iota-and kappa-carrageenases, cellulases, beta-glucosidases, sulfatases and amylases).

Conclusions
In an era where high throughput sequencing is mostly used to study bacterial communities, cultivation methods are underestimated. Here, we revealed that only ten percent of the cultivable bacteria on this brown alga could degrade algal polysaccharides, which lead to asking us; who and what are the 90 other percents doing there? Furthermore, by this cultivation method we could also identify putative new bacterial strains/species, which are screened for polysaccharidas. Novel glycoside hydrolases from unknown marine bacteria represent great biotechnological potential as they should have original industrial properties.
COMBINING MORPHOLOGICAL, BIOCHEMICAL AND PHYSIOLOGICAL ASPECTS TO COMPARE TWO STRAINS OF MICROCYSTIS AERUGINOSA (CYANOBACTERIA) DIFFERING IN THE PRODUCTION OF MICROCYSTIN

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Background

Microcystis aeruginosa is a bloom forming cyanobacteria impacting freshwater environments worldwide. Blooms are formed by a mix of strains, some able to produce a hepatotoxic peptide called microcystin, others not.

Objectives

Our objective was to compare two M. aeruginosa strains, a microcystin producing and a non producing one, isolated from reservoirs in São Paulo, Brazil.

Methods

The strains were cultured (23±2 °C, 40-50 µmol photons m⁻².s⁻¹, 14-10h light–dark cycle, ASM-1 medium), cells collected at exponential growth phase and morphological and physiological aspects were investigated. Proteomic shotgun analysis was performed using iTRAQ and 2DLC-MS/MS.

Conclusions

The strains were similar in chlorophyll-a and phycocyanin content, the non toxic strain had higher carotenoid content. The toxic strain was richer in antioxidants. It formed floating colonies with numerous cells embedded in mucilage (diameter 150-800µm). In contrast, the non toxic strain exhibited small colonies (100µm) with few cells dispersed in thick mucilage, not floating. Electronic microscopy revealed thylakoids and polyphosphate granules equally present in both strains, aerotopes more abundant in the toxic one, cyanophycin granules and carboxysomes more evident in the non toxic strain. Sixty nine differentially expressed proteins were found. Both strains expressed proteins related to photosynthesis, energy metabolism and translation. Gas vesicle and stress response proteins were more abundant in the toxic strain while transport and protein folding functions were more expressed in the non toxic one. These results are discussed in relation to the other physiological aspects investigated. These differences probably represent distinct ecological strategies adopted by each strain.
BIODEGRADATION OF MICROCYSTIN-LR BY BACTERIA FROM THE SEDIMENT OF JACAREPAGUA LAGOON (RIO DE JANEIRO, BRAZIL)

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Background
The release of effluents into aquatic systems leads to an increase in nutrients, a process known as eutrophication. This process promotes large proliferations of cyanobacteria (blooms) that can be harmful to the environment and to humans due to the production of cyanotoxins. The cyclic peptide microcystin (MC) is the prevalent and most studied cyanotoxin in blooms, with more than 80 variants. It has been reported consistently since 1996 in Jacarepagua Lagoon (RJ). MC is rarely found dissolved in the water column, but adsorbs to particles in suspension which settle at the bottom sediment or undergo biodegradation by microorganisms.

Objectives
The objective of this study was to evaluate the potential for biodegradation of MC-LR by bacteria found in the lagoon sediment, testing both recently isolated strains and microbial assemblages.

Methods
MC-LR was incubated with isolated strains (15) or microbial assemblages for 1 and 7 days, and quantified by LCMS.

Conclusions
After 7 days only one strain showed degradation of MC. The microbial assemblage extracted directly from the sediment was very efficient in degradation, decreasing (by ~50%) the added MC. Part of the observed decrease in MC amount was recognized as adsorption to sediment particles. The 15 isolated strains were identified as Ralstonia or Bacillus by 16SrDNA sequencing. This indicated that the culture medium led to selection of microorganisms, probably eliminating those able to degrade MC. On the other hand, MC degradation by microbial assemblages reflects the metabolic diversity of this sample and points to a possible cooperative activity to complete the process of biodegradation.
MOLECULAR CHARACTERIZATION OF PSEUDOMONAS AERUGINOSA POPULATION IN AQUATIC ENVIRONMENTS OF GREECE

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Background

Recent isolation of Ps. aeruginosa strains from Greek aquatic environments and subsequent PFGE analysis demonstrated that these strains have a unique genotype. Environmental isolates may also have a unique phylogenetic position. Literature has revealed that the behavior of Ps. aeruginosa during typing is affected by its habitat and by stress factors such as antibiotics, biofilm formation e.t.c.

Objectives

Considering the advantages of MLST, as a typing method that focuses strictly on 7 conserved housekeeping genes, it was chosen for typing and discrimination of Ps. aeruginosa strains isolated from water samples from all over Greece. Additional information regarding the resistant phenotypes circulating in these environments is cited.

Methods

The activity of 14 antibiotics was tested against all isolates by the disk diffusion method, where additional phenotypical tests were performed according to published protocols. MLST was performed as described by Curran et al, 2004 with some alterations concerning the annealing temperatures of the seven housekeeping genes. The control strains Pseudomonas aeruginosa ATCC 27853, clinical control from HPA/NEQAS (External Quality Control) and PAO1 were used as standards of reference during the MLST and for reproducibility experiments.

Conclusions

The majority of the isolates exhibited the intrinsic antimicrobial resistance, while a significant proportion presented additional resistant mechanisms. The amended protocol of MLST seemed to produce allelic profiles for all the isolates so far tested. Thus this typing method is going to be used in a larger sampling program to obtain more data and to conclude in a reliable phylogenetic analysis for the first time in aquatic environments of Greece.
PHOTOCHEMICAL AND MICROBIAL ALTERATIONS OF DOM SPECTROSCOPIC PROPERTIES IN THE ESTUARINE SYSTEM RIA DE AVEIRO

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Background
Photochemical transformations of chromophoric dissolved organic matter (CDOM) influence microbial communities in aquatic systems.

Objectives
This work aimed to evaluate this influence in the estuary Ria de Aveiro. In order to achieve that, two sites representative of the marine (MZ) and brackish water (BZ) zones of the estuary were regularly surveyed.

Methods
Optical parameters of CDOM indicative of aromaticity and molecular weight were used to establish CDOM sources, and microbial abundance and activity was characterized. Additionally, microcosm experiments were performed in order to simulate photochemical reactions of CDOM and to evaluate microbial responses to changes in CDOM composition.

Conclusions
The CDOM of the two zones showed different spectral characteristics, with significantly higher values of the specific ultra-violet absorbance at 254 nm and of the absorption coefficient at 350 nm and lower SR ratio at BZ than at the MZ, reflecting the different amounts and prevailing sources of organic matter, as well as distinct riverine and oceanic influences. At the MZ, the abundance of bacteria and the aminopeptidase activity correlated with absorbance, suggesting a microbial contribution to the HMW CDOM pool. The irradiation of DOM resulted in loss of color and increase of its bioavailability. However, the extent of photoinduced transformations and microbial responses was dependent on the initial characteristics of CDOM. In Ria de Aveiro both photochemical and microbial processes yielded optical changes in CDOM and the overall results of these combined processes determine the fate of CDOM in the estuary and have an influence on local productivity and in adjacent coastal areas.
IN SITU DETECTION AND LOCALIZATION OF THE PROBIOTIC PEDIOCOCCUS SP. AB1 IN THE GUT OF ABALONE HALIOTIS GIGANTEA

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Background
We previously reported that host-derived \textit{Pediococcus} sp. Ab1 can colonize the gut of abalone \textit{Haliotis gigantea} and that the addition of Ab1 to commercial feed can alter gut microflora and increase alginate lyase and VSCFAs in the gut. From those viewpoints, addition of Ab1 can provide valuable effects in abalone aquaculture as well as in other aquaculture animals.

Objectives
In this study, to revealing the dynamics of probiotic \textit{Pediococcus} Ab1, we tried to establish an optimum FISH protocol, and tried to \textit{in situ} detection of this bacterium.

Methods
TAMRA-labeled Rpt probe was constructed and samples were fixed by 4\% paraformaldehyde and hybridized at 40 °C for 3 h. In the case of described condition, we could discriminate clearly Ab1 from other control bacterial strains (\textit{Enterococcus}, \textit{Lactobacillus}). Fluorescence signals of Rpt probe-positive bacteria were easily distinguished from any detectable background autofluorescence.

Conclusions
Rpt probe-positive bacteria were detected in both probiotics supplemented animal and non-probiotics supplemented animal. Rpt probe-positive bacteria formed microbial colonies only in the probiotics supplemented animal. The mean numbers of Rpt probe-positive bacteria in the gut-attached samples of probiotic supplemented animal was $1.94 \times 10^7$ cells/g-gut, and those of non-probiotic supplemented animal were $6.85 \times 10^5$ cells/g-gut, respectively. From the thin section FISH analysis, Rpt positive cells were observed from gut samples of probiotics supplemented animal. Interestingly, Rpt positive cells were also observed from gills samples of them. From these results indicated that probiotic \textit{Pediococcus} sp. ab1 is colonized and make a micro-colony in their host gut.
Background
The proposed study is to reduce biofilm formation using nano-hydrophobic coating on cooling tower fill materials. Consequently, enhancing cooling performance, lengthen the material life, reducing clogging and biofilm associated pathogen bacteria.

Objectives
Primary aim is to reduce the slimy biofilm formation on fill material, which will lead to better cooling of water, longer material life and less clogging of the fills. It is widely known that cooling towers’ maintenance is heavily neglected. This end ups in shorter material life, expensive maintenance charges, inefficient cooling, excessive energy consumption and public health risk due to Legionella bacteria dissemination. While novel anti-biofilm approaches are still in nascent phases of development, our efforts are devoted for sustainable management of cooling towers and public health safety, where cooling towers are often associated with deadly Legionnaires’ disease.

Methods
Test surfaces were placed into biofilm reactor along with the untreated control coupons up to 6-months period for biofilm maturation. Natural bacterial communities were monitored to analyze the impact to mimic the real-life conditions. Surfaces were monthly analyzed in situ for their microbial load using epifluorescence microscopy.

Conclusions
Wettability is known to play a key role in biofilm formation on surfaces, because characteristics of surface properties affect the bacterial adhesion. Results showed that surface-conditioning with nano-silica significantly reduce (up to 90%) biofilm formation. Easy coating process is a facile and low cost method to prepare hydrophobic surface without any kinds of expensive compounds or methods.
THE REGULATION OF DIMETHYLSULFONIOPROPIONATE METABOLISM IN RUEGERIA POMEROYI DSS-3

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Background

Dimethylsulfoniopropionate (DMSP) plays a key role in the global sulfur cycle and marine microbial sulfur and carbon metabolism. The marine roseobacter Ruegeria pomeroyi DSS-3 is capable of metabolizing DMSP by either the cleavage or demethylation pathways. The cleavage pathway forms the volatile gas dimethylsulfide (DMS), the largest natural source of sulfur to the atmosphere. The demethylation pathway produces methanethiol (MeSH), which is readily assimilated or oxidized.

Objectives

A model for the regulation of DMSP metabolism has been developed for R. pomeroyi where the availability and turnover of methyl-tetrahydrofolate (THF) is a major regulatory point.

Methods

A combination of RNA-Seq, enzyme assays and growth experiments were performed to test predictions of the model.

Conclusions

According to RNA-Seq, the genes involved in THF biosynthesis and methyl-THF metabolism were significantly up-regulated during growth on DMSP as compared to acetate. Similarly, the specific activities of the methylene-THF reductase and formate dehydrogenase were two-fold higher in cell-free extracts of DMSP-grown cells. During growth on DMSP, treatment of cultures with low concentrations of the dihydrofolate reductase inhibitor trimethoprim increased production of DMS without inhibiting growth. This result was consistent with a model where high levels of methyl-THF metabolism allow DmdA to function at its maximal rate, leading to sufficient MMPA accumulation to overcome the DMSP-based inhibition of RPO_DmdB2, one of the two isozymes for the second step in the pathway. Based on the current
knowledge of DMSP metabolism, the regulation of the two pathways in *R. pomeroyi*
appears to be complex and multifaceted.
EFFECT OF BOTANICAL EXTRACTS & ESSENTIAL OILS IN FEED ON LAYING HENS PERFORMANCE & SALMONELLA, E.COLI & TOTAL BACTERIAL COUNT IN FECES

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Background

Ph.d Research idea was to explore plant extracts effects

Objectives
The aim of the present study was to investigate the effect of Botanical Extracts and Essential oils on performance of laying hens

Methods
One hundred and twelve, 40 weeks old, white Novagin laying hens were randomly assigned to seven groups equally (n = 16). Each treatment (group) was replicated four times of four birds per replicate. Experimental diets were prepared by adding A: positive control, antibiotic lincomysin 4.4%, 120mg/kg of feed, antioxidant seldox (BHA, BHT, ethoxiquine and citric acid) 120mg/kg of feed, acetic acid 99.5% pure 0.15ml/kg of feed), B: negative control (N.C) , no antibiotic, antioxidant and acetic acid, C: N.C + black tea poly phenolic extract 1ml/kg of feed, D: N.C + black cumin seed poly phenolic extract 1ml/kg of feed, E: N.C + fenugreek seed poly phenolic extract 1ml/kg of feed, F: N.C + black cumin seed oil 1ml/kg of feed, and G: N.C + fenugreek seed oil 1ml/kg of feed.

Conclusions
Weekly egg production percentage of positive control is significantly higher than negative control (P<0.05) but plant extracts and oils has no significant difference between each other (P>0.05). On the other hand Total bacterial count significantly reduced with increasing age in all treatments except negative control but there is no significant difference between treatments when compared for presence of salmonella and E. coli (P>0.05) in feces.
EFFECT OF ROOT EXUDATES AND DIFFERENT CARBON SOURCES ON BACTERIAL GROWTH OF BACILLUS AMYLOLIQUEFACIENS FZB42 AND BACILLUS SUBTILIS BBG131 AND THEIR LIPOPEPTIDE PRODUCTION

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Background

Rhizosphere is the region that is closed to the roots of plant. Plant root exudates consist of a complex mixture of organic acids, sugars and amino acids, which are used as substrates for the growth of bacteria colonizing this rhizosphere.

Objectives

we highlighted on the effect of root exudates and some of carbon sources which found in the root exudates, on the bacterial growth and lipopeptides production

Methods

Ten of carbon sources (glucose, fructose, sucrose, maltose, xylose, glutamic acid, citric acid, succinic acid, oxalic acid, fumaric acid), and root exudates were used to study bacterial growth and lipopeptides production

Conclusions

Different growth kinetics were observed depending of the substrates. For both strains, growth on oxalic acid is very slow and glucose is the best substrate. As a conclusion, the different behavior of the two strains to consume the root exudates and the different carbon sources, which reflex to the growth and lipopeptides production, may be causing the different ability to colonize the rhizosphere.
FEMS-3013
Plant/microbes interactions

ABUNDANCE AND DIVERSITY OF FUNGAL ENDOPHYTES FROM THE
GOTJAWAL FORESTS ON JEJU ISLAND IN KOREA
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Background

Endophytes are microorganisms that inhabit plant tissues by colonizing internal plant tissues without causing apparent harm to host. Particularly, some fungal endophytes have beneficial roles on plant growth and plant tolerances to environmental stresses such as abiotic and biotic stresses. Accordingly, identifying their abundance and diversity from unique forest ecosystem is an interested subject to discovery the beneficial fungal endophytes.

Objectives

The objective of this study is to construct endophytic fungal resources for discovering their beneficial functions, by collecting diverse fungal endophytes from the Gotjawal forest (Jeju, Korea), a unique forest ecosystem, and identifying their abundance and diversity.

Methods

Various plant tissues were collected from Gotjawal area on Jeju Island in Korea. They were sterilized and then, subjected to the endophytic fungal isolation. Subsequently, they were further pure-cultured and then subjected to the long-term storage in liquid nitrogen and the DNA isolation for the molecular identification, mediated by various DNA barcode markers.

Conclusions

Forty seven plant samples were collected from five Gotjawal forests on Jeju Island and composed of 34 species. Through the molecular identification, 196 fungal isolates were obtained from the plant tissues and classified as 3 classes, 11 orders, 17 families and 29 genera. Especially, fifty fungal MOTUs (molecular operational taxonomic units) were identified and classified further into four genera such as Colletitrichum, Xylaria, Pestalothiopsis, Fusarium. The fungal collection, obtained from Gotjawal forests, was deposited with classification information in National
Institute of Biological Resources as stocks and can become a useful resource to
discovery the beneficial functions of fungal endophytes.
DOES INTERACTION OF THE INVASIVE CORDGRASS SPARTINA DENSIFLORA WITH PLANT GROWTH-PROMOTING RHIZOBACTERIA EXPLAIN ITS TOLERANCE TO PHYSICOCHEMICAL PROPERTIES OF MARSHES SOILS?

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Background
Microbiota in the niches of the rhizosphere zones can affect plant growth and responses to environmental stress conditions via mutualistic interactions with host plants.

Objectives
A glasshouse experiment was designed to investigate the role of a bacterial consortium (Pseudomonas composti SDT3, Aeromonas aquariorum SDT 13 and Bacillus sp. SDT14) isolated from the rhizosphere of Spartina densiflora in its growth and physiological tolerance to the physicochemical properties of marshes soils.

Methods
Plants of S. densiflora were randomly assigned to two soil types with different physicochemical characteristics with and without inoculum for 50 days. Plant responses were examined using growth analysis, combined with measurements of gas exchange, efficiency of PSII biochemistry, total content of photosynthetic pigments and leaf water content. In addition the accumulation of nutrients in roots and leaves were determined.

Conclusions
The inoculation improved growth of S. densiflora through a beneficial effect on its photochemical apparatus due to its impact on chlorophyll concentration. This growth enhancement happened under both soil conditions and was mainly reflected in a greater length and diameter of roots. Modifications of pigment concentrations were linked to an increase in leaf magnesium content. Also, inoculation favoured LWC through the decline in gs and increment in root-to-shoot ratio. Moreover, this consortium was able to stimulate ions uptake in roots and leaves. Plant growth-promoting rhizobacteria of S. densiflora appears to play a significant role in its growth response and tolerance to the physicochemical properties of soils, through diverse protective effects on the photosynthetic apparatus, WUE and mineral nutrient balance.
Background

The plants growing under salinity stress conditions increase ethylene production, which induces root elongation inhibition. The bacterial enzyme 1-aminocyclopropane-1-carboxylate deaminase (ACCD) cleave ethylene precursor, the 1-aminocyclopropane-1-carboxylate, thus mitigating ethylene effects. Some strains also produce phytohormone indol acetic acid (IAA), which increases cell elongation.

Objectives

To determine the effects of inoculation with consortia formulated with IAA- and ACCD-producing endophytic (EB) and rhizospheric bacteria (RB) on salinity stress mitigation of wheat plants.

To compare the effects of EB and RB inoculation, as well as, different levels of bacterial IAA and ACCD production on salinity stress mitigation of wheat plants.

Methods

Twelve bacterial strains were isolated from avocado plants, and four consortia were formulated: 1) EB with higher ACCD and IAA production; 2) EB with lower ACCD and IAA production and 3) RB with higher ACCD and IAA production; 4) RB with lower ACCD and IAA production. Wheat seeds were inoculated with the bacterial consortia, and then seeds were grown under salt stress conditions. Length, dry weight and superoxide dismutase (SOD) activity of wheat shoot and roots were determined.

Conclusions

At lower levels of bacterial IAA and ACCD production, the EB were more efficient than RB consortia mitigating salt stress effects. Between RB consortia, only those with higher production are able to promote the growth of stressed plants. Both EB and RB consortia with higher production are able to increase SOD activity. Finally,
bacterial strains isolated from avocado plants mitigate plant stress and therefore have the potential to be used as commercial inoculum of avocado plants.
Background
Huanglongbing (HLB) is a destructive disease of citrus caused by phloem-limited bacteria, *Candidatus* Liberibacter spp. Although no known HLB-resistant citrus species or varieties have been identified, several studies report the group of *Poncirus trifoliata* and some of its hybrids, as more tolerant to the disease. One of the early manifestations of HLB is excessive starch accumulation in leaf chloroplasts.

Objectives
We hypothesize that callose deposition in the phloem may intervene photoassimilate exportation, causing the starch to over-accumulate. Thus, we examined citrus leaf phloem by microscopy to characterize plant responses to infection.

Methods
We have studied 3 genotypes (*C. sinensis*, *C. sunki* and *P. trifoliata*) and 7 hybrids obtained from crosses between *C. sunki* and *P. trifoliata*. All genotypes (3 replicates) were graft inoculated with budwood from HLB-infected plants and infection was confirmed by qPCR (TaqMan®) 8 months after plants inoculation.

Conclusions
We observed callose deposition in leaf petioles and accumulation of starch in leaves in all genotypes and hybrids, when compared with mock-inoculated controls. However, accumulation of starch and callose deposition were more abundant in *C. sinensis*, *C. sunki* and three of the hybrids. *P. trifoliata* and four of the hybrids showed the lowest starch accumulation and lower deposition of callose, when compared with others genotypes. Our results indicate that Liberibacter infection is accompanied by callose deposition in different level among the genotypes and the deposition of callose is higher in susceptible genotypes suggesting that the phloem plugging by callose inhibits phloem transport, contributing to the development of HLB symptoms.
Background

In many tropical areas rainforests are cleared in order to transform them into rubber and oil palm plantations, although massive transformations are a major threat for biodiversity. Most of the tree species in rainforests are associated with fungi that form mutualistic interactions with roots and function as key link for nutrient exchange between the above- and belowground compartment. Therefore, in addition to the loss in tree species, transformation of primary forests into intensely managed plantations may lead as well to changes in the fungal community structure of soils or even to a loss of species.

Objectives

The goal of this study is to identify potential differences in fungal community structures in soils of tropical lowland rainforests and plantations sites. Furthermore, soil-borne plant stress will be evaluated by analyzing arbuscular mycorrhizal (AM) spore density and morphology.

Methods

The study is implemented in one of the largest regions of tropical lowland rainforest in Southeast Asia, the Jambi Province (Sumatra, Indonesia). Two landscapes, National Park Bukit Duabelas and Harapan Rainforest, were investigated. Soils of different transformation systems (lowland rainforest, jungle rubber (extensive), intensive rubber and oil palm plantations) were investigated using the 454 Pyrosequencing approach. Fungal soil communities were characterized using internal transcribed spacer regions ITS1 and ITS2.

Conclusions

A strong increase of AM spore density in plantations compared to rainforest sites was detected supporting that monoculture tree crops may suffer from soil-born stress.
Currently we are investigating if transformation of tree species rich tropical rainforests to species poor plantations correlates with a loss of fungal diversity in soil.
Background

MicroRNAs (miRNAs) are endogenous small-RNAs transcribed from non-coding DNA, matching a target messenger RNA to repress translation or induce cleavage. They act in almost every biological plant activity, e.g. development, abiotic stress tolerance, signal transduction, and in defense from pathogens or parasites.

Objectives

To elucidate miRNAs role in plant-endophyte interactions, we constructed libraries from roots of Solanum lycopersicum endophytically colonized (Pmi) or not (Pm) by the hyphomycete Pochonia chlamydosporia. This fungus shows endophytic behaviour with growth promotion or nematode biocontrol effects. No data are available on tomato miRNAs role and targets in the endophytic interaction.

Methods

Illumina™ NGS of small-RNAs yielded $9 \times 10^6$ (Pmi) and $12 \times 10^6$ (Pm) reads per library. CLC Genomics Workbench was used for trimming, counting, annotation and data analysis.

Conclusions

Non-redundant, unique small-RNAs (869178 in Pmi, 958026 in Pm), were produced. MiRNAs expression was affected by endophytism. Analyses of tomato miRNAs (miRBase, rel.21), revealed miR156 and miR168 (conserved across higher plants), as most abundant in roots. Four further miRNAs (miR169a, miR169c, miR9473 and miR9476), out of 75 known in tomato, were expressed only in Pmi, with seven further (miR169d, miR1917, miR169e, miR394, miR167a, miR5300 and miR9475) over-expressed and 27 down-regulated (fold change range: 1.2–4.8). 37 remaining miRNAs were equally expressed in both conditions. A Pmi comparative analysis showed that 1732 out of 5055 Pmi down-regulated genes were miRNA targets,
involved in structural protein, metabolism, transcription factor, growth and
development, stress-related, signaling pathways, storage and other processes.
CHARACTERIZATION OF THREE ENSIFIER MELILOTI BACTERIOPHAGES FROM SOILS SUBJECTED TO ABIOTIC STRESS.

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Background

The presence of bacteriophages in soils was demonstrated as early as 1935. The presence of rhizobiophages in soils suggests that through selection or elimination of certain types of rhizobia, the rhizobiophages influence the evolution of bacterial populations.

Objectives

In this work, we isolated 3 \textit{Ensifer meliloti} bacteriophages (EMph1, EMph2 and EMph3) from the rhizosphere soil of \textit{Medicago marina} collected in two areas of the South of Spain. This study is part of a project in which the diversity of rhizobia strains that nodulate \textit{M. marina} was studied in the Odiel river marshes (Huelva) and in the dunes of San Fernando beach (Cádiz), soils subjected to pH and salt stress.

Methods

Bacteriophages were isolated from rhizosphere soil samples by the enrichment technique of Barnet (1972). Phages were purified by three successive isolation of single plaques. The basic DNA manipulations and molecular techniques used have been described elsewhere. To study their host range, phages were assayed on 22 different bacteria, 13 of them were strains isolated from \textit{M. marina} nodules, along with additional bacteria, including strains of \textit{E. meliloti}, \textit{E. medicae}, \textit{E. fredii}, \textit{R. tropici}, \textit{R. etli}, \textit{R. leguminosarum} bv. \textit{viciae}, \textit{R.I.} bv. \textit{trifolii} and \textit{Agrobacterium tumefaciens}.

Conclusions

The 3 phages were resistant to restriction with many enzymes tested. EMph1 and EMph3 displayed a wide host range within the whole bacteria tested. On the contrary, phage EMph2 exhibited a narrow host range which was capable of killing only 5 out of 22 bacteria tested.

Background
Many phytopathogenic bacteria colonize heterogeneous interior of their host consisting of various tissues that form diverse microniches for microorganisms. However, the dynamics of bacterial population structure in such heterogeneous and changing system as plant organism is poorly understood.

Objectives
In our investigations we described the dissociation of Pectobacterium atrosepticum SCR1043 (Pba) populations and the formation of various subpopulations in tobacco plants.

Methods
Microbiological methods; RT PCR; light, transmission electron microscopy; immunocytochemistry; chromatography.

The proliferative potential and bacteria distribution within different plant tissues were analyzed from inoculation time till long-term preservation of microorganisms in dead plant debris. We have revealed new structures totally occluding xylem vessels of the host plant and termed them "bacterial emboli". These structures are composed of tightly packed bacterial cells having a predominant spatial orientation and a peculiar way of formation. Using immunocytochemistry and biochemical methods it was shown that high molecular weight products of pectic compounds degradation and Pba exopolysaccharides play an important role in the bacterial emboli formation. Such structures were formed in the plant vessels both in the pronounced and inapparent disease symptoms after Pba infection. Bacterial emboli are likely to form conditions for bacterial downward migration through the xylem vessels resulting in rhizosphere colonization. After plant death bacteria were transformed to viable but non-culturable state.
Conclusions
Our investigation demonstrates that pectobacteria realize different strategies in colonization of various plant compartments and are able to pass through the life cycle symptomatically and asymptomatically.
This study was supported by RFBR (14-04-01750_A), RSF (15-14-10022), MK-7359.2015.4.
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Conclusions
Our investigation demonstrates that *pectobacteria* realize different strategies in colonization of various plant compartments and are able to pass through the life cycle
symptomatically and asymptomatically.
COMPOSING PLANT-MICROBIAL ASSOCIATION RESISTANT TO SOIL SALINIZATION

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Background
Potash mining by Belaruskali concern poses a grave environmental threat in terms of soil pollution with sodium, potassium and calcium chlorides. Salt-induced stress affects growth and productivity of cultivars. A promising method for salinized soil remediation is field introduction of legume crops, like alfalfa (*Medicago sativa*) upgrading soil fertility due to atmospheric nitrogen fixation of selected rhizobial strains and PGRP bacteria. This work was aimed at isolation of beneficial microorganisms enhancing alfalfa resistance to elevated salt concentrations in soil.

Objectives
Halophilic phosphate-solubilizing and nodulating bacteria.

Methods
Recovery and selection of phosphate-solubilizing bacteria was conducted on TY medium containing chlorides of sodium, potassium, calcium in concentration range 3-15 %. Taxonomic affiliation of isolates was based on physiological-biochemical characterization using VITEK2 identification system.

Conclusions
Four halophilic bacterial cultures able to grow on TY medium comprising 15% NaCl were isolated from specimens of solid saliferous wastes sampled at Starobin potash deposit. The examined isolates were capable to solubilize phosphates and exert favorable influence on seed germination, growth and development of alfalfa. Treatment of alfalfa seeds by strain FM-3 under saline conditions increased germination rate by 50% as compared to control. Nitrogen-fixing strain *Ensifer meliloti* Mst 3-2 isolated from alfalfa nodules displayed good growth on TY medium containing 2% NaCl. Investigation allowed to refer three phosphate-solubilizing isolates to genus *Bacillus*. The isolated strains of nitrogen-fixing and phosphate-solubilizing bacteria are especially attractive as components of plant-microbial association resistant to soil salinization.
CULTIVATING BIOFUEL CROPS ON MARGINAL LAND: PLANT-ASSOCIATED BACTERIA OF LIGNIN-REDUCED (GMO) ARABIDOPSIS THALIANA AND THEIR CAPACITY TO FACILITATE GROWTH.

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Background

Producing second generation biofuels out of lignocellulosic biomass can contribute to reduce the food-fuel competition on fertile agricultural soils. However, the recalcitrance of lignin polymers is a limitation during processing the biomass to pure cellulose fibers. The efficiency of this process can be increased by using genetically modified crops with lower lignin-contents. Though, the genetic modification can have effects on the phenolic compounds inside the xylem which can influence the endophytic bacterial population.

To even further reduce the food-fuel competition, biomass can be cultivated on marginal land (containing e.g. cadmium (Cd)). An additional advantage can be the reduction of contaminants in these soils by making use of phytoremediation, a process that can be optimised by inoculating bacteria with plant growth promoting capacity (e.g. siderophores, IAA, organic acid and ACC deaminase production).

Objectives

We want to identify the effects of the genetic modification and/or Cd-exposure on the endophytic bacterial population and determine the endophytic growth promoting capacity.

Methods

The endophytes of different tissues of Arabidopsis thaliana wildtype and reduced lignin mutants, grown in a hydroponic system with and without 3µM CdSO₄, were isolated and characterised using 16S rDNA amplification/sequencing. Their in vitro plant growth promoting capacity was tested using colorimetric tests.

Conclusions
Differences in bacterial populations due to the genetic modification and Cd-exposure were observed in stems and seeds but they seemed to be less pronounced in leaves. The root endophytic populations were more or less identical. However, among all tissues differences in growth promoting capacity were found.
Background

Plants lack an immune system in the sense that it exists in animals. However, they are able to recognize potential pathogens. Lipoxygenase signaling pathway plays an important role in regulation of plant growth and development, cell signaling and defense. Lipoxygenase initiates synthesis of group of bioactive compounds collectively called oxylipins (fatty acid hydroperoxides, hydroxy-, oxo-, or keto-fatty acids, divinyl ethers, volatile aldehydes, or jasmonates). Usually oxylipins are not preliminary formed but synthesized de novo in response to mechanical damage, infection and other stress factors. The main role in the biosynthesis of plant oxylipins belongs to the CYP74 enzymes that are non-classical cytochromes P450: allene oxide synthases (AOS), hydroperoxide lyases (HPL), divinyl ether synthases (DES). A number of AOSs and HPLs have been cloned and characterized from various plant species. DESs are less studied. However, divinyl ethers were found in a large number of organisms.

Objectives

We studied interaction between Linum usitatissimum, Ranunculus acris and Selaginella moellendorffii plants with pathogen bacteria Pectobacterium atrosepticum SCRI1043.

Methods

Methods of microbiology, molecular biology, biochemistry, mass spectrometry, NMR and UV spectroscopy.

Conclusions

We cloned and characterized DESs from L.usitatissimum (LuDES), R.acris (RaDES) and S.moellendorffii (SmDES1 and SmDES2). LuDES, RaDES and SmDES2 convert
substrates into divinyl ethers (omega5Z)-etherolenic and (omega5Z)-etheroleic acids, respectively. The main reaction product of SmDES1 is (11Z)-etherolenic acid. (Omega5Z)-etherolenic acid possesses bactericide properties, whereas other divinyl ethers – bacteriostatic properties. Expression of DESs genes after infection is 50 times higher than in control. Thus, DES branch of lipoxygenase cascade involved in plant defense against pathogens.
Background
Cells of a bacterial pathogen *Ralstonia solanacearum* strain OE1-1 (OE1-1) form biofilms on surfaces of tomato cells adjacent to intercellular spaces after invading tomato plants, and cause bacterial wilt. OE1-1 cells in intercellular fluids but not xylem fluids from tomato plants form mushroom-type biofilms. The production of major extracellular polysaccharide, EPS I, positively regulated by a multi-transcription regulator PhcA is involved in the biofilm formation by OE1-1 cells.

Objectives
We analyzed involvement of biofilm formation by OE1-1 cells in its virulence.

Methods
Among PhcA-positively regulated genes, we created *ralA* encoding furanone synthetase-deleted mutant. Biofilm formation of OE1-1 and the mutant incubated in intercellular fluids from tomato plants were observed under the scanning electron microscope. EPS I production and expression of EPS I production-related genes, *xpsR* and *epsB*, were analyzed. Furthermore, virulence of the strains on tomato plants was analyzed.

Conclusions
The mushroom-type biofilm formation of the *ralA*-deleted mutant significantly reduced, compared to those of OE1-1. Furthermore, expression of *xpsR* and *epsB*, and EPS I production of the mutant significantly reduced. Inoculation with the mutant into tomato plants using root dipping resulted in a significant reduction of bacterial growth in intercellular spaces and loss of its virulence. On the contrary, tomato plants inoculated with the mutant through petiole wilted. The transformation with native *ralA* resulted in recovery of not only EPS I production and biofilm formation but also virulence. These results suggest that biofilm formation of which regulation is positively influenced by *ralA* may be involved in OE1-1 virulence.
EFFECTS OF RHIZOBACTERIAL ACC DEAMINASE ACTIVITY ON THE GROWTH OF SOYBEAN UNDER SALINE CONDITION

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Background
1-aminocyclopropane-1-carboxylate (ACC) deaminase is an enzyme that degrades the precursor of plant hormone ethylene. Plant growth-promoting bacteria that ACC-deaminase facilitate plant growth and development under stress conditions by decreasing plant ethylene levels.

Objectives
This study was conducted to analyse the changes in root architecture and root hair length of soybean induced by three rhizobacteria Pseudomonas putida TSAU1, Pseudomonas aureantiaca TSAU22, Pseudomonas sp.. NUU8 strains containing ACC-deaminase.

Methods
ACC-deaminase activity was determined by monitoring the amount of ammonia generated due to hydrolysis of ACC by the rhizobacterial isolates containing ACC-deaminase. Four salinity levels (5.0, 7.5, 10.0 and 12.5 dSm⁻¹) were maintained in the gnotobiotic system using NaCl salt.

Conclusions
Results showed that the salinity adversely affected on the root length of soybean decreasing by 79% the heighest NaCl concentration (12.0 dSm⁻¹). The bacterial strains could utilize ACC an N source indicating the presence of ACC-deaminase that play a role in reducing ethylene levels in plants. The inoculation of seeds with the P.putida TSAU1, P. aureantiaca TSAU22, Pseudomonas sp. NUU8, increased the root and shoot length of soybean at 5.0, 7.5 and 10.0 dSm⁻¹ up to 58% compared to uninoculated plants exposed to salt stress. In summary, based on the results of our work we recommend utilization of rhizobacteria having capacity to produce ACC-deaminase to alleviate salt stress of soybean grown in the saline soils.
CO-INOCULATION OF BRADYRHIZOBIUM WITH PSEUDOMONAS STRAINS TO IMPROVE GROWTH AND YIELD OF SOYBEAN UNDER SALINE SOIL CONDITION

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Background
Salinity stress is one of the most serious factors limiting the productivity of agriculture, especially the negative effects on yield and nitrogen fixation of Leguminous plants. However, there are several reports on the positive impacts of co-inoculation with Pseudomonas and Rhizobium spp., but less attention has been paid how co-inoculation of root-colonising and PGPR bacteria will affect Rhizobium-Legume interactions under salinity stress.

Objectives
The objectives of this research were to observe if the co-inoculation of soybean with Bradyrhizobium japonicum NU1 and Pseudomonas sp.NUU8 can enhance salt tolerance, nodulation, plant growth, pod yield and grain yield of soybean under saline soil conditions.

Methods
The effects of bacterial inoculation on growth, nodulation, pod yield and grain yield of soybean plant grown under saline soil conditions were studied in field experiments. Plants were grown in saline soil of Sherobod district, Surkhandarya province, Uzbekistan.

Conclusions
The results showed that co-inoculation of with B.Japonicum NU1 and Pseudomonas sp. NUU8 gave more benifits in nodulation, plant growth, pod yield and grain yield of soybean compared to plants inoculated with B.japonicum NU1 alone. Under field condition, co-inoculation of B. japonicum NU1 and Pseudomonas sp. NUU8 strains significantly improved shoot dry weight by 38% and root dry weight by 58% of soybean compared with the uninoculated control. The synergistic use of B.japonicum NU1 and Pseudomonas sp. NUU8 also improved the nodulation, plant growth, pod yield and grain yield under salt-stress. The results suggested that these strains could be used to formulate a biofertilizer for sustainable production of soybean under salt stressed field conditions.
Background
Plant growth promoting rhizobacteria, Azospirillum brasilense SM releases phytohormone-Indole-3-acetic acid (IAA) and other plant growth regulators into the rhizosphere which enhances plant development. A crosstalk between IAA and gasotransmitter, Nitric oxide (NO) is speculated which may further benefit the plants.

Objectives
To identify NO production by strain SM and unravel potential rhizospheric crosstalk between IAA and NO.

Methods
The study involved creating mutant strains which overexpress essential NO metabolism genes, NO Fluorescence assay, IAA quantification by HPLC, determining plant-bacterial association by SEM, and Real time PCR for gene expression.

Conclusions
Production of NO and presence of NO metabolism genes i.e. nitrous oxide reductase (nosZ), nitrous oxide reductase regulator (nosR) and nitric oxide reductase (norB) were identified by fluorescence assay and PCR sequencing. Improved PGP response of their overexpressing mutant strains was mediated by increased NO and IAA levels. Surface colonization of strain SM on sorghum roots was established by electron microscopy and improved plant development was observed with the mutants. Quantitative IAA estimation suggested that nosR and norBC influences regulation of IAA biosynthesis in A. brasilense SM. The NO quencher, inhibitor and donor reduced or blocked IAA biosynthesis in wild type and mutants, emphasizing a common regulatory role of these molecules in IAA biosynthesis. Expression studies by qPCR showed positive influence of Tryptophan and Arginine on NO genes. IAA biosynthesis gene, indole-3-pyruvate decarboxylase (ipdC) was influenced by Tryptophan but not significantly by Arginine. These results impress some shared signalling mechanism or potential crosstalk involving IAA and NO in strain SM.
IMPORTANCE OF METHANE FOR SPHAGNUM-ASSOCIATED MICROBIAL NITROGEN FIXATION

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Background

Pristine Sphagnum-dominated bogs are N-limited and depend on N\textsubscript{2} fixation by diazotrophs or atmospheric N-deposition for their N supply. Sphagnum mosses harbor a very diverse microbiome including numerous methanotrophs that contribute to the carbon supply of the mosses. It has been hypothesized that methane might also stimulate Sphagnum-associated N\textsubscript{2} fixation during early stages of peat development, thereby increasing Sphagnum growth and N content, although other studies did not see this effect.

Objectives

The objectives are to elucidate whether methane indeed does stimulate N\textsubscript{2} fixation in Sphagnum mosses from ombro-, oligo- and mesotrophic field sites and to test whether this is affected by oxygen level.

Methods

N\textsubscript{2} fixation and CH\textsubscript{4} oxidation activity were studied by incubating mosses with $^{15}$N\textsubscript{2}, $^{15}$N\textsubscript{2} + $^{13}$CH\textsubscript{4} or no additions. Furthermore, different oxygen regimes (aerobic, microaerobic (either N\textsubscript{2}-He atmosphere or submerged) were applied. DNA and RNA are to be extracted and 16S rRNA and nitrogenase (nifH) genes quantified and analyzed by qPCR and high-throughput sequencing of amplicons.

Conclusions

The isotope tracer-studies showed that only in oligotrophic sites the diazotrophic communities associated with Sphagnum reacted positively to methane addition and microaerobic conditions. For mosses from ombrotrophic and mesotrophic sites, diazotrophic activity was neither affected by methane addition, nor by reduced oxygen concentration. Combined, these results indicate that methane dependent nitrogen fixation may only be important under certain environmental conditions in Sphagnum-dominated peatlands. Conditions proven to be important for
methanotrophy (pH, water level) and diazotrophic community composition are likely to affect diazotrophy as well, and will be subject of future studies.
Background
Algae live in close association with microorganisms. However, our knowledge on the physiology and metabolism of complex microbial communities associated with algae is very limited.

Objectives
A main goal of our research is to understand the underlying molecular mechanism of species divergence and adaptive processes in microalgae and bacterial systems. According to the fundamental questions “Who’s there?” and “What are they doing?”, bacterial communities associated with plants and their functions should be analyzed in more detail. We have analyzed the bacterial populations of different microalga and we speculate that these bacteria have evolved a common mechanism to communicate and manipulate their hosts.

Methods
The diversity and population dynamic of the bacteria were examined using scanning electron microscope, 16S rRNA gene analysis, MS and metagenome analyses. Moreover, the current work is focused on transcriptome analysis.

Conclusions
A comprehensive understanding of microalgal and bacteria interactions requires knowledge of the associated gene expression changes in both the microalgae and the bacteria. So, we will analyze the role of common signaling pathways and signals from eukaryotic and prokaryotic sites. The results will help us to understand overall principles of microbial adaptation and signal exchange in more detail.
Background
Bacteria are able to colonize a wide spectrum of habitats including the rhizosphere of plants. In such association bacteria may utilize plant root exudates components as carbon and energy source, but also other processes may take place, like degradation of toxics, competition with pathogenic bacteria, phytohormones synthesis, nitrogen fixation, quorum sensing control, and several others. Despite the relevance for agronomical applications, the molecular mechanisms underlying microbe-plant root exudates interactions are poorly understood.

Objectives
To improve understanding of plant-microbe interactions through transcriptomica and metabolomics profiling of selected bacteria exposed to plant root exudates when plants have been grown under different nutritional and stress conditions.

Methods
Cell cultures of two different Burkholderiales species able to associate with Arabidopsis (one plant growth promoting rhizobacterium –PGPR- and the other one a non-PGPR) were exposed to plant root exudates obtained from plants grown on control, N-limitation, and saline or chemical stress conditions, and gene expression profiling: Real Time PCR analysis for specific genes, and complete transcriptomic profile analysis by High-Seq Illumina platform, were obtained. Metabolomic profiling of plant root exudates was done by HPLC-MS.

Conclusions
Plant root exudates exhibit different composition profile according to the growth conditions of the plant. This may explain differential gene expression profiles observed with the PGPR and the non-PGPR species.
Background

Apple cultivation a prominent industry in Himachal Pradesh, India occupies an area of 1,01,485 hectares and production reaching 2,11,295 metric tonnes. Today, the six decade old apple orchards are being replaced by new plantations. However, their survival is a major challenge which results in indiscriminate use of chemical inputs. This poses potential threat to environment/human health and therefore managing plant rhizosphere is critical.

Objectives

Work presented in here, highlights novelty of Bacillus sp. strain CKA1 (apple roots endophyte) in being used as rhizosphere engineer.

Methods

Microbial formulation was evaluated under field conditions for its effect on plant establishment; early growth; yield and rejuvenation of diseased apple trees. Whole genome sequencing of the bacterium helped in deciphering molecular mechanisms underlying the multifunctional plant growth promoting potential. Application of microbial formulation resulted in: increased plant biomass (75.40-80.11%); increased Nitrogen (2.42%), Phosphorus (0.58%) and Potassium (1.33%); increased fruit yields (35 to 45%); rejuvenation of diseased apple trees infected with Dematophora necatrix in farmer’s field over a three year trial. Comparative genomics paved the way for understanding molecular mechanisms involved in the strains ability to function as multifunctional plant growth promoter.

Conclusions
Apple orchard rejuvenation projects are being ambitiously launched by State agencies for restoring six decade old planted apple orchards. Integrated nutrient management systems comprising biological systems especially, managing rhizosphere using endophytes such as strain CKA1 opens up new avenues not only for improving crop yield but also in sustaining soil health.
Background

Endophytic fungi are prominent producers of putative phytochemicals and their analogues which can be exploited as therapeutic interventions/or pharmaceutical agents.

Objectives

The current investigation deals with isolation, purification and characterization of fibrinolytic enzymes produced by endophytic fungal isolates derived from different medicinal plants of Western Ghats of India.

Methods

In preliminary screening assays, 17% of the endophytic fungal isolates expressed fibrinolytic activity whereas 26% of the isolates exhibited proteolytic activity. Maximum fibrinolytic and proteolytic activity was shown by endophytic fungal isolate #37 CRSTBRT which exhibited a halo formation of 113.04 mm$^2$ on fibrin clot assay. The fibrinolytic enzyme (VM 22) from fungus was purified to electrophoretic homogeneity with the methods including ammonium sulphate precipitation, anion exchange and gel filtration chromatography. The molecular weight of the purified enzyme was estimated to be 35 KDa by SDS-PAGE, fibrin zymography and gel filtration chromatography. The enzyme was stable between 5.5 – 8.5 pH and below 50°C. The optimal pH for the enzymatic activity was 7.8 at 35°C. The purified enzyme also exhibited an 18 fold increase in the enzyme activity. In vitro studies also revealed that the enzyme could effectively catalyses the blood clot lysis. Further the endophytic fungal isolate #37 CRSTBRT was identified as a *Xylaria curta* species based on morphological and molecular taxonomic tools.

Conclusions

The fibrinolytic enzyme VM 22 directly lyse the fibrin clot and not by plasminogen activators indicating that this enzyme could be useful in thrombolytic therapy.
EFFECT OF GENE SILENCING OF THIOREDOXIN H DURING THE COMPATIBLE INTERACTION OF CAPSICUM ANNUUM EUPHORBIA MOSAIC VIRUS-YUCATAN PENINSULA

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Background
There are evidence of the participation of salicylic acid (SA) pathway in the plant/virus interaction. Thioredoxins (Trxs) participate as a regulator in this mechanism, and in plants, the h-type play a fundamental role in tolerance of oxidative stress and defense responses against pathogens.

Objectives
The objective of this work was to analyze the effect of silenced CaTRXh1 in plants of Capsicum annuum during the compatible interaction with the begomovirus Euphorbia mosaic virus-Yucatán Peninsula (EuMV-YP).

Methods
CaTrxh1 was silenced in plants of Capsicum annuum using a VIGs vector based on the TRV. 15 days post-silencing, plants were infected with the EuMV-YP. A time course experiments was done. Plant samples were collected at different times post-inoculation. Gene expression of thioredoxin h, NPR1, and PR10, and EuMV-YP replication were evaluated on the time course by Real Time PCR.

Conclusions

NPR1 and PR10 genes increase their expression at 14 and 7 days post inoculation (DPI), respectively. In the silenced and infected plants, the expression of NPR1 decreases in approximately 41% at 28 DPI, while PR10 gene maintains the same expression at 7 DPI. At 4 hour post inoculation (HPI) it was observed an increase in the SA content in the plant infected with the EuMV-YP and at 1 HPI in the pTRV2:CaTRX/EuMV-YP, compared with the control plants. Also, the viral DNA accumulation was higher in the plants silenced and infected with the begomovirus. These results suggest the participation of CaTRXh1 on plants of pepper in the mechanism of defense during the compatible interaction to EuMV-YP.
IN Volvement of Biofilm Formation of Ralstonia Solanacearum in Its Colonization in Intercellular Spaces

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Background
Ralstonia solanacearum is a soilborne bacterial pathogen and is the causal agent of bacterial wilt of more than 200 plant species, limiting the crop production around the world. The colonization of R. solanacearum strain OE1-1 (OE1-1) in intercellular spaces after invasion through wounds is required for its virulence.

Objectives
We analyzed colonization mechanism of OE1-1 in intercellular spaces.

Methods
The behavior of GFP-labeled OE1-1 cells in intercellular spaces of tomato plants was observed under the fluorescence microscope. OE1-1 cells in intercellular spaces were then observed under the scanning electron microscope (SEM). OE1-1 cells in intercellular fluids and xylem fluids from tomato plants were observed under the SEM. Furthermore, biofilm formation of the major extracellular polysaccharide, EPS I, productivity-deficient mutants was also analyzed.

Conclusions
The observation under the fluorescence microscope showed aggregation of fluorescence from GFP-labeled OE1-1 cells in intercellular spaces. The SEM observation showed attachment of OE1-1 cells on surfaces of tomato cells adjacent to intercellular spaces and biofilm-like structures by OE1-1 cells surrounded by an extracellular matrix. Interestingly, the SEM observation showed that OE1-1 cells incubated in intercellular fluids from tomato plants formed mushroom-type biofilms. On the contrary, incubation in xylem fluids led to a reduction of biofilm formation by OE1-1 cells. Furthermore, EPS I productivity-deficient mutants incubated in intercellular fluids significantly reduced their biofilm formation. Together, colonization of OE1-1 cells in intercellular spaces of tomato plants may consist of their attachment and biofilm formation on surfaces of tomato cells adjacent to intercellular spaces, in which EPS I productivity is involved.
LEAF SPRAYING WITH RHIZOBACTERIA FOR FOR BIOLOGICAL CONTROL OF XANTHOMONAS AXONOPODIS PV. PHASEOLI

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Background
The bean common blight (BCB) is the most important bacterial disease in the tropics. The chemical control of the BCB is neither efficient nor economic. Therefore, is important to develop new approaches to control CBB. Previous studies using seed microbiolization showed that DFs strains and combinations were effective in control leaf, root and vascular diseases (CORRÊA et al., 2014) and changed enzymatic activities (SILVA et al., 2009).

Objectives
So, here these rhizobacteria were evaluated by spraying them in leaves inoculated with Xanthomonas axonopodis pv. phaseoli.

Methods
The treatments were Bacillus (DFs093, DFs348 and DFs769), Pseudomonas (DFs513 and DFs831) and their combinations (DFs93+DFs769+DFs831 and DFs348+DFs769+DFs831) or water (test) sprayed 48 or 24 h before, 24 or 48 h after inoculation of the pathogen. The experiment was conducted in a greenhouse, in a design completely randomized, with six repetitions. The BCB severity was assessed after 96 hours each two days using a diagrammatic scale (0 to 6).

Conclusions
The test plants were 100% symptomatic after 120 hours and the final severity was between 5.35 and 5.48. Only the spraying 48 h before the pathogen inoculation result in a significant difference between the bacterial treatments. The treatments DFs513, DFs769, and the combination DFs348+DFs769+DFs831 showed a smaller area under disease progress curve (AUDPC) (20.6, 26.8 and 22.8% respectively). This result suggests the occurrence of induced resistance, since it requires previous spraying, as occurs when these treatments were used to treat seeds.

CORRÊA et al., 2014. Biological Control, 72: 71-75.

THE RHIZOSPHERE MICROBIOME OF SEAGRASSES AND THEIR ROLE IN SULFUR PROCESSES

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Background

Seagrass meadows grow in coastal, biogeochemically active sediments. These productive ecosystems are distributed from tropical to temperate areas and are dominated by seagrasses, a group of marine angiosperms that is often threatened by the presence of hydrogen sulfide, a phytotoxic gas produced by belowground bacterial communities. The seagrass rhizosphere is still poorly described, however the occurrence of die-off events caused by high levels of sulfide stresses the need to understand these plants and bacterial communities present in their rhizosphere – rhizobiome.

Objectives

The aim of this research was to obtain insight into the seagrass rhizobiome and to answer the following questions: a) Is the rhizobiome plant-specific?; b) Does the rhizobiome co-evolve with its host?; and c) Who are the core species of the seagrass rhizobiome?

Methods

Rhizosphere samples of Zostera marina, Z. noltii and Cymodocea nodosa, bulk sediments and seawater were collected from Portugal, and the former two rhizospheres were additionally collected from France. Next Generation Sequencing of 16S rDNA amplicons was performed using MiSeq Illumina platform.

Conclusions

The rhizobiome of seagrasses differs significantly from their surrounding environment. Although they don't vary at a local scale, the rhizobiomes were significantly different between plants from different geographical locations. The core rhizobiome of seagrasses is mainly composed of OTUs involved in the sulfur cycle (sulfate reduction and sulfur oxidation), and known for their ability to fix nitrogen.
Moreover, our results point to a niche differentiation of sulfur bacteria, in which sulfide oxidation is performed by different taxa.
EFFECT OF GFP-LABELLED PAENIBACILLUS POLYMYXA ON GROWTH OF AGRICULTURAL CROPS
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Background
Paenibacillus polymyxa strain P2b-2R isolated from internal stem tissue of a naturally regenerating pine seedling fixes nitrogen in association with lodgepole pine seedlings and promotes their growth. It has been shown to colonize lodgepole pine seedling tissues endophytically using a green fluorescent protein (GFP)-labelled derivative of P2b-2R.

Objectives
We wanted to see if the GFP labelled derivative of P2b-2R would fix N and promote growth of corn and canola in ways similar to the wild type strain.

Methods
We inoculated corn and canola seeds with wild type P2b-2R or the GFP labelled derivative of P2b-2R and seedlings were grown for 40 days in a N-limited soil mix. Seedlings were harvested 20, 30 and 40 days after inoculation and evaluated for biological nitrogen fixation and growth promotion.

Conclusions
Seedlings inoculated with the GFP labelled P2b-2R strain derived small amounts of N from the atmosphere (upto 17%) but grew significantly larger than seedlings inoculated with wild type P2b-2R. Seedlings growth was promoted by inoculation with GFP labeled P2b-2R with an increase of upto 40% in height and 70% in biomass as compared to wild type P2b-2R. Thus, we concluded that GFP modification of strain P2b-2R resulted in a significant enhancement of its growth promotion efficacy of corn and canola.
Background
Several bacterial strains of *Paenibacillus* that possessed N-fixing ability were isolated from extracts of surface-sterilized lodgepole pine seedling and tree tissues. One strain, *Paenibacillus Polymyxa* P2b-2R, was found to fix high amounts of nitrogen when reintroduced to lodgepole pine and western red cedar (gymnosperms). But can this bacterial strain fix nitrogen if introduced into agricultural crops?

Objectives
We wanted to determine if this bacterial strain could fix N and promote plant growth while living inside or in rhizosphere of agricultural crops (Corn and Canola).

Methods
We inoculated corn and canola seeds with P2b-2R and grew seedlings for 40-60 days. Corn seedlings were harvested 10, 20 and 30 days after inoculation and canola seedlings were harvested after 20, 40 and 60 days after inoculation. Seedlings were evaluated for biological nitrogen fixation and growth promotion.

Conclusions
Seedling growth was promoted significantly by inoculation with P2b-2R with an increase of upto 35% in height and 30% in biomass from control. P2b-2R also fixed more than 20% of atmospheric nitrogen while living both inside the plant and in the rhizosphere. These results suggest that this bacterial strain has a broad range of hosts and is successful in N-fixation and growth promotion in agricultural crops.
EVALUATION OF TEMPERATURE, PH AND CARBON SOURCE IN INDOLE ACETIC ACID PRODUCTION BY YEASTS ISOLATED FROM RIZOSPHERE AND PHYLLOPLANE

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Background
It is possible to find naturally in rhizosphere and phylloplane some yeasts species capable of producing phytohormones, solubilize phosphates and other minerals, as well as acting as biological control agent. Despite that, scarce information is known about this microorganism group as plant growth promoters.

Objectives
Considering these, the aim of this study was evaluate the production of phytohormone auxin (indole acetic acid (IAA)) by yeasts, with variations of culture conditions.

Methods
The yeasts evaluated were Rhodotorula mucilaginosa (2F32) and Trichosporon asahii (3S44), isolated from phylloplane and rhizosphere of sugar cane, respectively, in Brazil, São Paulo State. Yeasts were cultivated in Potato Broth medium, with 20 g/L of glucose or sucrose, as carbon source; the cultivation of yeasts was carried out at temperatures 22°C, 25°C or 30°C; pH was modified to 3.0, 4.5 or 6.0. All cultures were analyzed by colorimetric technique with reaction of broth and Salkowsky reagent; the cells count was realized to evaluate the development of yeasts.

Conclusions
The results for T. asahii showed that IAA production with sucrose was superior at pH 6.0, and glucose was superior at pH 4.5; these results indicate direct relation between carbon source and pH medium. Rh. mucilaginosa presented a high IAA production if compared with T. asahii or even with microorganisms related in literature; the higher production (655 μg/ml) was obtained at pH 6.0 and glucose as carbon source. Temperature was not able to influence IAA production, significantly. Variations of culture conditions did not affect the cell growth of yeasts.
**Background**
Rhizobia are nitrogen-fixing bacteria that establish a symbiotic relationship with leguminous plants. After entering the root cells, rhizobia differentiate into bacteroids within the root nodule of the host plant, and provide the plant with fixed nitrogen. Inside nodule, it have been believed that bacteroids construct optimal environment for nitrogen fixation, but the details are still unknown. We have previously evaluated the intercellular proteome of rhizobia

**Objectives**
To understand the mechanism by which rhizobia alter their metabolism to establish nitrogen-fixing symbiotic relationship with hosts, time-course quantitative proteome analysis of *Mesorhizobium loti*, one of the model rhizobia, was performed during the nodule maturation.

**Methods**
*Lotus japonicus* was inoculated with *M. loti*. Nodules were harvested at 2, 3, and 4 weeks post-inoculation. Bacteroids were extracted from nodules, and their proteins were extracted, digested by trypsin, and labeled by Tandem Mass Tag. The prepared samples were analyzed by LC-MS/MS equipped with a 500 cm-long monolithic silica capillary column.

**Conclusions**
Using a quantitative proteomics approach, we identified and quantified 537 proteins in *M. loti* bacteroids. The results revealed significant changes in the carbon and amino acid metabolisms upon differentiating into bacteroids. Furthermore, our findings suggest that *M. loti* enters a nitrogen-deficient condition during the early stages of nodule development, and changes to a nitrogen-rich condition in the intermediate stages.

**Reference**
1) Tatsukami Y. et al., *BMC Microbiol.*, 13, 180 (2013)
3) Nambu M. et al., submitted
FUNCTIONAL GENOMICS ANALYSIS OF SALMONELLA-TOMATO INTERACTIONS REVEALS MICROBIAL ADAPTATIONS AND PLANT RESPONSES TO THE INFECTION BY A HUMAN PATHOGEN

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Background
As the number and severity of produce-associated gastroenteritis outbreaks increase, we are coming to recognize that we know little about the ecology of *Salmonella* and enterovirulent *E. coli* outside of their animal hosts.

Objectives
The objective of this work was to delineate mechanisms behind interactions of a model human pathogen, *Salmonella enterica*, and its alternate plant host.

Methods
TnSeq and RNAseq analyses of *Salmonella* behavior in tomatoes and tomato responses to the colonization by the pathogen were used to reveal key mechanisms of bacterial adaptation to persistence within tomato and plant responses to the colonization by the human pathogen. Expression of individual genes was confirmed by qPCR and Recombinase-based *in vivo* Technology (RIVET).

Conclusions
*Salmonella* virulence genes located on the Pathogenicity Islands were not involved in persistence within tomato fruit. We identified important functions for the regulators of capsule synthesis (*yihT, rcsB*) in the phenotype. *Salmonella* genes involved in *de novo* synthesis of amino acids were critical to persistence within tomato fruits. *Salmonella* gene expression depended on the host maturity and genotype, for example, RIVET assays confirmed that *cysB* was expressed strongly in a tomato cultivar known to be resistant to a tomato pathogen *Ralstonia solanacearum*, while *fadH* was responsive to the maturity-dependent accumulation of specific fatty acids. Proliferation of the pathogen was restricted in the plant mutants defective in ethylene synthesis and response (*rin, Nr, nor*). Tomato genes involved in responses to plant pathogens were activated in response to *Salmonella* infection, however, typical responses to infection were only observed in ethylene mutants.
ENDOPHYTIC COMMUNITIES OF ARABIDOPSIS THALIANA: DOES CULTIVATION SUBSTRATE MATTER?

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Background

Bacterial endophytes colonize the internal tissues of plants without causing harm. An important subgroup are seed endophytes that are transferred from one generation to the next and that are already present during the early plant growth stages. Several studies suggest that seeds can serve as a vector for beneficial bacteria and that these bacteria could improve plant growth.

Objectives

We want to determine the effect of different cultivation substrates, which are frequently used during bulking of Arabidopsis thaliana seeds, on the seed endophytic population of this model plant.

Methods

Arabidopsis thaliana was cultivated on sand (bacteria-poor) and on a mixture of sand with potting soil (bacteria-rich). Seeds harvested on both substrates were sown again on the same substrate until the radicle emerged or until there were 3 week-old leaves. Cultivable endophytes were isolated from soil, seeds, radicles and leaves, and identified using 16S rDNA amplification and sequencing. Total bacterial populations were identified by a direct DNA extraction on soil, seeds, radicles and leaves, followed by amplification and 454 pyrosequencing of the V5-V7 hypervariable region of the 16S rDNA.

Conclusions

Despite large differences in the soil bacterial populations, the seed endophytic populations were very similar, which indicates that plants are able to select which bacteria can become seed endophytes. This can impact on the establishment of the endophyte community of the next generation of plants.
COMPARATIVE EFFECT OF BIO AND NPK FERTILIZERS ON THE GROWTH OF LETTUCE (LACTUCA SATIVA L.)

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Background
The use of inorganic fertilizers has been established to cause environmental problems.

Objectives
The study investigates the effect of a biofertilizer, *Trichoderma longibrachiatum* NGJ167 and NPK fertilizer on the growth of two varieties of lettuce (Greatlake and Eden).

Methods
The study was carried out in the screenhouse by inoculating soils with mycelial plugs of *T. longibrachiatum* NGJ167 and compared with NPK-fertilizer. Thereafter, *in-vitro* and conventionally propagated lettuce were planted on the treated soils and plant growth parameters observed.

Conclusions
Results showed that the micro-propagated Greatlake lettuce treated with *T. longibrachiatum* had higher plant height (22.23 cm) while the *in-vitro* propagated Greatlake lettuce treated with NPK-fertilizer had mean plant height value of 14.17 cm. The conventional propagated Greatlake and Eden treated with *T. longibrachiatum* had mean plant heights of 17.23 cm and 15.07 cm respectively while the controls had average values of 2.67 cm and 12.4 cm respectively. The average number of leaves of the *in-vitro* propagated Greatlake and Eden lettuce treated with *T. longibrachiatum* (7 and 4 respectively) were higher than those of the NPK-fertilized Greatlake and Eden lettuce (6 and 5 respectively). The result of the fresh biomass revealed that the conventional propagated Eden lettuce treated with *T. longibrachiatum* had an average value of 19.77 g while the NPK-fertilized plants had a mean of 15 g. The implication of these results showed that *T. longibrachiatum* NGJ167 was better than NPK fertilizer with respect to the growth of lettuce. The study concluded that the use of *T. longibrachiatum* improved the growth of lettuce.
MOLECULAR CHAPERONES OF THE TYPE III SECRETION SYSTEM ARE NOT REQUIRED FOR SECRETION OF THEIR PARTNER SUBSTRATES, BUT CAN BLOCK EFECTOR TRANSPORT IN PECTOBACTERIUM CAROTOVORUM

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Background

*Pectobacterium carotovorum* (*Pca*) is a plant pathogenic soft rot bacterium that can use the type III secretion system (T3SS) to suppress host defence. T3SS is utilized by many Gram negative bacterial pathogens to inject bacterial virulence proteins into eukaryotic cells, where they can manipulate host cell processes to pathogen advantage. T3SS chaperones are involved in protein transport, but their exact function is still unclear in many cases. In *Pca* two potential T3SS chaperones are coded for by the divergent transcriptional units, *dspEF* and *hrpWshcW*. Chaperone function was suggested for the DspF protein in related bacterium *Erwinia amylovora*, but not in *Pca*, while the ShcW protein was not studied before.

Objectives

Determine the role of DspF and ShcW in T3SS-dependent protein transport in *Pca*.

Methods

PCR, molecular cloning, directed mutagenesis, DNA sequencing, far-western blotting, two-hybrid screening.

Conclusions

We have found no evidence for chaperone requirement for stability or secretion of the DspE and HrpW effector proteins. On the other hand, introduction of additional copies of the *dspF* or *shcW* genes into *Pca* cells blocks their ability to induce hypersensitive response on nonhost plants. This phenotype is caused by a severe defect in translocation of effectors into plant cells. Translocation of the DspE-Cya fusion appeared to be completely blocked while translocation of AvrPto-Cya – reduced from *Pca* cells carrying *dspF* on a plasmid. Thus, additional molecules of T3SS chaperones can block effector translocation, while their native amounts are not required for secretion/translocation. This suggests that the main function of the DspF and ShcW chaperones may be to repress premature effector transport.
CYCLIC β-(1,2)-GLUCAN FROM XANTHOMONAS CAMPESTRIS PV. CAMPESTRIS SUPPRESSES ARABIDOPSIS THALIANA IMMUNITY BY MODULATING FLAGELLIN INDUCED DEFENSE.

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Background

Plants are constantly exposed to microbes, such as bacteria, fungi or viruses and most plants are resistant to most microbes. To be successful, a pathogen must overcome constitutive defenses or suppress induced defenses. Our group previously demonstrated that cyclic β-(1,2)-glucan, a polysaccharide synthesized by Xanthomonas campestris pv. campestris, suppresses the accumulation of callose deposition, as well as the local and systemic expression of PR1, a defense-related gene associated with salicylic acid responses (The Plant Cell. 2007: 2077-2089).

Objectives

To gain knowledge about the mechanism of cyclic glucan in the modulation of plant defense

Methods

We performed Xanthomonas campestris pv. campestris infection assays on A. thaliana leaves pretreated with flagellin (flg22) and purified cyclic glucan or both. Measurement of WRKY22, WRKY33 and MPK3 expression was done by RT-PCR and binding assays was made using Arabidopsis membranes and labeled 14C-cyclic glucans.

Conclusions

We observed that cyclic glucans of X. campestris suppresses partially the immunity elicited by flg22 in Arabidopsis thaliana. In addition, this compound downregulates WRKY22, WRKY33 and MPK3 expression, early defense genes induced by this elicitor. In order to get further insights into how CG modulates flg22 responses, we performed binding assays with Arabidopsis membranes and labeled 14C-CG. The radiolabeled glucan, 14C-CG, bound specifically to wild-type plant extracts, suggesting the existence of direct interaction between CG and an still unknown Arabidopsis receptor. The present results shed light on the mechanism by which Xcc CG hijacks, at least partially, plan immune response.
XBMR, A NOVEL TRANSCRIPTION FACTOR REGULATES CHEMOTAXIS, BIOFILM FORMATION AND VIRULENCE IN XANTHOMONAS CITRI SUBSP. CITRI

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Background

Citrus canker is one of the most important and aggressive bacterial diseases of citrus trees. The causal agent of this disease is Xanthomonas citri subsp. citri (Xcc). Biofilm formation on citrus leaves plays an important role in epiphytic survival of Xcc. Previous work from our laboratory described a genetic screen for biofilm formation-defective mutants in Xcc, this work identified that a mutant with a transposon insertion in XAC3733 (xbmR) had significantly reduced attachment to a polystyrene surface (Microbiology. 2013: 159, 1911–1919).

Objectives

To gain knowledge about the regulatory mechanisms of Xcc infection

Methods

For in vitro analysis of biofilm formation, we used confocal laser scanning microscopy. For pathogenicity assays grapefruits leaves was used as the host plant for Xcc. Gene expression levels was obtained by qRT-PCR. Intracellular alteration in c-di-GMP was achieved by exogenous expression of either the diguanylate cyclase WspR19 from P. fluorescens or the c-di-GMP phosphodiesterase PA2567 from P. aeruginosa.

Conclusions

A knock-out of xbmR led to a substantial downregulation of fliA, which encodes a σ²⁸ transcription factor, as well as fliC and XAC0350 which are potential member of the σ²⁸ regulon. XAC0350 encodes an HD-GYP domain cyclic di-GMP phosphodiesterase. These findings suggest that XbmR is a key regulator of flagellar-
dependent motility and chemotaxis exerting its action through a regulatory pathway that involves FliA and c-di-GMP.
FEMS-1036
Pollutant degradation

MICROBIAL RESOURCES TO BIODEGRADE COMPLEX TAR AFTER STEAM GASIFICATION PROCESSES
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Background

Biomasses gasification produces toxic compounds (tar), mainly composed by polycyclic aromatic hydrocarbons (PAH). Up to now the problem of the tar disposal is not solved. Bioremediation of tar with a mixed microbial consortium could be a highly promising and innovative approach for the handling of this by-product.

Objectives

The aim of this study was to assess the tar-degradation process in a laboratory-scale microcosm.

Methods

Triplicate tar-containing microcosms (PAH 13,904 mg/L) were incubated for 20 days with a microbial consortium selected from pinewood and polluted soils. After 20 days HPLC and GC/MS showed a significant reduction of all the PAH compounds to about 150 mg/L whereas PAH concentration in the negative controls was about 8,400 mg/L. Reliability of distinct replicated microcosms was very high since microbial communities showed 97% of similarity. Automated fingerprints and ribosomal gene pyrosequencing showed, at first, a noticeable lag-phase (0-2 days), where fungi were the leading part of the community. A second phase was evident between 3 and 10 days of degradation with specific bacterial genera. After 15 days, a stable phase with 24 bacteria genera and only one fungal genus was detected. Overall, a total of 59 bacterial and 22 fungal genera participated in the degradation.

Conclusions

Bacterial species grew according to a “cross-feeding” behavior. Natural and polluted soils proved to successfully provide valuable microbial taxa for innovative services such as tar degradation.
COMPARISON OF BACTERIOPLANKTON COMMUNITIES AND DIESEL BIOREMEDIATION ABILITY OF NATIVE BACTERIA FROM DIFFERENT MEDITERRANEAN TOURIST PORTS

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Background

Tourist ports are subjected to seasonal impacts determining high pollution by petroleum hydrocarbons. Bioremediation is an emerging clean-up technique based on the addition of amendments or microorganisms able to stimulate the degradation activity of the autochthonous bacteria.

Objectives

This study was carried out within MAPMED, a multidisciplinary project aimed to improve the environmental sustainability of tourist ports in the Mediterranean Sea with regard to hydrocarbon pollution. Cagliari (Italy), El Kantaoui (Tunisia), and Heraklion (Greece) were selected as case study sites. The present study aims to compare bacterioplankton, hydrocarbon degraders, and the effects of different bioremediation additives on the autochthonous communities among the selected sites.

Methods

The structures of bacterioplankton were compared by 16S rRNA T-RFLP among sites, sections with different usage and seasons (winter, beginning and end of the tourist season). Cultivable hydrocarbon degraders were characterized regarding phylogenetic position and catabolic abilities. Different bioremediation treatments (biostimulation with nutrients and bioaugmentation with selected strains) were tested in seawater microcosms supplemented with diesel. The monitoring parameters are the titles of heterotrophs and diesel degraders, and the hydrocarbon removal efficiency. At the end of treatments, the bacterioplankton communities are compared by 16S rRNA gene analysis.

Conclusions

The structure analysis highlights a clear seasonal variation in bacterioplankton and differentiates Heraklion communities from those found in Cagliari and El Kantaoui. Degraders exhibiting different ecological strategies dominate the three communities, copiotrophics in Cagliari and hydrocarbonoclastic bacteria in El Kantaoui and
Heraklion. Comparison of the effects of different bioremediation treatments is currently in progress.
EXTRACELLULAR DNA AS BIOSCAFFOLD FOR THE SYNTHESIS OF SUPERIOR PALLADIUM(0) NANOCATALYSTS HIGHLY ACTIVE IN THE DETOXIFICATION OF PERSISTENT ORGANOHALOGEN POLLUTANTS

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Background
In a search for new aqueous-phase systems for catalyzing reactions of environmental and industrial importance, we prepared novel palladium(0) nanocatalysts in the presence of Shewanella oneidensis strains. Especially a nuclease-deficient mutant of Shewanella oneidensis MR-1 showed an increased tolerance against elevated heavy-metal stress, obviously as a result of high levels of extracellular DNA (eDNA) and additional adaptive response mechanisms.

Objectives
The main objective of our study was to assess whether eDNA is able to protect S. oneidensis cells from toxic Pd(II) ions and can simultaneously serve as a scaffold for nanocatalyst synthesis.

Methods
With eDNA as nucleation site, it was possible to retrieve superior nanocatalysts, bearing small sizes, a monodisperse size distribution and low aggregation behaviour which are important prerequisites for high catalytic activity. Furthermore, in comparison to catalysts produced by the Shewanella oneidensis wildtype, the nanocatalysts synthesized by the mutant were more resistant against catalyst transformation, deactivation and poisoning. The catalytic properties of the palladium(0) nanocatalysts were tested using a newly developed real-time PTR-ToF-MS analytical technique, which is able to follow the nanocatalyst-mediated detoxification of highly-persistent anthropogenic pollutants at environmentally relevant concentrations. The palladium(0) nanocatalysts synthesized in the presence of the nuclease-deficient mutant of Shewanella oneidensis MR-1 showed a high catalytic activity for the dehalogenation of hexachlorobenzene and triclosan.

Conclusions
Further tests with "old" and emerging halogenated persistent organic pollutants are under way and will demonstrate the high potential of Pd(0) nanocatalysts for clean-up of drinking water, aquifers and specific industrial effluents, as well as the suitability of PTR-ToF-MS to assess the respective transformation and detoxification reactions.
COMPARATIVE STUDY OF THE CAPACITY OF CARRIERS: ANOXKALDNES K1, POLYPROPYLENE PADS AND GRANULAR CORKSORB TO FORM HYDROCARBON-DEGRADING BIOFILMS AND REMOVE HYDROCARBONS FROM DIESEL POLLUTED WATER

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Background
Hydrocarbons are a widely distributed pollutant that reaches the environment from multiple sources and involve significant risks to the environment that must be minimized.

Objectives
To design a new treatment system for hydrocarbon contaminated water based on biofilm technology, we studied three different carriers 1) AnoxKaldnes™ (a plastic carrier). 2) Polypropylene pads, these are oil absorbent pads composed of 100% meltblown polypropylene that absorb oil only and not water. 3) Granular thermal treated hydrophobic cork which absorbs oil and solvents without absorbing water.

Methods
Using 1L bioreactor with 400 ml of water and 200 ml of carrier, kinetics of growth and adherence to carriers were determined by enumeration of viable cells. Development of biofilm was visualized by scanning electron microscopy. TPH, n-alkanes, branched-alkanes and naphthalene removal were determined by GC/MS at the beginning and at the end of the assays. Pseudoalteromonas elyakovii strain W18 was inoculated to study biofilm formation. Waters with and without 1% (v/v) diesel, were used to analyze the characteristics of formed biofilm and the efficiency of hydrocarbon removal by indigenous microorganisms.

Conclusions
No inhibitory effect of carriers was observed, even in bioreactors with high hydrocarbon contamination. SEM analyses showed that Polypropylene pads and Granular CorkSorb allow microbial biofilm formation. GC/SM analyses showed that Polypropylene pads not only enhance microbial growth but also retain high amount of hydrocarbons.

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NOVEL ANAEROBE OBTAINED FROM A HEXADECANE-DEGRADING CONSORTIUM

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Background

Aliphatic hydrocarbons (AHC) are abundant in crude oil and fuels, and are frequent contaminants of water, soil and sediments. There is potential for AHC bioremediation using sulfate as electron acceptor, due to its abundance in marine environments and natural presence in soils and groundwater.

Objectives

In this work sulfate-reducing anaerobic microorganisms involved in AHC biodegradation were studied.

Methods

Anaerobic sludge was incubated at 37°C with hexadecane (1mM) and sulfate (20mM) in serum vials. Cultures were successively transferred to fresh medium until a stable enrichment was obtained (monitored by microscopy and PCR-DGGE of 16S rRNA gene). For isolation of AHC-degrading bacteria, serial dilutions and successive transfers are now running using palmitate (1mM) as an easier substrate.

Conclusions

Cultures growing on palmitate show two main bacterial cell types: a rod-shaped bacterium closely related to Desulfomonile limimaris (94% identity) was predominant in the first 30 days of incubation, when 83% of the added palmitate was degraded coupled to 4 mM sulfate reduction (suggesting stoichiometric palmitate conversion to acetate); and an oval-shaped bacterium related to Desulforhabdus amnigena (99% identity) that mainly developed when incubations where extended and a total of 11.5 mM sulfate was reduced. Growth of Desulforhabdus was stimulated when incubated with acetate. The role of the Desulfomonile in AHC degradation will be further discussed in the presentation, as well as its halorespiring ability, a characteristic of
the Desulfomonile genera. Further characterization of this novel bacterium is important due to its high potential for bioremediation of hydrocarbons, fats and halogenated pollutants.
APPLICATION OF HIGHLY EFFECTIVE MICROBIAL DEGRADERS OF OILS AND FATS IN WASTEWATERS DECONTAMINATION

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Background

Disposal of municipal sewage and industrial effluents remains a top priority challenge. Annual discharge of wastewaters containing lipid substances in Belarus reaches 10-12 mln ton. It is recommended to utilize high-efficient microbial strains decomposing oils and fats for their remediation.

Objectives

Searching and examination of microorganisms-degraders of lipid compounds.

Methods

Isolation of microbial strains was carried out by enrichment culture technique. Lipase activity was evaluated by Ota-Yamada method. Dynamics of lipid dissimilation was monitored on model water comprising fats (lard, dairy) and oils (sunflower and olive) in concentration 1000 mg/l as the sole carbon sources.

Conclusions

Four superactive bacterial strains *Rhodococcus ruber* 2B, *Rhodococcus sp.* R1-3FN, *Bacillus subtilis* 6/2-APF1, *Pseudomonas putida* 10AP – degraders of fatty substrates showing lipase activity levels in the range 0.65-0.70 u/mg protein were derived by adaptive selection method. It was found that the selected strains were capable to consume 83.4-94.2 % of animal fats and 82.3-90.4 % of vegetable oils in 7 days. Nutrient medium composition was defined and optimized in terms of nitrogen, potassium, phosphorus and carbon sources. It allowed to attain maximum biomass concentrations (3.0-4.8 g/l by 48 h of fermentation) with elevated lipase activity.

Based on the obtained data technology of manufacturing microbial preparation to accelerate disposal of lipid pollutants in wastewaters was developed.
Introduction of new microbial product into biodecontamination scheme promoted 15.9 \% decline in chemical oxygen demand, increased degree of lipid removal by 59 \% and improved quality of activated sludge.
APPLICATION OF CARBON STABLE ISOTOPE ANALYSIS (CSIA) TO INVESTIGATE BIODEGRADATION AND DIRECT PHOTOLYSIS OF ANTIBIOTIC SULFAMETHOXAZOLE

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Background

Sulfamethoxazole (SMX) represents sulfonamide antibiotics (SAs) widely used in veterinary and human medicine. SMX is incompletely metabolized and enters the environment with wastewater discharge, animal manure and aquaculture. SMX is frequently detected in wastewater treatment plants, surface and ground water. In the environment it undergoes different degradation processes, such as biodegradation and photodegradation.

Understanding of SA’s removal routes is crucial for an assessment of their environmental impact. Compound specific stable isotope analysis (CSIA) can provide additional information on the organic contaminants' transformation processes in complex environments. In CSIA, the changes in isotope composition of the parent compound are monitored during (bio)transformation processes and the isotope enrichment of the investigated contaminant provides an evidence for its (bio)degradation without a need of metabolite analysis.

Objectives

This research aims to evaluate the applicability of CSIA for the assessment of SMX transformation pathways. Therefore, the isotope fractionation of SMX during biotic degradation by Microbacterium sp. strain BR1 and abiotic transformation via direct photolysis was determined.

Methods

Bio- and photodegradation assays; HPLC; LC-IRMS; quantification of enrichment factors

Conclusions
A significant difference in isotope fractionation during biotic and abiotic SMX decomposition was observed, showing that CSIA has a potential for distinguishing these two degradation processes. Isotope fractionation during direct photolysis was variable and depended on pH of the solution.

This work shows a new application for CSIA - monitoring of sinks, sources and environmental behaviour of water-soluble contaminants, among which pharmaceuticals are of particular concern.
Background

Exploration and drilling of oil requires the use of drilling fluid to lubricate and cool the drill bit. Oil based muds (OBMs) containing diesel, are preferred drilling fluids. After mixing with drill cuttings, OBMs form complex industrial wastes containing hydrocarbons and brine. Bioremediation is a promising alternative to physicochemical methods for cleaning up drilling wastes.

Objectives

The goal of this study was to isolate halophilic bacterial consortium capable of degrading diesel oil in drilling muds and evaluating their applicability in the lab scale drill cuttings microcosm.

Methods

Between 10 microbial consortia isolated from different saline environments, the best consortium was isolated from Qom sample which grew very well on Bushnell Haas medium containing diesel with the bacterial count about $1.9 \times 10^{15}$ CFU/ml after 96h of incubation. For evaluating the applicability of the consortium in bioremediation of drill cuttings, it was mixed by 50% of fine sands or biological active soil in the lab scale microcosms and the effect of consortium inoculation was compared with corresponding un-inoculated control for three month.

Conclusions

The Measurement of the amount of remaining pollutant (TPH) after 3 month showed that the most degradation rate (40%) belonged to the microcosm containing 50% fine sand and consortia inoculation. Sequencing of 16S rRNA gene revealed that the dominant strain of the consortium belonged to the *Dietzia* genus. It could be concluded that bioaugmentation is useful for remediation of drill cuttings when the cuttings mixed with fine sand which has a weak microbial flora and allowing the better growth of inoculated consortium.
ISOLATION AND CHARACTERIZATION OF CONFLUENTIMICROBIUM SP. NS6, A NEW NAPHTHALENE-DEGRADING BACTERIUM, ISOLATED FROM AN OIL-CONTAMINATED TIDAL FLAT

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Background
Polycyclic aromatic hydrocarbons (PAHs) are great environmental concerns because of their persistence, toxicity, and carcinogenicity. Many studies for bacteria capable of PAH degradation have been reported in seawater, but PAH-biodegrading bacteria in tidal flats have been rarely explored.

Objectives
The purpose of this study was to isolate strains NS6 as a novel PAH degrader capable of PAH degradation in tidal flats and characterize its physiological and PAH-biodegrading properties.

Methods
We established a slurry type enrichment system based on tidal flat sediment and seawater using naphthalene as a sole carbon and energy source. The enrichment culture was spread on marine agar (MA) and incubated at 25°C for 2 days. Colonies grown on MA were characterized phylogenetically based on their 16S rRNA gene sequences and the naphthalene degradation abilities were evaluated in seawater, tidal flat slurry, and ONR7a. Finally, a strain showing a good naphthalene degradation property was isolated and its PAH degradation properties and genes were characterized.

Conclusions
A naphthalene degrading bacterium, designated strain NS6, was isolated from the slurry type enrichment and classified into a member of Confluentimicrobium based on its 16S rRNA gene sequence. Confluentimicrobium sp. NS6 degraded PAH compounds quickly in all tested media. Naphthalene dioxygenase gene of Confluentimicrobium sp. NS6 was successfully amplified by only NDO 201 primer set, which suggested that strain NS6 may metabolize naphthalene via a nah catabolic pathway alike Rhodococcus. In addition, the PAH degradation properties, physiologies, and PAH degradation gene structure of strain NS6 will be investigated and discussed in detail in the poster section.
Background
The use of white rot fungi such as *Pleurotus ostreatus* and their lignin-modifying enzymes has become an effective treatment for various organic soil and water pollutants. The manganese peroxidase gene family (mnp genes) is a major part of the ligninolytic system of *P. ostreatus*. This gene family is comprised of nine genes encoding short manganese peroxidases (short-MnPs) or versatile peroxidases (VPs). The VPs contain unique active sites which are responsible of direct oxidation (in the absence of Mn$^{2+}$) of various aromatic compounds.

Objectives
Here we study the oxidation mechanisms of aromatic compounds by VP1 of *P. ostreatus*.

Methods
We show that in Mn$^{2+}$-deficient GP medium *vp1* (encoding VP1) has a key and non-redundant function. We used the azo-dye Orange II (OII) as a model contaminant, its decolorization occurs only during the idiophase where the abundance transcripts of mnp genes indicate that *vp1* is the predominantly expressed and a Δ*vp1* strain showed a drastic reduction in the decolorization. Three degradation metabolites were identified by LC-MS indicating both asymmetric and symmetric enzymatic cleavage of the azo bond. The presence of asymmetric cleavage diminishes the toxicity level of the degradation products. To better understand mechanisms of degradation and detoxification we purified and characterized VP1. The purified enzyme degraded 60% of OII (50µM) within the first min and continued up 90% within 6 min.

Conclusions
The non-specific oxidation properties of VP1 and its unique ability to degrade organic compound in the absence of Mn$^{2+}$ suggest that it may have potential applications for treatment of contaminated water.
Background

Accumulation of synthetic polymers in the environment is cause for concern, as they have been associated with various negative effects on ecosystem health, especially marine systems. Knowledge on biodegradation pathways of conventional polymers is very limited, but it is known that these compounds are hardly biodegradable.

Objectives

Here, we investigated whether fungi causing white and brown rot of wood are capable of degrading the widespread polymer polystyrene (PS) as well as its water-soluble analogue polystyrene sulfonate (PSS). Their well-known extracellular oxidation capabilities made them promising candidates for the degradation of these recalcitrant substrates.

Methods

PSS analysis by size exclusion chromatography revealed that it was practically inert to white-rot fungi, but vulnerable to various brown-rot strains, which caused strong depolymerisation leading to molecular mass reductions of more than 90%. Detailed investigations pointed to Fenton reactions driven by fungal hydroquinones as agents of depolymerisation. Solid PS films were significantly more recalcitrant, as no changes in their bulk properties were observable after long periods of fungal treatment. Indications of biodegradation only came from surface property measurements, which revealed increased hydrophilicity through slightly decreased water contact angles. Investigations by X-ray photoelectron spectroscopy revealed that surface oxygen content had increased significantly in fungi-treated samples.

Conclusions
These results demonstrate that biodegradation of recalcitrant synthetic polymers is, in principle, possible. However, the different observations between PS and PSS indicate that biological attack on the solid polymer proceeds extremely slowly, raising questions about the feasibility of PS waste biodegradation.
FUNCTIONAL ANALYSIS OF A PLASMID-BORNE ALKYL SULFATE DEGRADATION MODULE OF PSYCHROBACTER SP.

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Background

Plasmid pP62BP1 (34.5 kb), harboured by strain Psychrobacter sp. DAB_AL62B isolated from ornithogenic deposits in Spitsbergen (Svalbard Archipelago, Norway), was found to carry a phenotypic module, named SLF, which consists of four open reading frames (slfCHSL) encoding putative catabolic enzymes and a gene (slfR) for transcriptional regulator of AraC/XylS family. Based on the comparative in silico analyses, the module was predicted to take part in the metabolism of alkyl sulfates, e.g. sodium dodecyl sulfate (SDS), a popular anionic surfactant.

\begin{align*}
\text{dodecyl sulfate} & \xrightarrow{\text{SlfS}} \text{dodecanol} \\
& \xrightarrow{\text{SlfH}} \text{dodecanal} \\
& \xrightarrow{\text{SlfC}} \text{dodecanoic acid} \\
& \xrightarrow{\text{SlfL}} \text{dodecyl-CoA}
\end{align*}

Objectives

The aim of our work was to perform a functional characterization of the SLF module both in native host and in other bacterial strains as well as to elucidate the regulatory mechanism of the slfRCHSL genes expression.

Methods

Qualitative and quantitative SDS degradation assays were designed based on the properties of Stains-All, a carbocyanine dye. Promoter strength was examined in β-galactosidase activity assays. Transcriptional organization of the module was characterized with the use of reverse transcription PCR.

Conclusions

Growth experiments revealed that 0.17 mM concentration of SDS in the medium is sufficient to exert bacteriostatic effect on several tested Psychrobacter spp. strains. Nonetheless, the pP62BP1 containing strain was capable of the complete SDS degradation in these conditions. The cloning of the whole SLF module and its slfR-deficient derivative into Escherichia coli TG1 resulted in obtaining transformants able to degrade SDS. The molecular studies showed that the activity of the slfCHSL operon is dependent on SlfR protein which acts as a negative transcriptional regulator.
FEMS-1870
Pollutant degradation

MOLINATE BIOREMEDIATION STRATEGY USING A MICROENCAPSULATION APPROACH
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Background

Molinate is a thiocarbamate herbicide used in rice cultivation to control grass weeds, however its use had led to environmental contamination, being critical the establishment of remediation procedures. A mixed culture has been described as being able to mineralize molinate as the sole source of carbon, nitrogen and energy [1, 2], primarily, by the activity of molinate hydrolase (MolA) [3], encoded by Gulosibacter molinativorax ON4T. Previous studies, using soil microcosms, have demonstrated that a bioaugmentation strategy using the mixed culture was a feasible approach to bioremediate paddy soils contaminated with molinate [4].

Objectives

To improve the bioremediation strategy, we aimed at microencapsulating ON4T cells or MolA, using a spray-drying process that uses biopolymers as encapsulating agent [5].

Methods

Several encapsulating agents (sodium and calcium alginate, arabic gum, chitosan and modified chitosan) were used. The spray-drying product yield was about 50% for all the agents. The metabolic activity of the microencapsulated cells was proven by a qualitative colorimetric method using a redox dye (tetrazolium violet).

Conclusions

There was a linear correlation between the amount of encapsulated cells and the colour development intensity. The feasibility of using optimized microencapsulated biomaterial will be tested in contaminated soil and water using a microcosm approach.


Background

With the emergence of microorganisms resistant to multiple antimicrobial agents there is increased request for promotion of disinfection methods.

Objectives

Since ZnO nanoparticles (ZnO-NPs) exhibit strong antibacterial activities on a broad spectrum of bacteria the aim of this study was to evaluate the antimicrobial activity of ZnO-NPs against *pseudomonas aeruginosa* as a model for gram-negative bacteria.

Methods

Muller Hinton broth was used as a growing medium for *pseudomonas aeruginosa*. Photocatalytic experiment was carried out in a laboratory-scale batch reactor with low pressure ultraviolet irradiation (380 nm). Different experimental parameters such as amount of ZnO-NPs, contact time, inorganic and organic substances and pH on photocatalytic inactivation of *pseudomonas aeruginosa* cells have been studied. An initial *pseudomonas aeruginosa* concentration of $10^8$ CFU/mL was used for all experiments.

Conclusions

Result showed that, almost all the initial *pseudomonas aeruginosa* cell ($10^8$ CFU/ml) was inactivated in 60 min in the presence of 2 g/l ZnO-NPs. Photocatalytic inactivation of bacteria was found to follow first order kinetics. The initial pH of the water did not play an important role on the inactivation rate within a range of 6–8 pH units. The amount of photocatalyst also plays an important role in photocatalytic inactivation rate. As the result showed increasing the photocatalyst amount provided more rapid inactivation. Addition of some inorganic ions to the suspension affects the sensitivity of *pseudomonas aeruginosa* and caused to retard the inactivation rates.
Since the sensitivity of *Pseudomonas aeruginosa* to photocatalytic treatment was fairly good, it is therefore, recommended to use this nano-particle for water treatment.
ADAPTATION TO AROMATIC HYDROCARBONS INVOLVES A NEW HYBRID TWO-COMPONENT REGULATORY SYSTEM IN AZOARCUS SP. CIB

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Background

The catabolic processes performed in anaerobiosis are becoming of great environmental interest because many anoxic ecosystems are frequently contaminated with aromatic compounds. The facultative anaerobe β-proteobacterium Azoarcus sp.CIB has been used as model system to study basic aspects on the biochemistry and genetics underlying the anaerobic metabolism of several aromatic compounds. Within the operon responsible for the anaerobic degradation of toluene, we identified an orphan gene, tolR, without homologues. The TolR protein shows an atypical architecture which corresponds to a new hybrid two-component system (HTCS), where the sensor histidine-kinase and the c-di-GMP phosphodiesterase-containing response regulator (RR) components are fused in a single polypeptide.

Objectives

The main objective was to demonstrate that TolR constitutes a new HTCS involved in anaerobic adaptation to aromatic hydrocarbons in Azoarcus sp.CIB.

Methods

An in vivo approach for confirming the c-di-GMP phosphodiesterase activity of the tolR gene was done using a lacZ-based P. aeruginosa reporter strain. In vitro approaches by using purified TolR and truncated forms were used to demonstrate toluene-induced autokinase and transphosphorylation activity. Transcriptomic studies were done to identify genes involved in the TolR-mediated response to aromatic hydrocarbons.

Conclusions

TolR encodes a unique HTCS whose RR is not a transcriptional regulator but a c-di-GMP phosphodiesterase. We demonstrate for the first time the effector-dependent induction of the intramolecular phosphorelay in HTCS. Transcriptomic studies confirmed that TolR is involved in a signalling network that controls the morphological, metabolic and stress response programs. The toluene-dependent control of c-di-GMP levels represents an unprecedented mechanism of bacterial adaptation to aromatic hydrocarbons.
BIODEGRADATION POTENTIAL OF PENICILLIUM SP. ISOLATED FROM SOUTH CHINA SEA

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Background
Some halophiles fungal genera were found from sea sediment samples are potentially play an important role in nutrient regeneration cycles as decomposers.

Objectives
In this study, three genera of microfungi derived from South China Sea were isolated as Penicillium, Aspergillus and Talaromyces in order to identify the fungal saprotrophic properties.

Methods
For the first stage of investigation by using dermatophyte test medium (DTM), the results showed that only the ascomycete Penicillium sp. highly potential as saprophytic microfungi. This fungus was used to observe their early thallus development in vitro and to determine a degradation potential of the textile dyes Trypan blue (TB), Methyl orange (MO), Sudan III (S3), Cango red (CR) and Bromocresolgree (BG) in broth culture.

Conclusions
Penicillium sp. displays a distinctive morphological growth, produced an orange pigment in all different media as an additional features of the strain. In contrast, there were significant differences in physiological tests of Penicillium sp. such as thermophilic osmomophilic, pH, salinity, xerotolarent and nitrogen stress. In the presence of textile dyes in liquid media was able decolorized by Penicillium sp. Thus, this study shows that halophilic Penicillium sp. from sediment of South China Sea is hereby reported for the first time and will be used as bio-indicator of water pollution and also in biological wastewater treatment. In additional this investigation also demonstrated the need of extra and intracellular enzymes associated to decolorization and biodegradation assays.
STUDIES ON HYDROGEN SULFIDE OXIDASE FROM PSEUDOMONAS SP.
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Background
Biogas resultant from the anaerobic treatment of sludge is composed mainly of CH₄ and CO₂. H₂S, a minor component, is of particular concern due to its corrosive and environmentally hazardous properties. Some microorganisms are able to perform enzymatic oxidation of H₂S to sulfur and/or sulfate. Biological treatment using these microorganisms can be an alternative to physical/chemical processes, avoiding the production of secondary waste.

Objectives
This work aims at the characterization of enzyme(s) responsible for H₂S oxidation in Pseudomonas sp.

Methods
Isolates A9, B9 and C1, identified as Pseudomonas spp., were isolated through enrichment from wastewater treatment plant deodorant bioreactor supplied with H₂S streams. These strains were screened for the presence of genes responsible for H₂S oxidation by PCR using degenerated primers for group II sulfide quinone:oxidoreductase. Fragments with the expected size were purified from the gel and subjected to sequencing. Translated sequences were compared using the BLAST software. Total protein profiles were screened by SDS-PAGE, before and after the addition of H₂S. Activity tests were conducted using crude cell extract as catalyst and measuring the formation of sulfate as the reaction product.

Conclusions
The amplified fragment from isolate A9 showed 51% identity with an oxidoreductase from Pseudomonas sp. M1, indicating that this bacterium has the machinery required to the desired activity. The protein profile of the strains when grown in the presence H₂S differ from the profile of the same strain grown without sulfide.
Background

The biodegradability of PBAT (poly(butylene adipate-co-butylene terephthalate)) under aerobic conditions has been examined in several studies. However, there is considerably less known about PBAT biodegradation in anaerobic environments.

Objectives

Amongst others Clostridium species are known to be typically present during naturally occurring anaerobic degradation processes of polymers (e.g. in biogas plants). Hence, various Clostridium species were in-silico screened for esterases that are possibly active on synthetic polyesters like PBAT.

Methods

First, a selection of identified hydrolases from Clostridium species were successfully expressed in E. coli BL21-Gold(DE3). The activity of these esterases was confirmed on the soluble standard substrate p-nitrophenyl butyrate as well as on the PBAT polymer. All esterases were characterized in detail including the determination of their crystal structure or modeling of the proteins. The crystal structure of one promising esterase revealed the presence of a metal ion that lies deep beneath the protein surface. Furthermore, Clostridium cultures were tested for their ability to express these active and naturally occurring enzymes in situ and degrade the PBAT polymer.
Conclusions

We were able to prove that the novel hydrolases from *C. botulinum* and *C. hathewayi* hydrolyze the aliphatic-aromatic polyester PBAT. These enzymes are promising candidates for industrial applications or pollutant degradation. Furthermore the study provides information about these anaerobic strains and their naturally occurring enzymes.
USE AND IMPACT OF MNO2 MICROPARTICLES FOR SULFIDE SCAVENGING IN MICROBIAL BROTHS

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Background

Hydrogen sulfide (HS⁻) is ubiquitously present in many waste streams originating from industrial activities as well as in sewage. It needs to be removed, as it can be toxic to bacteria, induces concrete corrosion and forms an odour nuisance. MnO₂ has been proposed for HS⁻ adsorption and oxidation, the addition of such particles to microbial broths may enable selective sulfide scavenging. However potentially negative effects of addition of MnO₂ microparticles (MPs) on microbial metabolism may occur.

Objectives

Therefore, we evaluated the efficiency of this MnO₂ MPs based HS⁻ removal strategy and test the effect of the particles and the sulfide scavenging on bacterial growth and microbial community activity.

Methods

Abiotic batch tests were set up to test impact of pH on HS⁻ removal. HS⁻ removal rate after 24h at pH 7.2 was twice that at pH 10.5. Scanning electron microscopy with energy dispersive X-ray spectroscopy showed the presence of sulfur as well as MnS on the particle surface. The effect of HS⁻, MnO₂ MPs and mixtures of both on the growth of single bacterial strains, and mixed microbial community activity were assessed using an optical density microplate reader placed into an anaerobic chamber and biochemical methane potential assays respectively.

Conclusions

The presence of MnO₂ MPs did not affect bacterial growth under any of the conditions tested. Inhibitory effects due to presence of HS⁻ on bacterial growth were observed, upon addition of MnO₂ MPs normal growth could be restored. We finally observed that a mixture of HS⁻ and MnO₂ MPs positively impacted methanogenic activity.
Pollutant degradation

IN STREPTOMYCINES, STRONG ANTIBIOTIC PRODUCTION CORRELATES WITH EXTENSIVE STORAGE LIPIDS DEGRADATION AND HIGH ATP GENERATION.

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Background

Some *Streptomyces* strains produce antibiotics and others don’t. Why? For instance *S. lividans* and *S. coelicolor* are phylogenetically closely related strains possessing the same functional antibiotic biosynthetic pathways but *S. lividans* produces antibiotics at low level whereas *S. coelicolor* produces them at high levels. Interestingly, a dramatic overproduction of antibiotics occurred in *S. lividans* upon interruption of the ppk gene. This mutant strain experiences energetic stress (ATP deficit) since Ppk regenerates ATP from ADP and polyphosphate. However, the role of the Ppk in relation to the regulation of antibiotic biosynthesis remains unclear.

Objectives

To better understand it we assess the global impact of strong antibiotic production on the cellular metabolism.

Methods

To do so proteomic and lipidomic analysis were conducted and the cellular content in polyphosphate, ATP/ADP and free Pi was assayed throughout growth of the three strains of interest grown in phosphate limitation or proficiency.

Conclusions

Our results revealed that an active degradation of storage lipids (TriAcylGlycerol, TAG) was taking place in the antibiotic producing strains. TAG degradation generates precursors used directly or indirectly for antibiotics biosynthesis as well as numerous reduced co-factors (FADH2) whose re-oxidation by the respiratory chain yields high levels of ATP. Whereas the ppk mutant likely mobilizes its storage lipids to re-
establish its energetic balance, the reason of the extensive TAG degradation taking place in *S. coelicolor* is still not understood.

**References**

Le Maréchal P *et al.* (2013) Comparative proteomic analysis of the wild-type and the *ppk* mutant of *S. lividans* revealed the importance of storage lipids for antibiotic biosynthesis. AEM 79-19:5907-5917.
TOWARDS THE ANAEROBIC-AEROBIC BACTERIAL MINERALIZATION OF CHLORENDIC ACID

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Background

Chlorendic acid (1,4,5,6,7,7-hexachlorobicyclo[2.2.1]-hept-5-ene-2,3-dicarboxylic acid, known as HET acid, or Hetron 92) has been identified in the past as the dead-end product of the biodegradation of the cyclodiene pesticides Aldrin, Endrin, Dieldrin, Endosulfan, Chlordane, and Heptachlor. On the other hand, this compound is still being produced as a flame retardant, and used in respective materials and corrosion-resistant equipment. Chlorendic acid has been considered as non-biodegradable until today.

Objectives

Although the hexachlorocyclopentadiene moiety of the compound is highly halogenated and does not carry any hydrogen atom, its dichloroethene substructure may provide the side of bacterial attack by the mechanism of anoxic dehalogenation as known from tetrachloroethylene or polyhalogenated aromatics (halo-respiration), and thus represent the initial side of a biochemical reaction. Further steps of dehalogenation then may proceed aerobically as, for instance, in the catabolic pathway of hexachlorocyclohexane (HCH, Lindane).

Methods

We started enrichment experiments with sediments from the River Elbe (Hamburg, Germany) the former contamination of which by pesticides from production plants of chemicals has been well documented, and established a gradient bioreactor system with an anaerobic zone at the bottom towards an aerobic zone at the top. From the latter we could already isolate the dominant bacterium responsible for the mineralization of the (almost) dehalogenated carbon backbone. 16S rDNA analysis revealed this species as 98% identical to Pseudomonas aeruginosa.

Conclusions

Analyses of the 16S rRNA gene sequences in the anoxic and transition zones will determine the bacterial community structure involved in the catabolic sequence. Identification of intermediates of the combined anaerobic-aerobic pathway is underway.
MICROBIAL DEGRADATION OF EMERGING TRACE ORGANIC CONTAMINANTS: SULFAMETHOXAZOLE, PIRACETAM AND RITALINIC ACID

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Background

Contamination with pharmaceuticals is an emerging environmental problem. Most pharmaceuticals are only partially eliminated in biological wastewater treatment processes, leading to contamination of ground- and surface water. Among them, antibiotics are of particular concern due to potential spread of antibiotic resistance. Sulfamethoxazole (SMX) represents sulfonamides, which belong to most prescribed and consumed antibiotics worldwide. Only a few microbial strains using SMX as a sole carbon source have been isolated and metabolic pathways for most of them have not been described.

The nootropic drugs are a new group of emerging contaminants, very popular these days because of their increasing use as pharmaceuticals and illicit drugs. Some of them, e.g. piracetam and ritalinic acid, have been recently detected in drinking water but so far their potential biodegradation has received very little attention.

Objectives

This research aims at isolation and characterization of novel microbial strains capable to degrade SMX, piracetam and ritalinic acid.

Methods

Enrichment and isolation of strains was carried out under aerobic conditions using various environmental samples as inocula: wastewater, activated sludge, soil, birds feces etc. Degradation is monitored by HPLC-DAD; new method for ritalinic acid and piracetam have been established; 16S rRNA analysis was performed; radio assays with 14C labeled ritalinic acid will be carried out.

Conclusions
Two novel strains capable of SMX degradation have been isolated. Enrichment cultures with piracetam and ritalinic acid are active and the isolation of strains is ongoing. It is a first attempt to obtain the strains growing on nootropic drugs.
DEGRADATION OF TRICLOSAN AND TRICLOCARBAN BY A WASTEWATER MICROORGANISM

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Background
Since last several decades, biocides like triclosan and triclocarban have been used as a common ingredient in many synthetic products especially in industrial and personal care products. Hence, the widespread use of triclosan and triclocarban has raised major concern over their impact on eco-geological system.

Objectives
We have isolated and identified a triclosan-degrading bacterium from wastewater treatment plant (WWTP) named as Sphingomonas sp. strain YL-JM2C.

Methods
Response surface methodology was used to understand the suitable conditions for the degradation of triclosan in strain-YL JM2C. The bacterium can efficiently degrade triclosan (5 mg L⁻¹) with biomass between 0.1-0.3 g L⁻¹ at 30-35 °C with pH 7-8. By GC-MS analysis, the intermediates of triclosan biodegradation were identified as 2,4-dichlorophenol, 2-chlorohydroquinone and catechol. Tracking experiment using ¹³C labeled triclosan confirmed that the ¹³C labeled triclosan was completely mineralized into carbon dioxide by strain YL-JM2C, and the phospholipid fatty acids (PLFAs) extracted from bacterial cells showed that part of labeled carbon from ¹³C labeled triclosan was incorporated into the strain’s fatty acids.

Conclusions
These results indicated that the bacterium was able to utilize triclosan as a carbon source. We also observed that the bacterium was also able to degrade 30 to 35% of triclocarban (4 mg L⁻¹). In Sphingomonas sp. strain YL-JM2C, triclocarban was initially hydrolyzed into 3,4-dichloroaniline and 4-chloroaniline. 3,4-Dichloroaniline was further transformed to 4-chlorocatechol via 4-chloroaniline. As per our knowledge, this is the first report of bacterial degradation of triclocarban by a pure isolated bacterial strain.
COMPARATIVE AND TRANSCRIPTIONAL ANALYSIS OF THE PREDICTED SECRETOME IN THE LIGNOCELLULOSE DEGRADING BASIDIOMYCETE PLEUROTUS OSTREATUS

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Background

Fungi interact with their environment by means of secreted proteins to obtain nutrients, elicit responses and modify their surroundings. Consequently, lifestyle influences the set of fungal secreted proteins.

Objectives

To perform a combined bioinformatics-transcriptomics study of the secretome of the basidiomycete Pleurotus ostreatus and to explore the conservation of these secreted proteins across the Basidiomycota phylum

Methods

We identified bioinformatically the set of secretable proteins in two monokaryotic strains (haplotypes) of the white-rot basidiomycete P. ostreatus (PC9 and PC15) using the web pipeline SECRETOOL [1]. Then, we performed two RNA-seq analyses to study the relationship between the functional profile of the predicted secretome and the expression level of each group. Finally, we used the set of proteins secreted by P. ostreatus as a query to search for similar proteins in the fungal genomes released in JGI Mycosom[2]

Conclusions
538 and 554 protein models were predicted to be secreted (4.41% and 4.77% of PC9 and PC15 gene models, respectively). The functional annotation of these proteins revealed the unknown (37.2%), glycosyl hydrolases (26.5%) and red-ox enzymes (11.54%) as the main functional groups, in a similar distribution for the two strains. The expression level of these groups further enhances the relevance of the unknown group and was significantly different in the two strains (revealing different responses to the same environment). Furthermore, the presence of similar proteins to *P. ostreatus* secreted proteins in other basidiomycetes was used to cluster them into
groups coherent with their particular lifestyles rather than with their corresponding phylogenetic positions.
NEW INSIGHTS INTO THE T6SS OF PSEUDOMONAS AERUGINOSA

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Background

Pseudomonas aeruginosa is a gram-negative opportunistic pathogen capable of living in a wide variety of environmental niches and is the third most common cause of all hospital-acquired infections. P. aeruginosa utilises a range of secretion systems and virulence factors to enable it to prosper. The Type VI Secretion System (T6SS) is one such factor. This nanomachine can modulate invasion of epithelial cells and is widely known for its ability to facilitate the delivery of effector proteins into both Eukaryotic cells and Bacterial competitors.

Objectives

The majority of P. aeruginosa strains have three complete T6SS clusters [H1-, H2-, and H3-T6SS] however additional ‘orphan’ genes or short operons are present in the P. aeruginosa genomes, which have the characteristics of T6SS components. In this study we have employed a range of molecular microbial techniques to further investigate components of the T6SS and related systems.

Methods

Through the construction of lacZ fusions, transposon mutagenesis, specific gene deletions, complementation and labelling with V5 tags we have identified new secreted products and mechanistic insights that contribute to the function of the P. aeruginosa T6SS. We also characterized global regulatory networks that are instrumental to the expression of genes encoding either core components of the T6SS machine or cognate T6SS effectors.

Conclusions

Overall secretion systems are of vital importance for Bacteria to interact with their environment and cause infection. A detailed knowledge of the mechanisms of secretion combined with an understanding of the role of the secreted factors should provide the basis for the development of new therapies to combat bacterial infections.
EXPRESSION OF THE MENINGOCOCCAL AUTOTRANSPORTER AUTB AND ITS IMPACT ON BIOFILM FORMATION

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Background
Autotransporters are secreted proteins of Gram-negative bacteria often involved in virulence. They consist of a translocator domain that inserts as a β-barrel in the outer membrane and a fused passenger domain that is translocated across the outer membrane. Genomes of the human pathogen Neisseria meningitidis contain eight autotransporter genes. Seven have been characterized to a certain extent, but the autotransporter AutB remains poorly studied. Previous studies suggested that the autB gene is a pseudogene.

Objectives
Our objective was to analyze whether AutB can be expressed and to elucidate its possible function.

Methods
In this study we used a combination of genomic and proteomic assays to determine the expression of the autotransporter. Included were also diverse functional assays to analyze its function.

Conclusions
Bioinformatics analysis of available genome sequences indicated that AutB expression is prone to phase variation, and can be inhibited by the presence of premature stop codons or other genetic disruptions. However, several genome sequences contain an intact autB gene. Western blotting and RT-PCR assays demonstrated the expression of AutB in strains with an intact gene. Proteinase K-accessibility assays evidenced that AutB is secreted but its passenger remains attached to the cell surface. Functional assays revealed the involvement of AutB in biofilm formation in a strain-dependent mode. We conclude that autB is a pseudogene only in some strains and that AutB may have a relevant function in the formation of bacterial communities.
THE OLOGOMERIZATION STATE OF THE TYPE III EFFECTOR/CHAPERONE COMPLEX CAN PROMOTE ITS RECOGNITION BY THE TYPE III SECRETION SYSTEM IN SALMONELLA.

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Background

Many bacterial pathogens use Type Three Secretion System (T3SS) to inject virulence factors, named effectors, directly into the cytoplasm of target eukaryotic cells. Before being translocated, effectors are maintained in a secretion-competent state by interacting with specific chaperones. The molecular mechanism of the recognition process between the effector/chaperone complex and the T3SS is still unclear but a highly conserved hexameric-ring-shaped ATPase localized at the basis of the needle complex has a critical role in the specific recognition process of chaperone-effector complex.

Objectives

As previous works showed that chaperones and effectors share structural motifs that could be at the origin of the recognition by TTSS, we investigated the structure of the complex between SopB, and its cognate chaperone SigE that is the form recognized by TTSS.

Methods

In this work we performed a biochemical and structural characterization of Salmonella SopB/SigE chaperone/effector complex by SAXS.

Conclusions

Our results showed that the SopB/SigE complex is assembled in dynamic homo-hexameric-ring-shaped structures with an internal tunnel. In this ring, the chaperone maintains a disordered N-terminal end of SopB molecules, in good position to be reached and processed by the T3SS. This ring dimensionally fits the ring-organized molecules of the injectisome including ATPase hexameric ring; this organization suggests that this structural feature is important for the ATPase recognition by T3SS. As effectors share neither sequence nor structural identity, the quaternary oligomeric structure could constitute a strategy evolved to promote the specificity and efficiency of T3SS recognition.
Background
VirB4-like proteins are associated with all bacterial Type 4 Secretion Systems (T4SS) described to date. These signature ATPases function in assembly of the T4SS channel and biogenesis of extracellular pili. They are also required for translocation of secretion substrates and nucleoprotein uptake during pilus-mediated phage infection.

Objectives
Very little is known about the regulation of VirB4-like ATPases in protein trafficking. Our paradigm to study control of VirB4-like ATPases is TraC of the F-like conjugative plasmid R1. Here we test the hypotheses that ATPase activity is controlled by (i) oligomeric state and (ii) intra-molecularly, by auto-inhibition.

Methods
Full length and truncated alleles of traC were modified with a strep-tag and evaluated functionally by complementation of conjugative transfer of a ΔtraC derivative of R1. Multimer formation, nucleotide binding and hydrolysis by purified TraC proteins were measured under various conditions in the presence and absence of protein interaction partners using NMR and biochemical techniques.

Conclusions
Full length TraC behaves as a monomer in solution with and without a 20-fold molar excess of non-hydrolysable ATP. This form binds but does not hydrolyze nucleotide. Preliminary data indicates that proteolytic processing of the protein stimulates both the formation of apparent hexamers and enzyme activity.

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MOLECULAR INVESTIGATION OF THE BASEPLATE ASSEMBLY OF THE TYPE VI SECRETION SYSTEM OF PSEUDOMONAS AERUGINOSA

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Background

The type VI secretion system (T6SS) involves ~13 core proteins and shares similarities with the tail of the bacteriophage T4. The current model of the structural organization of the T6SS suggests that its core components are assembled in several subcomplexes that are associated to form a dynamic phage-like machinery. A first set of T6SS components forms a trans-envelope complex which consists of the two inner membrane proteins TssM and TssL, and the outer membrane lipoprotein TssJ. A second group of T6SS proteins forms the bacteriophage tail-like structure. It includes the components Hcp and VgrG, and the sheath-like structure made of the two proteins TssB and TssC. Hcp proteins are hexameric rings which are able to form a rigid tube capped by the puncturing device made by VgrG proteins. Contraction of the TssB/TssC sheath propels the Hcp-VgrG structure, which ultimately results in the injection of toxins into target cells.

Objectives

The remaining T6SS core components are likely to form what is known as the baseplate structure in the phage. In the T6SS, it connects the tail-like structure to the membrane subcomplex. Functional and structural information about these T6SS proteins as well as their assembly within the machinery remain poorly described.

Methods

Here, we used biochemical and electron microscopy techniques to determine the structure and function of individual components of the T6SS in Pseudomonas aeruginosa. Additionally, using a systematic analysis of protein-protein interaction, we determined the interaction network between T6SS proteins.
Conclusions

Overall these data led to propose a comprehensive working model of the T6SS.
FEMS-1550
Secretion

TYPE VI SECRETION SYSTEM AS A VIRULENCE MECHANISM OF MAMMARY PATHOGENIC ESCHERICHIA COLI

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Background
Mastitis, infection and inflammation of the mammary gland, is a well-known problem in the dairy industry, affecting cows worldwide and causing considerable financial losses. Multiple bacterial species can cause mastitis and \textit{E. coli} is often involved. Despite many years of mastitis research, no efficient measures exist to prevent or treat the disease, and only little is known about specific virulence factors of the bacteria.

Objectives
Our goal is to understand the molecular mechanisms of host-pathogen interactions in the mammary gland and relate them to disease processes, in hope that understanding these mechanisms will lead to development of novel tools to combat \textit{E. coli} mastitis.

Methods
Using genome sequencing and analysis of six clinical isolates, we found that type VI secretion system (T6SS) gene clusters are present in all. Furthermore, using unbiased screening of strains for reduced colonization, fitness and virulence in our murine mastitis model, we have identified in P4-NR strain a new pathogenicity island encoding the core components of T6SS and its hallmark effectors Hcp, VgrG and Rhs. Next, we have shown that specific deletions of T6SS genes reduced \textit{in vivo} pathogenicity.

Conclusions
Based on our results we hypothesize that T6SS is an important virulence mechanism of MPEC. To our knowledge, we are the first to describe relevance of T6SS in the pathogenesis of mastitis. We intend to validate our findings in dairy cows and field strains and study the molecular mechanisms of T6SS associated with MPEC virulence. The identified mechanism may provide new targets for diagnostic, preventive and therapeutic intervention.
BACTERIAL KILLING VIA A TYPE IV SECRETION SYSTEM

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Background

Type IV secretion systems (T4SSs) are multiprotein complexes that transport effector proteins and protein-DNA complexes through bacterial membranes to the extracellular milieu or directly into the cytoplasm of other cells. They are essential for host colonization by many medically important microbes as well as for horizontal transfer of genetic material between bacteria and from bacteria to plants. Many bacteria of the family Xanthomonadaceae, that occupy diverse environmental niches, carry a T4SS with unknown function but with several characteristics that distinguishes it from other T4SSs.

Objectives

The aim of this study is to determine the function of the Xanthomonadaceae T4SS.

Methods

We have used structural biology, spectroscopy, enzymology and novel bacterial competition and secretion experiments to address this question.

Conclusions

The Xanthomonas citri (Xac) T4SS provides these cells the capacity to kill other gram-negative bacterial species in a contact-dependent manner. The secretion of one Type IV bacterial effector protein is shown to require a conserved C-terminal domain and its bacteriolytic activity is neutralized by a cognate immunity protein whose 3D structure is similar to peptidoglycan hydrolase inhibitors. This is the first demonstration of the involvement of a T4SS in bacterial killing and points to this special class of T4SS as a mediator of both antagonistic and cooperative interbacterial interactions and therefore an important driving force in the evolution of bacterial species.
STUDIES ON THE REGULATION OF TYPE VI SECRETION SYSTEMS IN ESCHERICHIA COLI

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Background

Type VI secretion systems (T6SS) are complex protein transport machineries and widespread among pathogenic Gram-negative bacteria. Accordingly, one could assume that they may contribute directly or indirectly to pathogenesis; for example, by targeting eukaryotic host cells or by competing with other bacteria in microbial populations.

Objectives

The genomes of the uropathogenic Escherichia coli (UPEC) strains often harbor one or more genomic islands encoding putative T6SSs. As the expression of T6SSs seems to be tightly regulated, most of the secretion systems are inactive under standard laboratory conditions. The screening for potential regulators directing gene expression of T6SSs is a first step to get a better understanding under which conditions these secretion systems may be active and contribute to the fitness and/or pathogenicity of E. coli.

Methods

To elucidate the transcriptional regulation of the T6SSs in UPEC strains, chromosomal and plasmid-based promoter-reporter gene fusions to core genes of both genomic islands have been constructed. The promoter activity has been tested in different E. coli K-12 deletion backgrounds as well as in various wild type E. coli strains.

Conclusions

The screening using the promoter-reporter gene fusion demonstrated that the availability of nucleoid-associated proteins and the growth phase, i.e. the stationary phase, affect transcription of genes coding for core elements of T6SSs.
FEMS-0660
Secretion

TOWARDS A BETTER UNDERSTANDING OF THE BACTERIAL TYPE II SECRETION PATHWAY
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Background
The bacterial type II secretion system (T2SS) is unique in its ability to promote the transport of large folded and sometimes multimeric proteins. In this secretion process, exoproteins are first translocated into the periplasm. The final release into the medium requires a multiprotein complex called the secreton.

Objectives
Although the 12 individual components of the secreton have been identified, its mode of action remains obscure.

Methods
We set up various dedicated in vitro and in vivo protein-protein interaction experiments to identify the Pseudomonas aeruginosa Xcp T2SS periplasmic interactome.

Conclusions
BIAcore experiments revealed that three Xcp components, XcpP, the secretin XcpQ, and the pseudopilus tip, directly and specifically interact with secreted exoproteins. Affinity chromatography co-purification indicated that the XcpY periplasmic domain interacts with the secreted substrate and a component of the pseudopilus tip XcpW. Interestingly, the periplasmic domain of another member of the Xcp inner membrane platform, XcpZ co-elutes with the XcpY/substrate and the XcpY/XcpW complexes during affinity chromatography. Finally the direct interaction between the secreted substrate and XcpY was confirmed by in situ photo-crosslinking. All together, our results allowed us to propose the most advanced integrative model of Xcp T2SS assembly and function.
BACTERIAL TYPE III SECRETION NEEDLE COMPLEX: A STOICHIOMETRIC ANALYSIS

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Background

Bacterial type III secretion systems are cell-envelope spanning effector protein-delivery machines employed by Gram negative pathogens and symbionts. The membrane-embedded core unit of these secretion systems is termed needle complex. It can be divided into a base that anchors the complex to the inner and outer membranes, a hollow filament composed of inner rod and needle subunits that serves as conduit for substrate proteins, and an export apparatus facilitating substrate translocation located at the center of the base.

Objectives

While the low resolution structural analysis of the base has revealed the stoichiometry of this large ring-forming complex composed of three different proteins, the stoichiometries of the five hydrophobic export apparatus components and of the inner rod are largely unknown.

Methods

We employed a mass-spectrometry based peptide-concatenated standard strategy to evaluate the stoichiometry of the entire needle complex.

Conclusions

Using this strategy, we could validate the hitherto suggested stoichiometries of the base components and major export apparatus protein. We further propose a stoichiometry of 5:1:1:1 for the minor export apparatus components SpaP, SpaQ, and SpaR and for the switch-protein SpaS of the type III secretion system located on pathogenicity island 1 of Salmonella Typhimurium. The herein presented data also suggest that the inner rod is merely a rod but rather a disc because of the low stoichiometry observed.

Even though the complexity and hydrophobic character of the evaluated components allowed only the obtainment of approximate stoichiometries in this study, the gained results provide the first complete picture and a valuable framework for further investigations.
CHARACTERIZATION OF A NEWLY DISCOVERED TOXIN-ANTITOXIN FAMILY PREDICTED TO HAVE AN ADP-RIBOSYLATING TOXIN

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Background

Toxin-antitoxin (TA) systems are highly abundant in bacteria and are typically composed of adjacent linked genes that encode both stable toxin and its unstable ‘antidote’. TA systems were shown to have a diverse set of physiological roles that range from phage defense to bacterial persistence. We recently reported the discovery of six new types of TA systems (Sberro et al. Discovery of functional toxin/antitoxin systems in bacteria by shotgun cloning. Mol Cell. 2013 50(1):136-48).

Objectives

Here, we aimed in characterizing one of these newly discovered TA systems, Pmen TA. We propose that this toxin is involved in ADP-ribosylation, a post-transcriptional modification of crucial components within the bacterial cell. This mechanism is observed in a variety of bacterial exotoxins.

Methods

We use a combination of bioinformatics tools, molecular biology and biochemistry in order to attain initial characterization of this TA system after expressing it in E.coli.

Conclusions

The Pmen Toxin is predicted to have an NAD-binding domain that allows ADP-ribosylation activity. The anti-toxin is unstable and physically binds the toxin. It has an N-terminal domain, known to bind ADP-ribose, and its removal eliminates the rescue effect against the toxin. The conserved residues of the toxin resemble the core active site residues in other ADP-ribosylating toxins, such as Cholera toxin. Substitution of the most conserved active site residue leads to abolishment of toxicity. Possible common evolutionary origin between toxin-antitoxin systems and lethal exotoxins suggests that TA systems may be a genetic source for the emergence of pathogen-related toxins.
Background
Most prokaryotic genomes, including human pathogens, code for toxin-antitoxin (TA) systems, usually consisting of a toxin and its cognate antitoxin, which counteracts toxin activity. TA systems have been associated with a variety of biological functions, including general stress response, biofilm formation and pathogenicity. *Acinetobacter baumannii* is multidrug-resistant pathogen causing serious hospital-acquired infections worldwide. The internally encoded TA systems can be a useful tool in understanding and preventing the spread of multi-drug resistant *A. baumannii*.

Objectives
Recently, at least five functional *A. baumannii* TA systems (RelBE, HicAB, HigBA, SplTA, CheTA) were discovered. Their toxins were shown to inhibit translation in *Escherichia coli* cells. In order to characterize the *A. baumannii* toxins in more detail, we have investigated their cellular localization and ribosome-association.

Methods
Series of inducible *E. coli* expression vectors encoding *A. baumannii* TA components, fused with fluorescent proteins have been constructed. The functionality of fusion proteins in *E. coli* was confirmed using the kill-rescue assay. The ability of toxins to interact with ribosome was tested using ribosome fractionation, cellular localization was accessed by fluorescence microscopy.

Conclusions
Microscopy of *E. coli* where the fusion toxins, alone or with their cognate antitoxins, were expressed, has shown either polar or whole cell localization pattern. The analysis of fluorescence profiles of *E. coli* ribosomal fractions has demonstrated only RelE associated with ribosome, while association with the ribosome is unlikely for HicA, HigB and SplT toxins of *A. baumannii*. The latter toxins might cleave RNA independently from ribosome, or inhibit translation by other mechanisms.
Background
Microcystins, produced primarily by cyanobacterium *Microcystis aeruginosa*, are the most commonly studied cyanotoxins (1). Most studies have focused only on the toxic effects of microcystins on humans and animals while their physiological or ecological roles have not been elucidated. Therefore, there is a considerable interest in understanding the importance of cyanotoxins in natural environments (2).

Objectives
The aim of the present study was to clarify the ecological significance of microcystins in algal succession.

Methods
*Desmodesmus subspicatus* was cultured in a medium together with dialysis tubing containing *M. aeruginosa* 7806. Cultures were incubated under 30 μEm\(^{-2}\)s\(^{-1}\) continuous irradiation at 23°C for 4 weeks. Microcystin-LR was quantified using mass spectrophotometer and algal density was obtained by measuring the OD\(_{750}\).

Conclusions
Microcystin-LR was determined in the media after two weeks but there was no change in *D. subspicatus* growth rate. After 4 weeks incubation, *D. subspicatus* growth decreased. Using co-cultivation method gave the opportunity to investigate the algal interactions at continuous toxin production. By continuing the growth of *M. aeruginosa*, toxin concentration increased and caused a decrease in *D. subspicatus* growth. Results provided evidences to suppose the role of toxin as an allelochemical in algal interactions.

References:


Background

Campylobacter fetus cause human infection and are important pathogens. Recent comparative genomics of C. fetus subspecies revealed Fic loci encoding Fido superfamily proteins, which may contribute to niche adaptation and pathogenicity.

Objectives

We analysed function of 4 Fic loci organizes as toxin-antitoxin (TA) modules on the chromosome and ICE of C. fetus subsp. Venerealis 84-112.

Methods

The C. fetus proteins and mutant variants were expressed ectopically in E. coli, yeast and in transiently transfected HeLa cells. Prevalence of fic genes in 102 C. fetus isolates was surveyed with PCR.

Conclusions

We show that Fic proteins are cytotoxic to human cells but not S. cerevisiae. The fic loci are organized as TA modules on the chromosome and ICE of C. fetus subsp. venerealis 84-112. Expression in E. coli validated the cytotoxic and neutralizing activities of the proteins, providing the first functional evidence for TA systems in Campylobacter. Reversal of fic-mediated filamentation and growth inhibition in E. coli also revealed antitoxin crossreactivity between loci. Key active site residues involved in adenylylation by Fic proteins are conserved in Fic1, Fic3 and Fic4, but degenerated in Fic2. We show the non-canonical Fic2 disrupts assembly of E. coli ribosomes. fic genes are prevalent in C. fetus subsp. venerealis but not generally conserved among Campylobacters. Strikingly, homologous genes are found in some Campylobacters and unrelated pathogens adapted to the human and animal
urogenital tract. *C. fetus* *fic* genes thus appear to be important to adaptation and virulence in this niche.
TOXIN-ANTITOXIN SYSTEMS ON ANTIBIOTIC RESISTANCE PLASMIDS: JUST PLASMID MAINTENANCE?

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Background

Toxin-Antitoxin (TA) systems are small genetic elements composed of a toxin gene and its cognate antitoxin. They were originally discovered as plasmid-borne loci that promote plasmid maintenance by killing daughter cells that have lost their TA encoding plasmid, a phenomenon known as post-segregational killing. Nowadays it is known that a great diversity of plasmids, including the antibiotic resistance (AbR) plasmids, encodes one or even more TA systems. The widespread dissemination of antibiotic resistance genes is ascribed to the spread of bacterial plasmids encoding these genes and this successful dissemination it is attributed in part to TA systems and its participation in plasmid maintenance. However, there are some lights indicating that plasmidial TA systems could participate in functions other than plasmid maintenance.

Objectives

Our main aim was to elucidate the diversity and function of TA systems encoded on AbR plasmids. Specifically to characterize a new putative TA system encoded on an IncX4 plasmid.

Methods

By bioinformatic tools a novel putative TA system was identified in pJIE143, an IncX4 plasmid carrying blaCTX-M-15 from an Escherichia coli ST131 isolate. We analyzed the effect of the expression of this system on E. coli growth and its participation on plasmid maintenance.

Conclusions

pJIE143 encodes a novel TA system that appears to be related exclusively to IncX plasmids. The characteristics and putative functions of this novel TA system will be discussed in this work. These results reveal that AbR plasmids could contain an underappreciated diversity of TA systems, which could have functions further of plasmid maintenance.
Background
The German cockroach, Blattella germanica (L.) has been recognized as a serious health problem throughout the world. Control failures due to insecticide resistance and chemical contamination of environment have led some researchers focus on the other alternative strategy controls. Microbial insecticides such as those containing entomopathogenic fungi could be of high significance. Lecanicillium muscarium and Beauveria bassiana grow naturally in soils throughout the world and act as a parasite on various arthropod species, causing white muscardine disease. Thus, these two species could be considered as entomopathogenic fungi.

Objectives
The current study conducted to evaluate the toxicity of Beauveria bassiana and Lecanicillium muscarium against German cockroach, Blattella germanica.

Methods
Conidial formulations of L. muscarium (PTCC 5184) and B. bassiana (PTCC5197) were prepared in aqueous suspensions with Tween 20. Bioassays were performed using two methods including submersion of cockroaches in conidial suspension and baiting. Data were analyzed by Probit program and LC50 and LC90 were estimated.

Conclusions
The obtained results indicated that both fungi species were toxic against German cockroach however; Beauveria bassiana was significantly 4.8 fold more toxic than L. muscarium against German cockroach using submersion method.
REGULATION OF TOXIN-ANTITOxin SYSTEMS WITHIN THE SOS REGULON IN E. COLI

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Background

TA systems (TAs) encode a stable toxic protein and its cognate unstable antitoxin that is either an antisense RNA (type I) or a protein (type II). Under steady-state conditions, both components are produced and cells are growing normally. Under conditions in which the antitoxin:toxin ratio is perturbed in favor of toxin, cell growth can be impaired. Therefore, maintaining an appropriate ratio is essential for viability. While negative autoregulation is observed for type II systems, few information is available for type I. Some of these TAs (symER, tisAB-istR and dinQ-agr) are part of the SOS regulon, as well as two type II TAs (dinJ-yafQ and yafNO).

Objectives

Our objective is to investigate the transcriptional regulation of these TAs at the population and single cell levels under steady-state growth and SOS conditions.

Methods

Approaches based on fluorescent reporters fused to TA promoters combined with flow cytometry and microscopy analysis are currently being developed.

Conclusions

Our data confirmed that the dinJ-yafQ and symE promoters are activated during SOS response, although at different timing and levels. Interestingly, the dinJ-yafQ promoter is active both in wild-type and dinJ-yafQ mutant strains, indicating that SOS regulation prevails on autoregulation. Furthermore, heterogeneity in promoter activity was observed at the single cell level, suggesting that phenotypic heterogeneity might play a role in the SOS response. This will be further investigated.
EVOLUTIONARY RELATIONSHIPS BETWEEN TYPE II TOXIN-ANTITOXIN SYSTEMS AND POLYMORPHIC TOXIN SYSTEMS

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Background

Type II toxin-antitoxin systems (TAs) are small bicistronic modules encoding a toxic protein and its cognate antitoxin protein. TAs are widespread in bacterial and archeal genomes.

Objectives

Our hypothesis is that TAs might constitute a reservoir of toxic domains that are recruited by polymorphic toxin systems.

Methods

Polymorphic toxin systems, such as CDI and Rhs, are composed of large conserved proteins containing variable carboxy-terminal (CdiA-CT or RhsA-CT) domains showing toxic activity and small immunity proteins. Genetic organization of these systems is analogous to that of TAs: a toxic gene or domain followed by an antitoxin or immunity gene. A bioinformatics approach is currently developed to evaluate whether CdiA-CT and RhsA-CT toxic domains are found in TAs. Domains are collected and HMM profiles are built. Bacterial genomes are scrutinized for the presence of these domains under TA organization. Functionality of these systems is tested in *E. coli*. Toxin activity and targets are then investigated.

Conclusions

We identified and experimentally validated novel toxic domains that are found in TAs organization and in CDI and/or Rhs systems. Upon overexpression, toxins from TAs and CdiA-CT/Rhs-CT domains are toxic and antagonized by the expression of cognate antitoxin and/or immunity proteins. These domains were shown to inhibit translation. These data indicate that toxic domains are indeed shared by TAs and polymorphic toxin systems. Recruitment of toxic domains encoded by TAs by more
sophisticated systems might constitute a strong selective pressure on TAs and might explain why these small modules are evolutionary successful.
Background
Type II toxin-antitoxin (TA) systems are small genetic loci coding for a toxic protein and its antidote. Such systems are widespread in bacterial and archaeal genomes. These systems stabilize mobile genetic elements and may act as stress response elements. Toxins of type II TA systems are most commonly mRNAses, with the exception of gyrase inhibitors and several families that have more elaborate enzymatic functions. We have found novel class of toxins that possess acetyltransferase domain and constitute typical type II TA systems.

Objectives
To functionally describe TA modules of this novel family and explore mechanism of toxicity of acetyltransferase domain toxin.

Methods
TA systems from Escherichia coli and Vibrio cholerae have been functionally validated by killing/rescue assay. Activity of toxin has been tested by measuring rates of replication, transcription and translation, mRNAses activity by Northern blots analysis. Mutational analysis on acetyltransferase active site has been performed. TA complex has been purified and separated by affinity chromatography. Bioinformatics analysis has been performed to describe the prevalence and diversity of this novel family.

Conclusions
Small acetyltransferase domain proteins found in pair with RHH-domain antitoxins constitute functional TA pairs. When expressed together they form a heteromultimeric complex of 2 toxins and 2 antitoxins. When expressed alone toxins possessing acetyltransferase domain inhibit translation without degrading mRNAs and therefore exhibit novel toxicity mechanism. Mutations in the acetyltransferase active site abolish toxicity, strongly indicating that toxicity relies on target(s) acetylation. Detailed analysis of translation inhibition in vitro is currently under progress and should reveal the target(s) of the toxin.
Background

Anthrax toxin, composed of protective antigen (PA) and lethal (LF) and edema (EF) factors, is a model system for transmembrane protein translocation. The PA component self-assembles into a proton motive force (PMF)-driven channel, which translocates LF and EF into target cells. The channel contains multiple nonspecific polypeptide-clamp sites, which catalyze unfolding and translocation. It is unknown how clamps facilitate transport while avoiding tightly bound kinetic traps.

Objectives

As the α clamp, specifically recognizes α helix, we asked how the conformational dynamics of the translocating chain might avoid kinetic traps during translocation.

Methods

~50-residue peptides (based upon the sequence of LF) were synthesized with identical sequences but variable stereochemistries to control for side-chain chemistry while manipulating helical sense and backbone flexibility. Single-channel and ensemble planar lipid bilayer electrophysiology monitored peptide binding, dissociation, and translocation.

Conclusions

Cooperative binding and allosteric modulation of translocation were only observed with an isotactic peptide synthesized only with l-enantiomers (αL). Cooperativity depended on acidic pH and the channel's α-clamp and ϕ-clamp sites. Incorporation of d-enantiomers disrupted cooperativity, allosteric control of translocation, and slowed the rate of translocation. The translocation of αL occurs via a subsecond series of consecutive irreversible transitions and is unlike the translocation of a syndiotactic
peptide, synthesized with alternating L- and D-enantiomers (αld), which translocates 100-times slower (as slow as 776-residue LF). Therefore, to avoid kinetic traps, which may otherwise impede translocation, the channel's clamps operate allosterically through efficient, repeated, stereospecific contact along the length of the translocating peptide chain.
Background

The symbiotic interaction between the bacterium *Sinorhizobium meliloti* and the legume *Medicago sp* results in the development of new root organs, the nodules, where the differentiated bacteria (the bacteroids) reduce atmospheric $N_2$ to ammonia. A few weeks after symbiosis establishment, nodules present a premature senescence.

Objectives

Since VapBC-type Toxin-Antitoxin (TA) systems of pathogenic bacteria is involved in the survival of the bacteria, we examined the role of *S. meliloti* VapBC modules in bacteroid viability and nodule senescence. The *vapBC* genes form an operon negatively regulated by the TA complex. Free toxin (VapC) has an RNase activity that is neutralized by the antitoxin (VapB).

Methods

We showed that, during the symbiotic interaction, a mutation in the *vapC5* toxin of the VapBC5 module, leads to a higher nitrogen-fixing activity, plant yield increase and a delayed nodule senescent phenotype. Thus, inactivation of this toxin improves symbiotic efficiency (Lipuma et al., 2014). We obtained an opposite phenotype with another mutant of toxin deletion (*vapC7*): aberrant nodules, where nitrogen fixation is nearly abolished. Nodule ultrastructure analysis and flow cytometry demonstrated that bacteroid differentiation was followed by a rapid bacterial death leading to early senescence phenotype. These results demonstrate that this Toxin Antitoxin module is essential for bacteroid viability (in preparation).

Conclusions

We demonstrated the role of two Toxin Antitoxin systems in *S. meliloti* survival during *Medicago sp.* interaction. The two mutants analysed show different phenotypes. To a better understand the role of all VapBC systems; phylogenetic analyses were done to define the evolution origin of these genes.
VAPC FROM THE LEPTOSPIRAL VAPBC TOXIN-ANTITOXIN MODULE DISPLAYS SPECIFIC RIBONUCLEASE ACTIVITY ON THE INITIATOR TRNA. VAPC HIGH PRESSURE REFOLDING AND IN SILICO STRUCTURAL 3D STUDIES.

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Background

The most accepted hypothesis of the physiological function of the ubiquitous prokaryotic Toxin-Antitoxin (TA) operons is the reversible cessation of cellular growth under stress conditions. vapBC operons, present in Leptospira interrogans, are classified based on the presence of a ribonucleasic PIN domain in the VapC toxin. Their mechanisms of action remain mostly unknown. The expression of the leptospiral VapC in E. coli promotes a strong bacterial growth arrestment, making it difficult to obtain the recombinant protein.

Objectives

Produce soluble and active VapC in order to find its target and characterize its activity. Study and rank alternative 3D models of VapC.

Methods

vapBC locus, vapB and vapC of L. interrogans were cloned and expressed in E. coli. Proteins were purified by IMAC after refolding by pressurization (VapC). VapB and VapC interaction was tested by pull-down assay and affinity blotting. Ribonuclease activity was tested towards E. coli rRNA and tRNAfMet. Models of leptospiral VapC were generated and ranked using SWISS-MODEL.

Conclusions

VapC was refolded by high hydrostatic pressure, providing a new method to obtain the active toxin. VapB neutralizes the activity of VapC in vivo and in vitro. The 3D model of the leptospiral VapC closely matches the Shigella’s VapC X-ray structure. In agreement, leptospiral VapC shows no activity towards E. coli rRNA and was found to cleave tRNAfMet. This finding suggests that the cleavage of the initiator tRNA may
represent a common mechanism to a larger group of bacteria and potentially configures a mechanism of post-transcriptional regulation leading to the inhibition of global translation.
THE IDENTIFICATION METHOD FOR THE SEQUENCE PATTERN AT THE CLEAVAGE SITES OF MAZF FAMILY ENDOBONUCLEASES USING MASSIVE PARALLEL SEQUENCING

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Background
MazF family toxins, representative endoribonucleases that cleave single-stranded RNA (ssRNA) in a sequence-specific fashion, are distributed among bacterial and archaeal lineages. Since their sequence pattern of cleavage sites is highly diverse, they are of importance not only in terms of prokaryotic physiologies, but biotechnological applications.

Objectives
To elucidate physiological roles of MazF family toxins and/or to expand the biotechnological utilization of these endoribonucleases, we established a novel easy-to-use method to effectively determine the pattern of the sequences at their cleavage sites.

Methods
The sequences cleaved by MazF family toxins were determined by a modified RNA-Seq protocol. To evaluate our method, MazF, an ACA specific endoribonuclease derived from Escherichia coli, was selected as a model target. Besides, the sequence digested by an uncharacterized MazF homologue from Proteobacteria was also identified using this method. The sequences recognized by these endoribonucleases were further analyzed by using Fluorescence Resonance Energy Transfer (FRET) based assays.

Conclusions
The method developed in this study correctly inferred the sequence pattern at the cleavage sites of the model target, E. coli MazF, as ACA. Furthermore, the possible sequence pattern recognized by MazF homologue from Proteobacteria was consistent with the sequence confirmed by FRET based method, indicative that the present method is a useful and straightforward means to explore the potential sequence recognition pattern of these endoribonuclease. This method may be applicable to various toxins that catalyze ssRNA cleavage in a sequence-specific manner, facilitating subsequent physiological investigations and practical uses of these enzymes in the genetic engineering.
IN VIVO CHARACTERIZATION OF A BACTERIAL KILLER TYPE IV SECRETION SYSTEM EFFECTOR/IMMUNITY PROTEIN PAIR
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Background

Type IV secretion Systems (T4SS) are bacterial nanomachines that transfer proteins across the cell envelope and into target cells. We recently provided the first demonstration that the Xanthomonas citri (Xac) T4SS mediates the contact-dependent killing of other bacterial cells [1]. One Xanthomonas T4SS effector (X-Tfe) X-Tfe\(^{\text{XAC2609}}\) has \textit{in vitro} lysozyme-like lytic activity. This enzymatic activity is inhibited by the soluble domain of the putative Xanthomonas T4SS immunity protein (X-Tfi) X-Tfi\(^{\text{XAC2610}}\).


Objectives

Here, we set out to investigate the \textit{in vivo} function of X-Tfi\(^{\text{XAC2610}}\). Since X-Tfe\(^{\text{XAC2609}}\) is predicted to act in the bacterial periplasm and X-Tfi\(^{\text{XAC2610}}\) has a lipobox which directs it to the bacterial outer membrane, the hypothesis is that X-Tfi\(^{\text{XAC2610}}\) acts as an immunity factor against the possibly detrimental autolytic effects of X-Tfe\(^{\text{XAC2609}}\).

Methods

Xac mutants strains carrying single gene deletions (ΔX-Tfi\(^{\text{XAC2610}}\), ΔX-Tfi\(^{\text{XAC2609}}\) and ΔT4SS) and double mutants (ΔX-Tfi\(^{\text{XAC2610}}\)-ΔT4SS and ΔX-Tfi\(^{\text{XAC2610}}\)-ΔX-Tfi\(^{\text{XAC2609}}\)) were created. Colony phenotypes and cellular viabilities were compared for the above mutant and wild type Xac strains.

Conclusions

1) X-Tfi\(^{\text{XAC2610}}\) provides immunity against the \textit{in vivo} auto-lytic activity of X-Tfe\(^{\text{XAC2609}}\).

2) The \textit{in vivo} autolytic activity of X-Tfe\(^{\text{XAC2609}}\) does not depend on a functional T4SS.
3) X-Tfl$^{XAC_{2610}}$ inhibitory activity is also independent of the Xac T4SS.
PLASMID-BORNE MAZEF TOXIN-ANTITOXIN LOCI ARE WIDESPREAD IN ANTIBIOTIC-RESISTANT STAPHYLOCOCCUS AUREUS, AND TOxin ACTIVATION PROVIDES SELECTIVE KILLING

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Background
Antibiotic-resistant bacteria have become a global concern and new strategies to control pathogenic bacteria are urgently needed. In theory, activation of the toxin or inhibition of the antitoxin within a bacterial toxin-antitoxin (TA) system could provide a potent new antibiotic therapy.

Objectives
We aimed to identify the location of TA systems, to evaluate the functionality of TA systems in clinical isolates of antibiotic-resistant S. aureus, to determine the functionality of TA system and to evaluate the antitoxin as an attractive antimicrobial target for the eradication of antibiotic-resistant S. aureus.

Methods
To evaluate potential TA loci as therapeutic targets, we screened the plasmid and chromosome sequences of 1000 clinical isolates of S. aureus for the presence of TA loci.
Plasmid transformation, ATPase assay, Turbidity and PNA assay were performed.

Conclusions
Plasmid-borne mazEF TA loci were present in all tested, antibiotic-resistant S. aureus strains in Iran and Malaysia. A subset of strains expressed the mazE and mazF transcripts, and ATPase and growth assays revealed that the mazEF TA loci were functional. In addition, the plasmid-borne copies were stable in the absence of antibiotic selection. To activate toxin expression, we targeted the mazE antitoxin mRNA using peptide nucleic acid (PNA) oligomers. The anti-mazE oligomers were bactericidal against drug-resistant S. aureus containing mazEF and did not inhibit strains lacking mazEF. Therefore, mazEF TA loci are widespread in drug-resistant strains of S. aureus and are plasmid-borne, and activation of toxin activity by silencing of the antitoxin gene provides a means to selectively kill drug-resistant strains.
CHARACTERIZATION OF TOXIN-ANTITOXIN SYSTEMS IN THE HUMAN PATHOGEN STREPTOCOCCUS PYOGENES

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Background

The Gram-positive bacterium *Streptococcus pyogenes*, also known as group A *Streptococcus* (GAS), is an important human pathogen responsible for a wide spectrum of diseases such as pharyngitis, necrotizing fasciitis, and toxic shock syndrome. In addition to encoding multiple factors for adaptation and pathogenicity, GAS contains several predicted type II toxin-antitoxin (TA) loci. TA systems are widespread in bacterial pathogens and enable bacteria to adapt to rapidly changing environmental conditions, thereby contributing to the pathogenicity of the organisms.

Objectives

To understand the role in physiology and virulence of these putative TAs in *S. pyogenes*, we aim to characterize hypothetical toxin-antitoxins (TAs) and unravel the molecular mechanisms involved in their regulation and mode of action.

Methods

Operon architecture and expression of the predicted loci was analyzed by RT-PCR, RNA sequencing (RNA-seq), and reporter-fusions. Toxin and antitoxin activity and function was assessed by growth arrest assays, activity assays, Western blotting and microscopy.

Conclusions

A series of transcriptional fusions was constructed that enabled the confirmation of TAs annotation. Three out of four predicted TAs cause growth arrest upon overexpression. We show that two of the predicted toxins cause a reduction in CFUs but not in optical density, which can be explained by a cell division defect. Expression of the cognate antitoxins was able to relieve the growth arrests. Understanding the roles of TAs will increase our knowledge on the regulation of bacterial pathogenicity, and may reveal key targets for potential novel antibacterial strategies.
EXPOSURE TO TICKS AND SEROPREVALENCE OF BORRELIA BURGDORFERI LIVING IN THE PROVINCE OF ERZINCAN, TURKEY

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Background
Lyme borreliosis is a zoonosis caused by Borrelia burgdorferi transmitted by ticks, especially Ixodes species.

Objectives
The aim of this study was to determine the Borrelia Burgdorferi seroprevalence caused by ticks in the province of Erzincan, which has a high tick population due to its geographical position and climate conditions.

Methods
368 people, who were live in rural area in the province of Erzincan located in the east of Turkey were included in the study. In all the acquired serum samples, the B.burgdorferi IgG antibodies were screened by using the ELISA method (SERION ELISA classic Borrelia burgdorferi IgG). The positive and doubtful results obtained were confirmed using the Western blot method (The VIRO-BLOT Anti-Borrelia IgG test).

Conclusions
B. burgdorferi IgG antibodies were determined as positive (4.1%) in 15 of all cases and suspicious in 36 cases by using the ELISA method. B. burgdorferi IgG levels were examined by using the Western Blot method in totally 51 serum samples that were determined to be positive and doubtful according to the result of the SERION ELISA classic Borrelia burgdorferi IgG test. Evaluating all the results together; B. burgdorferi IgG positivity was found at the rate of 2.17 % in 8 of 368 cases. The story of tick contact was determined in 3 of cases that were determined to have positive B. burgdorferi IgG. This study proved the presence of Borrelia Burgdorferi in the province of Erzincan. It is thought to offer the preventive health services for those in the risk group in order to minimize the tick exposure.
Background

Crimean-Congo Haemorrhagic Fever (CCHF) is a zoonotic disease caused by a tick-borne CCHF of the genus Nairovirus of the family Bunyaviridae.

Objectives

The aim of this study was to determine seroprevalence of the CCHF in animal breeders in the province of Erzincan, for which there has been no seroprevalence study despite being accepted as an endemic region in terms of the CCHF by the Ministry of Health.

Methods

372 people, who were live in the province of Erzincan located in the east of Turkey were included in the study. In all the obtained 372 serum samples, the CCHF IgG antibodies were screened by using the ELISA method. The samples that were found to be CCHF IgG positive were examined with CCHF IgM. CCHF IgM-IgG ELISA kits were used in order to determine the antibody levels. IgM positive samples were processed for detection of viral RNA through RT-PCR.

Conclusions

In 13.9% (52/372) of all samples included in the study, the CCHF IgG was determined as positive. This ratio was found respectively as follows; 16.7% (29/174) in people who had a tick contact/bite, 12.4% (18/145) in people who animal breeders and had no tick contact and 9.4% (5/53) in people who were not animal breeders in the city center and had no tick contact. Seven samples were found positive for IgM whereas viral RNA was detected in 1 samples. In the province of Erzincan where many CCHF-related death cases are reported every year, the CCHF IgG antibody level was found to be high as expected.
Background
Cutaneous Leishmaniasis (CL) is a common endemic parasitological disease in Iran. This disease is always serious health problems in Esfahan provience specially Badrood city and its prevalence has been doubled over the last decade.

Objectives
This study was designed to determine the epidemiology and clinical characteristics of CL in Badrood in 2013.

Methods
This descriptive cross sectional study was on all detected patients with cutaneous leishmaniasis in the city Badrood by active detection within one year in 2014. Patients were visited by physician and a questionnaire including information about demographic and characteristics of wounds were filled through interview and examination. Diagnosis was confirmed by revealing of leishman body in smear of wounds.

Conclusions
We found 63 definitive case of CL. 55.6% of patients were male. Age group 15-30 years had the highest rate (23.8%) among the patients. 66.7% of patients had history of stay in Agha Ali Abbas region. All wounds were rural form. Most patients with cutaneous leishmaniasis in Badrood had a wound with diameter less than 3 cm, secretion, painless, itch and extremities was the most common site for infection. Based on the findings of this study, cutaneous leishmaniasis in Badrood city is rural form and most patient are men and older than 15 year. there was complete or partial healing after treatment in the most patients.
Background
Cutaneous Leishmaniasis (CL) is a common endemic parasitological disease in Iran. This disease is always a serious health problem in Isfahan province, especially in the city of Badrood, and its prevalence has doubled over the last decade.

Objectives
This study was designed to determine the epidemiology and clinical characteristics of CL in Badrood in 2013.

Methods
This descriptive cross-sectional study was on all detected patients with cutaneous leishmaniasis in the city of Badrood by active detection within one year in 2014. Patients were visited by a physician and a questionnaire including information about demographic and characteristics of wounds were filled through interview and examination. Diagnosis was confirmed by revealing of leishman body in smear of wounds.

Conclusions
We found 63 definitive cases of CL. 55.6% of patients were male. Age group 15-30 years had the highest rate (23.8%) among the patients. 66.7% of patients had a history of stay in Agha Ali Abbas region. All wounds were rural form. Most patients with cutaneous leishmaniasis in Badrood had a wound with diameter less than 3 cm, secretion, painless, itch, and extremities was the most common site for infection.

Based on the findings of this study, cutaneous leishmaniasis in Badrood city is rural form and most patients are men and older than 15 years. There was complete or partial healing after treatment in the most patients.
PREVALENCE OF INTESTINAL PARASITES AMONG PRIMARY SCHOOL
PUPILS IN ABAKALIKI METROPOLIS
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Background

Intestinal parasites are still major health challenges in tropical and sub-tropical areas. They are commonly found among people with low socio-economic status and in unhygienic environments. Children are usually more infected than adults due to easier fecal-oral transmission. Children with parasitic infections are usually undernourished, weak and may have complications like diarrhoea, nutritional disorders and potentially lethal systemic disease.

Objectives

To determine the prevalence of intestinal parasites among primary school children in Abakaliki metropolis.

Methods

A total of 180 stool samples were aseptically collected and examined macroscopically by wet preparation (saline and iodine) and then microscopically by formal ether concentration techniques. The study was approved by Anambra State University Teaching Hospital Review Board.

Conclusions

Out of the 180 stool samples examined, intestinal parasitic infection prevalence of 25.0% was recorded. Hookworm showed the highest of prevalence, followed by Ascaris lumbricoides and Gardia lamblia, while Entaemoba histolytica had the least prevalence. Prevalence in male was insignificantly higher than that of female (p>0.05). The highest prevalence was recorded amongst age group 5-7 years. There was no significant difference in the rate of parasitic infection in relation to age (p>0.05). Higher number of parasites was seen in the concentration technique as compared to other laboratory techniques (normal saline and Iodine method). Higher prevalence was seen among the children of farmers compared to other children. A well-structured control programme is needed to reduce mortality and morbidity from intestinal parasites and sustained commitment by the government and policy makers.
are needed to improve the quality of life of these children.
EVALUATION OF HAIR SAMPLES AS TARGET TO DIRECT DETECTION OF LEISHMANIA SPP. IN LEPORIDAE

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Background

Leishmaniasis is a parasitic zoonotic disease caused by protozoa of the genus Leishmania, responsible of high mortality in developing countries and considered one of the three major neglected parasitic diseases in developed countries. Wild reservoirs have a key role in certain epidemiological situations, i.e. Leporidae were significantly involved in the largest recorded European outbreak of leishmaniasis, in Madrid (Spain), still active. The possibility of monitoring Leishmania spp. using samples easy to obtain, with noninvasive techniques and stable for transport/storage is an essential key for surveillance programs in wild reservoirs. Previous studies have shown that hair samples are ideal for this purpose in dogs, mice and some wild species, but their suitability in wild Leporidae has not been studied before.

Objectives

Assessment of hair as a target for direct detection of Leishmania infantum in wild Leporidae.

Methods

Seventeen wild rabbits and nine hares from Madrid were included. Sera from all the animals were tested by immunofluorescence antibody test (IFAT). Moreover, hair, skin and spleen samples were subjected to DNA extraction and analyzed by real-time PCR for Leishmania infantum.

Conclusions
All animals sampled (n=26) were positive to at least one of the three classic samples (serum, skin, spleen): 23 individuals (88.5%, 95%CI: 77.3%-100%) were positive in IFAT and 25 (96.2%, 95%CI: 88.2%-100%) were positive in skin/spleen RT-PCR.

A total of 22 hair samples (84.6%, 95% CI: 69.7%-99.8%) were positive. Despite the limited sample size, our results demonstrate that hair could be considered an adequate sample for direct diagnosis of *Leishmania infantum* infection in wild *Leporidae*. 
A NEW SPECIES OF CHOLEOEIMERIA (APICOMPLEXA: EIMERIIDAE) PARASITIC IN THE ROUGH-TAILED GECKO CYRTOPODION SCABRUM (SAURIA: GEKKONIDAE) IN EGYPT

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Background
Cyrtopodion Fitzinger is a large genus of the Gekkonidae Gray (gecko) family with currently 28 described species. In contrast to the large diversity of this genus, only Eimeria lineri McAllister et al. 1988 has so far been described. In Egypt, the genus Cyrtopodion is represented only by Cyrtopodion scabrum Heyden 1827. Genus Choleoeimeria Paperna and Landsberg 1989 was proposed to incorporate eimeriid-like coccidia of elongate-ellipsoidal oocysts (L/W ratio 1.6–2.2), with sporocysts that lack a Stieda body and that develop in the epithelium of the gall bladder and bile duct of their hosts.

Objectives
The present work aims to establish the taxonomic status of an unknown Choleoeimeria from the gallbladder of the rough-tailed gecko Cyrtopodion scabrum using the characteristics of exogenous and endogenous stages of the parasite

Methods
A total of 15 rough-tailed geckoes, Cyrtopodion scabrum were collected and examined for coccidian infection. The presence of oocysts was determined by direct microscopic examination of bile contents. To study the endogenous stages, infected gallbladders were fixed in 10% neutral buffered formalin and embedded in paraffin. Sections were cut and stained with haematoxylin and eosin (H&E).

Conclusions
Based on the combination of the oocyst morphology, site and characters of the endogenous development; we assigned the present species to the genus Choleoeimeria and to which the name Choleoeimeria scabrumi n. sp. was suggested.
Background
This study was conducted to determine the load of pathogenic bacterial contamination and explore the prevalence of pathogenic bacterial species in play areas of fast food restaurants and shopping malls in Riyadh, Saudi Arabia. This novel study aims to pilot other studies in the area to explore bacterial contamination for purposes preventing pediatric disease.

Objectives
The study seeks to identify and determine the load and antibiotic sensitivity of bacterial organisms in the play areas present in fast food restaurants and shopping malls in Riyadh, Saudi Arabia.

Methods
The sample included three fast food restaurants’ play pits and two shopping mall play areas in Riyadh, Saudi Arabia during the month of March in 2012. Nineteen samples were isolated using sterile swabs from surfaces of balls in ball pits and different arcade games. Samples were inoculated onto blood agar and MacConkey agar and were incubated 24 hours at 37º C. Bacterial species were identified and tested for their antibiotic sensitivity. Bacteria isolated from a total of 19 samples included Klebsiella pneumoniae, Staphylococcus spp., Diphtheroids, Pantoea agglomerans, Enterococci spp., Streptococci viridans and two fungal growths that included Aspergillus spp. and Penicillium spp.

Conclusions
The results of this study confirm that toys in play areas and arcades could serve as media for the transmission of pathogenic microorganisms and fungal elements that pose a potential health risk for children.
EFFECTS OF ORAL ADMINISTRATION OF THE BUTYRIVIBRIO GROUP BACTERIUM ON THE INFLAMMATORY DRY SKIN CONDITION IN CHRONIC IRRITANT CONTACT DERMATITIS

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Background

The genus Butyrivibrio is one of the predominant residents in the rumen, and also inhibits the intestine of monogastric animals. We previously reported that oral administration of B. fibrisolvens to mice alleviated experimental enterocolitis and suppressed the formation of colorectal aberrant crypt foci. Although the preventive mechanisms of this bacterium have been still unknown, it might have the potential to improve immune responses. Thus, some strains of this bacterial group might be used for improving the dysfunction in inflammatory skin diseases such as irritant contact dermatitis (ICD).

Objectives

The newly strain of Butyrivibrio group to improve the skin barrier function was isolated and its effect on inflammatory dry skin was examined.

Methods

Several strains of the Butyrivibrio group bacteria were isolated from the goat rumen and confirmed by reading the nearly full-length 16S rRNA gene sequences. Some strains were examined as the candidates for probiotic bacteria that recover the skin barrier function. Chronic ICD was induced to mice with oxazolone at the ears by repeated application. The isolated strains were administrated as the freeze-dried cells. The beneficial effects on cutaneous inflammation were evaluated by the dermatitis severity score, lymph node weight, histological analysis, and mRNA expression of IL-1β and IL-6 in the lesional ear.

Conclusions

When the isolated strain was administrated, the development of skin roughness or exfoliation was alleviated and mRNA expression of the pro-inflammatory cytokines was suppressed. In conclusion, this newly isolated Butyrivibrio strain might be used as a probiotics for the restoring the skin barrier function.
Background
The dental field has been developed with the purpose of seeking new bioactive principles for the formulation of drugs with different applicability.

Objectives
Analyze the stability of an emulsion of copaiba oil (*Copaifera multijuga* – CM) for dental cavity cleaning. In this emulsion was performed physico-chemical tests and antibacterial test in different environments and periods.
Methods
The results of the pH and density determination were analyzed by ANOVA and Bonferroni’s test (p < 0.05). For other tests were given descriptive analysis. In the centrifugation, there was no phase separation at time 0, in 3 months (freezer, refrigerator and air conditioning) and in 6 months (in the freezer and refrigerator); in the pH, the incubator, the room temperature protected from light, the room temperature and the air conditioning had statistically significant difference from the control group (time 0); at density, the incubator had the lowest density values; in microbiological evaluation, there was no bacterial growth; for the evaluation of organoleptic characters, there was only change in color of the emulsion in 3 months (incubator) and 6 months (incubator, room temperature protected from light and room temperature). The CM showed bacteriostatic and bactericidal activity, respectively, in concentrations of 13.33μL/ml and 15 μL/ml for \textit{S. mutans}; 20 μL/ml and 23.33 μL/mL for \textit{S. salivarius} and 13.33 μL/ml, 15 μL/ml for \textit{L. paracasei}; 8.33μL/ml and 8.33μL/ml for \textit{S. oralis}.

Conclusions
The authors concluded that the CM demonstrated bactericidal activity; presented conditions of stability and quality without contaminants, the best storage environment is refrigerator.
A CASE OF CHRONIC OTITIS MEDIA DUE TO ACHROMOBACTER XYLOSOXIDANS IN AN IMMUNOCOMPETENT PATIENT

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Background

Achromobacter xylosoxidans is an aerobic, oxidase positive, non-fermentative, gram-negative bacillus and often misidentified as Pseudomonas aeruginosa by its oxidase positive feature. It may cause serious nosocomial and community-acquired infections especially in patients with immune deficiency.

Objectives

We present a case of chronic otitis media due to A.xylosoxidans which is rarely isolated from clinical materials in routine microbiology laboratory practice. In this report we emphasize clinical impact of A.xylosoxidans and its antimicrobial resistance profile.

Methods
A 20-year-old immunocompetent man was admitted to otorhinolaryngology service of our hospital with the complaints of left ear pain, difficulty in hearing, left ear discharge beginning from nearly three months his admission. His history revealed unsuccessful treatment attempts with various oral antimicrobials with the diagnosis of otitis media. Tympanic membrane perforation and mucopurulent yellow discharge was observed. He had no systemic symptoms. Microscopic examination of the Gram stained smear revealed PMNL and gram negative bacilli. *A. xylosoxidans* was isolated from discharge. Identification was performed by VITEK 2 automated system. Antimicrobial susceptibility was detected by disc diffusion method according to CLSI criteria for *Pseudomonas* spp. It was resistant to penicillins, most of the beta lactamases, fluoroquinolones, aminoglycosides and susceptible to piperacillin-tazobactam and imipenem.

**Conclusions**

*A. xylosoxidans* strains are often multidrug resistant. Empirical antibiotic therapy with piperacillin-tazobactam or a carbapenem is a reasonable choice until the results of susceptibility tests are available. It should be kept in mind that even an opportunistic and generally nosocomial pathogen like *A. xylosoxidans* can cause community-acquired infection like otitis media in a immunocompetent host.
EFFECT OF TRANS-CHALCONE ON TRANSCRIPTION OF GENES RELATED TO CELL WALL AND FATTY ACID SYNTHESIS IN THE DERMATOPHYTE TRICHOPHYTON RUBRUM

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Background

Trichophyton rubrum is the main causative agent of dermatophytosis worldwide. The infections caused by this dermatophyte are not lethal but are uncomfortable and difficult to treat. Moreover, invasive infections are rising due to modern medical interventions and immunosuppressive diseases. The development of fungal resistance limits the arsenal of antifungal drugs, which has a narrow spectrum of activity and often presents toxicity to the host. Therefore, novel drugs with more specific and effective mechanisms of action against dermatophytes are urgently needed. Chalcones drew attention by numerous pharmacological applications, including antifungal activity.

Objectives

The aim of this study was to evaluate the modulation of genes involved in cell wall and fatty acid synthesis of T. rubrum after the exposure to trans-chalcone.

Methods

The modulation of fatty acid synthesis genes (FAS 1p and FAS 2p subunits, acetyl-COA carboxylase 2p subunit) and genes of cell wall synthesis (beta-1,3-glucanosyltransferase and glycosylphosphatidylinositol anchored protein) was checked by qPCR after the exposure to MIC (7.8 µg/mL) and ¼ MIC (1.95 µg/mL) of trans-chalcone, respectively, for 16h for fatty acid genes and 7h for cell wall genes.

Conclusions

Trans-chalcone promoted a down-regulation of genes of fatty acid synthesis whereas cell wall genes were up-regulated. These results suggest that trans-chalcone may act on different fungal specific targets, which could be considered an advantage for an antifungal compound.
Background

Acidiplasma is the genus of acidophilic, ferrous iron-oxidizing, heterotrophic, cell wall-lacking, moderately thermophilic microorganisms belonging to the family Ferroploasmaceae. All representatives of the genus have high similarity of 16S rRNA gene (99-100%). The genus includes two species (A. aeolicum and A. cupricumulans) separated by DNA–DNA hybridization (DDH) values. However, complexity of DDH suggests the necessity of application of alternative tools for distinguishing representatives within the genus. Also, information about physiological traits of the genus may be incomplete. In our previous study, the new physiological trait (ability to oxidize elemental sulfur) was shown by the example of strain Acidiplasma MBA-1.

Objectives

The aims of this study were to apply rep-PCR fingerprinting technique for distinguishing Acidiplasma representatives and to investigate sulfur oxidation by the strains A. aeolicum VT, A. cupricumulans BH2T, Acidiplasma MBA-1, Ferroplasma acidiphilum YT.

Figure 1. Oxidation of sulfur by Acidiplasma strains and Ferroplasma acidiphilum YT.
Methods
The genomic fingerprints were obtained using BOXA1, BOXS1, ERIC and REP primers. Oxidation of sulfur was estimated by pH decreasing and increasing of sulfates-ions concentrations.

Conclusions
Sulfur was oxidized by all Acidiplasma strains, but not by F. acidiphilum Y^T (Fig. 1). It suggests sulfur oxidation can be widely distributed among Acidiplasma representatives, but not among Ferroplasma representatives. Each of Acidiplasma strains possess its unique genomic fingerprint (Fig. 2), it suggests genomic fingerprinting could be rapid, highly discriminatory screening techniques for Acidiplasma representatives. The work was supported by the Russian Foundation for Basic Research, project no. 14-04-31210 mol_a and Core Research Facility 'Bioengineering' equipment.
TREATMENT AGAINST THE MICROBIAL ACTIVITY WITH CINNAMON ESSENTIAL OIL IN ARTISTIC WORKS AND PACKAGING

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Background
The artwork and the packaging system used in all transfers and storage is a good substrate for the growth of microorganisms that can produce irreversible changes, as destruction or alteration of substrates on which microorganisms can multiply and that as a result of secondary metabolism can accumulate among them the indelible pigment.

Objectives
The aim of this study is to provide a preventive solution to the development of microorganisms without risk to the integrity of the artwork.

Methods
We proceeded through sterile swabs to collection of samples from artwork and packaging deposited in the MACBA. Samples were plated on TSA to determine the presence of bacteria and AS with antibiotic to determine fungi.

All artwork and packaging were submit an aspiration synthetic pallet and vacuum equipped with a HEPA filter.

Then, we applied by spraying a mixture of alcohol: water: cinnamon and elsewhere , to compare the results for the traditionally used mixture formed alcohol: water.

After a month of the application of the products we collected samples from all the treated areas in order to evaluate the efficacy of treatments. In order to assess the effect of treatment in cases of contamination, proceeded experimentally inoculated packaging materials and pieces of artwork who underwent both treatments indicated and to expose the action of an environment contaminated items artistic and packaging that did not have initially any pollution.

Conclusions
The results indicates that the mixture comprising cinnamon oil has a strong antimicrobial effect and avoid contamination of sensitive material in contact with elements altered by microorganisms.
THE EFFICACY OF PRTA TO PREVENT STREPTOCOCCUS PNEUMONIAE INFECTION

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Background

Streptococcus pneumoniae is a respiratory pathogen contributing to severe lung infection and/or bacteremic complications. Worldwide efforts were made to develop a conserved protein vaccine against S. pneumoniae and to provide a maximal and memory protection.

Objectives

The pneumococcal surface protein, PrtA, was identified by convalescent patient serum screening on a pneumococcal genomic expression library. The prtA gene is prevalent and conserved among S. pneumoniae strains; however, there was no report describing its protective efficacy.

Methods

Since IL-17A elicitation can improve the clearance of pneumococcal colonization, we combined a recombinant PrtA fragment (aa 144-1041) with curdlan, a strong inducer of IL-17A, to vaccinate three-wk old CD1 mice intranasally once a week for three weeks. It showed an elevated PrtA-specific Ab titer in mucosa and increased IL-17A production by splenocytes compared with sham group. Finally, the PrtA vaccination can successfully reduce S. pneumoniae D39 colonization in lung within 24 hr but failed to reduce the mortality after 12 days of infection. All of the experiments were approved by the Institutional Animal Care and Use Committee of the National Defense Medical Center Laboratory Animal Center. The animal experiments were performed in strict accordance with the Taiwan regulations of the Animal Protection Act and the course on Animal Care and Use in Research and Education from the American Association for Laboratory Animal Science.

Conclusions

Combined with PrtA fragment and curdlan adjuvant can enhance PrtA-specific humoral and cellular immunity; however, the vaccination was not sufficient to protect against S. pneumoniae infection.
Background

*Toxoplasma gondii* is an obligatory intracellular parasite that infects a wide range of warm-blooded animals and humans.

Objectives

Considering the severity of toxoplasmosis, side effects of current treatments, and the contribution of the ethnopharmacological knowledge for the treatment of parasitic infections, the aim of the present study was to investigate the efficacy of methanolic extracts of *Zea mays* and *Eryngium caucasicum* against tachyzoite of *T. gondii*.

Methods

Four concentrations (5, 10, 25 and 50 mg ml\(^{-1}\)) of extracts were incubated with infected macrophages for 30, 60, 120 and 180 min and then the viability of the tachyzoites were evaluated by trypan blue staining.

Conclusions

Concentrations of 10 and 25 mg ml\(^{-1}\) of *Z. mays* after 180 minutes, and also concentration 50 mg ml\(^{-1}\) after 120 minutes killed 100% of the tachyzoites. Also high anti-*Toxoplasma* activity was seen using *E. caucasicum* extract. The anti-*Toxoplasma* effects of the methanolic extracts from *Z. mays* and *E. caucasicum* did not show any significant difference in comparison with pyrimethamine (positive control). Findings of this research indicate that these extracts have acceptable anti-*Toxoplasma* efficacies *in vitro* and it can be used as candidate extracts for further research on treatment of toxoplasmosis *in vivo*. 
THE INHIBITORY EFFECT OF CROMOLYN SODIUM AND KETOTIFEN ON TOXOPLASMA GONDII ENTRANCE INTO HOST CELLS IN VITRO AND IN VIVO


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Background

Toxoplasma gondii is a protozoan with worldwide distribution and in spite of increasing information about its biology, treatment of toxoplasmosis is restricted to a few drugs and unfortunately using of each of drugs is associated with significant side effects in patients.

Objectives

This study was designed to evaluate the efficacy of cromolyn sodium and ketotifen as alternative drugs for the treatment of toxoplasmosis.

Methods

In vitro; in case group, concentrations of 1, 5, 10 and 15 μg/ml of ketotifen and cromolyn sodium were added to RPMI medium containing peritoneal macrophages. After 1 h incubation and adding tachyzoites to medium, efficacy rate of these drugs in entrance inhibition of Toxoplasma tachyzoites into macrophages were evaluated after 30 and 60 min. In vivo; case groups received ketotifen and cromolyn sodium with different concentrations at various times. Control groups received none of drugs.

Conclusions

We found that in vitro; after 60 min the best efficacy of these drugs in inhibition of cell entrance of Toxoplasma was observed at 15 μg/ml (78.9 ± 1.70 and 91.97 ±0.37 %, respectively) (P<0.05). In vivo; after 60 min ketotifen at 2 mg/kg in 3 h before tachyzoite injection (69.83 ± 2.25 %), and cromolyn sodium, at 10 mg/kg in 6 h before tachyzoite injection (80.47 ± 2/49 %) had the best effect on inhibition of Toxoplasma entry into the cells (P<0.05). Our findings show that ketotifen and cromolyn sodium are suitable drugs for entrance inhibition of tachyzoites into nucleated cells in vitro and in vivo.
Background

The Nagoya Protocol (NP) under the Convention on Biological Diversity (CBD) has entered into force on 12 October 2014. The NP covers the use of biological diversity in Research and Development. If not correctly translated by lawmakers or inefficiently apprehended by microbiologists it could heavily impact the activities of Culture Collections (CC) and their partners. The European lawmakers have already translated the NP into European Regulation.

Objectives

The challenge is to organize efficiently the access and the conveyance of transfer and utilization of the microorganisms at affordable cost.

Methods

CCs have anticipated these legal developments and have presented solutions during the 1st NP’s Meeting of the Parties.
Conclusions

CCs propose TRUST\(^\text{[i]}\), a system to help microbiologists dealing with this new set of rules on access and utilization of biological diversity, including micro-organisms.

Background
Saccharomyces cerevisiae trehalose synthesis complex is composed by Tps1 and Tps2 and two regulatory subunits, Tsl1 and Tps3. To protect membranes trehalose must be present on both sides of the bilayer.

Objectives
Our goal was to elucidate the protective mechanism of trehalose.

Methods
The absence of Tsl1 abolished the increase in Tps1 activity and accumulation of trehalose in response to a 40°C treatment, whereas deficiency of Tps3 only reduced Tps1 activity and trehalose. In extracts of heat stressed cells, Tps1 was inhibited by trehalose-6-phosphate and by cAMP. In contrast, cAMP-dependent phosphorylation did not inhibit Tps1 in tps3 cells, which accumulated a higher proportion of trehalose-6-phosphate after heating. Agt1 deficiency led to a reduced tolerance to heat shock, without interfering in trehalose accumulation, probably because the outside of the lipid bilayer was unprotected. ath1 cells still showed a high trehalose levels when they were shifted back from 40 to 28°C, as well as, increased tolerance to a subsequent heat stress.

Conclusions
We conclude that Agt1 plays a crucial role in transporting endogenous trehalose to the outer side of the plasma membrane when yeasts face adverse conditions. With the end of the stress, the intracellular trehalose pool would be hydrolyzed by Nth1 whereas the external molecules would be hydrolysed by Ath1. To stop readily trehalose synthesis during stress recovery, Tps3 would be phosphorylated by cAMP-dependent protein kinase, decreasing Tps2 activity and, consequently, increasing trehalose-6-phosphate levels which, in turn, would inhibit Tps1. We believe that these concepts are of great importance for medical and biotechnological applications.
INVESTIGATION OF ROSMARINUS OFFICINALIS L EXTRACT ON THE BACTERIA CAUSES GENITAL INFECTION

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Background
Some microbial causes in creating vaginitis are streptococcus group B and Gardnerella vaginalis which result in irrecoverable harms and increasing fatality, and providing infection in pregnancy period and transfer to the baby.

Objectives
The antibacterial effects some plants like Rosemary (Rosmarinus officinalis) on vaginitis we can identify these kinds of plants and then give them to the people in the form of plant drugs. This research is an investigation of Rosemary and its antibacterial effect on the bacteria and also comparing this effect with common antibacterial.

Methods
This study of 96 women who suffered from this disease, all samples confirms with phenotyping method. The Disk diffusion method was used for comparing the effect of antibacterial total extract with common antibiotics.

Conclusions
The MIC result for each strain showed respectively; streptococcus 1/64, Listeria 1/128, Candida Albicans 1/2, staph aureus 1/128, Gardnerella vaginalis 1/64. In the disk diffusion method, the results were in this form which streptococcus group B, staph aureus and Gardnerella vaginalis were more sensitive than antibiotic disk in comparing with extract, While Listeria monocytogenes was more sensitive than antibiotic disk to the extract.
Background

Lignin is one of the major components of plant cell wall and has significant impact on animal digestion of plants. It is known that Phanerochaete secretes a series of extracellular oxidases, which are encoded in multiple genes, to degrade lignin. However, Phanerochaete needs more stringent conditions to ferment. For animal husbandry application, it is challenge in genetic engineering to transfer all required oxidase genes from Phanerochaete into other strains that can ferment easily. Therefore, we chemically fuse protoplasts between *Pleurotus ostreatus*, a Phanerochaete strain, and *Saccharomyces cerevisiae*. To gain strains with the excellent characteristics of both *Pleurotus ostreatus* and *Saccharomyces cerevisiae*.

Objectives

We chemically fuse protoplasts between *Pleurotus ostreatus*, a Phanerochaete strain, and *Saccharomyces cerevisiae* to gain strains with the excellent characteristics of both *Pleurotus ostreatus* and *Saccharomyces cerevisiae*.

Methods

At first, the enzymatic composition, duration and temperature of emzymolysis, the types of osmotic stabilizer and the age of strains were screened to gain optimal protoplasts preparative condition through $L_{16}(4^5)$ orthogonal test. Next, *Pleurotus ostreatus* and *Saccharomyces cerevisiae* protoplasts were inactivated by heat and UV, respectively. Duration and temperature of inactivation were screened for optimal conditions. Then, the optimal chemical fusion conditions, including fusion temperature and duration, pH value, Ca$^{2+}$ concentration and polyethylene glycol-4000 concentration, were screened through $L_{16}(4^5)$ orthogonal test. At last, antagonism, morphological comparison of hypha and Random Amplified Polymorphic DNA were used to characterize the potential fusants, which was analyzed using NTSYS-PC software.
Conclusions

183 regenerative strains were obtained and, among them, 14 strains were proved to be acquired from fusing *Pleurotus ostreatus* and *Saccharomyces cerevisiae* protoplast.
ARE DOG ENZYMES INVOLVED IN THE FORMATION OF THE YEAST TPS2 DELETION MUTANT'S RESIDUAL TREHALOSE?

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Background
Trehalose is a disaccharide of which the concentration correlates very well with cellular stress resistance in yeast. It is synthesized in two enzymatic steps, were the intermediate trehalose-6-phosphate (Tre6P) is converted to trehalose by the enzyme Tps2. However, deletion of this gene does not completely abolish trehalose production. A possible explanation for this observation is that unspecific sugar phosphatases take over the function of the Tre6P phosphatase in a tps2Δ mutant.

Screening the Saccharomyces cerevisiae proteome for enzymes containing the phosphohydrolase motifs present in Tps2 identified Dog1 and Dog2 as possible candidates. These enzymes are known to dephosphorylate 2-deoxyglucose-6-phosphate, but the natural substrate remains unknown.

Objectives
Are Dog1 and Dog2 tre6P-phosphatase that are active in the absence of Tps2?

Methods
We generated S. cerevisiae DOG1 and DOG2 deletion and over-expression mutants in the wild type and a tps2Δ background and determined heat stress tolerance and trehalose levels in these strains.

Conclusions
The results obtained with the deletion strains seem to support our hypothesis. The dog1Δ dog2Δ tps2Δ mutant is more sensitive to heat stress than the tps2Δ mutant, which would indicate lower trehalose levels in the absence of the DOG genes. However, the results obtained with the DOG1 and DOG2 over-expression strains do not support the hypothesis, as trehalose levels and Tre6P-phosphatase activities are not increased in these strains. These results seem to indicate that the Dog1 and Dog2 may be involved in the expression of trehalose metabolism genes are may affect the enzymatic activity of these genes.
Background

One of the challenges of this century is to transform our economy into an eco-friendly and self-sustaining system. An innovative approach is the use of mycelium based materials.

Objectives

The mycelium of the mushroom forming fungus *Schizophyllum commune* has great potential for developing such materials.

Methods

Mycelium of *S. commune* was grown as a floating layer on liquid medium in the light or in the dark under ambient or a high concentration of CO₂. Mycelia were dried and subjected to increasing tensile force. Their tensile modulus (E), elongation (ε) and stress of the sample at breaking point (σ) were measured. These values were rather low for the wild-type strain 4-39 that had been grown in the light at low CO₂ (E=0.03±0.02 GPa, ε=0.75±0.28 %, σ: 0.44±0.31 MPa), resembling a crumble material that breaks easily. The properties of the wild-type strain 4.8b that had been grown under the same conditions resembled a flexible material (E: 0.22±0.08 GPa, ε=1.8±0.56 %, σ=3.5±0.9 MPa). Strain 4-39 and 4.8b grown in the light at high CO₂ had plastic-like and even stiffer properties, respectively (4-39: E=0.26±0.12 GPa, ε=1.15±0.49 %, σ=point 2.59±1.30 MPa; 4.8b: E=0.50±0.10 GPa, ε=1.13±0.21 %, σ=5.58±1.65 MPa). Similar results were obtained with 4.8b and 4-39 when grown in the dark, regardless of CO₂ concentration.

Conclusions

Taken together, physical properties of the mycelium is strain dependent and are affected by environmental growth conditions.
CHARACTERIZATION OF AIF5A (AEF5): AN EVOLUTIONARY CONSERVED TRANSLATION FACTOR

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Background

Protein synthesis is a complex process of fundamental importance in all cells during which a nucleotide sequence is translated into an amino acid sequence. The translation apparatus includes mRNA, ribosomes, aminoacyl-tRNAs and a number of translation factors, which assist all stages of the process. Some of the translation factors are conserved among Bacteria, Archaea and Eukarya. One of the universally conserved factors is eIF5A, highly conserved between Eukarya and Archaea, with an orthologue (EF-P) in bacteria. It was initially identified as a translation initiation factor, but recent studies have established a role for both eIF5A and EF-P in translation elongation specifically in promoting the translation of polyproline containing proteins. The two proteins are both characterized by particular post-translational modifications, which are essential for their function: hypusination for eIF5A and lysinylation for EF-P.

Objectives

Eukaryal and bacterial proteins have been extensively studied, while information on the archaeal homologues are extremely limited so in order to fill this gap we have started the characterization of the archaeal protein in Sulfolobaceae.

Methods

We have produced the recombinant protein from S. solfataricus using two different expression systems: E. coli and S. acidocaldarius and the purified protein was then used in several in vitro functional tests.

Conclusions

Results clarifying its function in the translation process will be presented and a molecular modelling will shed light on its interaction with the ribosome. In addition, we failed to obtain a knock-out strain in S. acidocaldarius proving that, as for the eukaryal and bacterial proteins, the gene product aIF5A is essential.
FEMS-2168
Free subjects - 2

AN INVESTIGATION OF BURKHOLDERIA CENOCEPACIA LIPOPOLYSACCHARIDE (LPS) MODIFICATION DURING CYSTIC FIBROSIS (CF) CHRONIC INFECTION AND ITS IMPACT ON HOST RESPONSE MODULATION
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Background
Bacterial species within the CF microbiome demonstrate adaptive strategies including altered expression of LPS. The structure of LPS governs its immuno-stimulatory potential. It is thus important to elucidate how LPS is modified during chronic infection and to determine its effects on host response modulation.

Objectives
We are investigating LPS extracted from four B. cenocepacia sequential isolates cultured from a CF patient during a 3.5 year infection to determine 1) if B. cenocepacia LPS is structurally modified during chronic infection and 2) the effect of any modification on the host response to LPS.

Methods
The LPS was extracted using hot phenol extraction and analysed via SDS-PAGE. The LPS was incubated with CFBE41o-, 16HBE14o- and HL60 cells. The pro-inflammatory cytokines were subsequently analysed by ELISA. The LPS mutant was generated by deletion of BCAL2405, an O-antigen biosynthesis gene using a tri-parental mating method.

Conclusions
The O-antigen domain was present in the LPS from the first isolate but absent from the three later isolates. The LPS from the later isolates induced a significantly greater pro-inflammatory response from CFBE41o- cells (P < 0.001), 16HBE14o- cells (P < 0.05) and HL60 cells (P < 0.001) when compared to LPS from the initial isolate. These data suggest that the loss of O-antigen in the later isolates may be responsible for the increased pro-inflammatory response. To verify this, we have successfully deleted the BCAL2405 gene from B. cenocepacia K56-2 which yielded LPS without the O-antigen and are currently investigating the pro-inflammatory potential of the mutant strain.
Background

Millions of species of microorganisms on the earth, a large amount of them still undiscovered, are the source of biological diversity. Microorganisms are not only indispensable for life; they are basic for many human activities. Microorganisms are an essential part of the day-to-day work not only for scientists, but also for people working in the area of e.g. biotechnology, agriculture, industry, or medicine. Therefore, their economic value is very high. A large percentage of this microbial material is preserved in culture collections (CCs), in which their stability and reproducibility can be protected and through which they are (in most cases) available for other researchers.

Objectives

The implementation of the strategy on improved communication between user and provide has to be organised in a coordinated way. By arranging a major part of the CCs pro-active communication through the MIRRI portal the users will get a facilitated access to all requested information.

Methods

The main objectives of CCs and microbial resource centres (MRCs) are, besides conserving biodiversity, sharing microbial resources and associated data.

Conclusions

MIRRI has prepared strategy for communication between providers, which are Culture Collections with status of Microbiological Resource Centre (MRC) and users of microbiological resources. MRC are public resource centers specialized on microbial
raw material. They are capable of providing for users with culturable organisms such as micro-organisms, plant, animal and human cells, replicable parts of these (genomes, plasmids, viruses, cDNAs), as well as databases containing molecular, physiological and structural information relevant to these collections and related bioinformatics.
ELECTROTAXIS AND DUROTAXIS INFLUENCE THE BEHAVIOUR OF SINGLE CELL PSEUDOMONAS AERUGINOSA

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Background

P. aeruginosa motility plays a pivotal role in the colonization of surfaces, especially during the formation of antibiotic resistant biofilm structures. Endogenous electrical signals represent an important signaling mechanism that guides cell migration during wound healing, ultimately regulating the direction of cell movement. We report an approach to study the motility of single P. aeruginosa cells in microfluidic channels possessing different structural geometries, all with the flexibility of being able to manipulate chemical concentration gradients and electric fields to investigate changes in motility in response to specific stimuli.

Objectives

This study is designed to examine the interplay between surface rigidity, mechanical, and electrical cues to pave the way for improvements in the design of anti-fouling surfaces for biomedical applications and to identify new ways to inhibit bacterial biofilm growth through motility restriction.

Methods

Three different microfluidic devices with varying geometries (circular, semi-circular shell, and square cut out respectively) were developed using soft-lithography and nanofabrication tools for the analysis of durotaxis and galvanotaxis of single cell P. aeruginosa.
Conclusions

With the use of fabricated microfluidic platforms, we studied the impact of spatial restrictions, rigidity of the substrate medium, and the impact of external electrical fields on Pseudomonas aeruginosa motility. Spatial restrictions resulted in a significant reduction in single-cell velocity and the distance travelled, but did not affect the trajectory directness. *Pseudomonas aeruginosa* single cells showed a preference for an environment with increased stiffness, which was independent of the time of exposure to the electric field.
Background

In Korea, approximately 100 *Penicillium* species have been recorded. Many of these species were isolated from soil, and some were found to be associated with post-harvest diseases of plant products. However, the diversity of marine-derived *Penicillium* in Korea is poorly understood relative to terrestrial species.

Objectives

We explored the diversity of marine-derived *Penicillium* in Korea by isolating *Penicillium* species from various marine substrates and identifying them using a multigene phylogenetic approach. We also evaluated the biological activity (extracellular enzyme activity and antifungal activity against the plant pathogens) of the strains.

Methods

The diversity of marine-derived *Penicillium* from Korea was investigated using morphological and multigene phylogenetic approaches (ITS, *BenA*, *RPB2*). We tested for the extracellular enzyme activity of alginase, endoglucanase, and beta-glucosidase, and antifungal activity against two plant pathogens (*Colletotrichum acutatum* and *Fusarium oxysporum*).

Conclusions

A total of 184 strains of 36 *Penicillium* species were isolated, with 27 species being identified. The most common species were *P. polonicum* (19.6 %), *P. rubens* (11.4 %), and *P. chrysogenum* (11.4 %). The diversity of *Penicillium* strains isolated from soil and marine macroorganisms was higher than the diversity of strains isolated from seawater. While many of the isolated strains showed alginase and beta-glucosidase activity, no endoglucanase activity was found. More than half the strains (50.5 %) showed antifungal activity against at least one of the plant pathogens tested. The
relatively high proportion of strains that showed antifungal and enzyme activity demonstrates that marine-derived *Penicillium* have great potential to be used in the production of natural bioactive products for pharmaceutical and/or industrial use.
Background
In fish farming the benefits of probiotics have been usually inferred appraising the effects observed on the host, namely growth, survival and immune response, and not through the direct assessment of probiotics dynamics and role in the host gut microbiota. Currently, there is the urgency to develop accurate tools to assess and weigh up the dynamics of autochthonous bacteria and probiotics in the gut microbiota, in order to better understand the effects of probiotics, prebiotics and synbiotics in aquaculture production.

Objectives
This work aimed to identify novel taxa-specific DNA markers for *B. licheniformis*, one of the most used probiotics in aquaculture, and develop culture-independent methods for detection and quantification of these bacteria and understand their modulation role of fish-gut microbiota.

Methods
Several putative *B. licheniformis*-specific DNA markers were selected using the
CUPID and Insignia databases. These utilities, combined with comprehensive genomic studies and dedicated experimental validation, allowed the selection of four DNA markers used for detection and quantification of *B. licheniformis* by multiplex PCR and qPCR, respectively, in digesta samples of fish gut samples.

**Conclusions**

The selected DNA markers were successfully applied for detection and quantification of *B. licheniformis* in fish gut. These results are a contribution for a better understanding of *B. licheniformis* dynamics in gut microbiota of farmed fish in response to different diets, but also during disease outbreaks. This knowledge is an added-value to optimize and implement best practices for aquaculture.
ICLIKVAL: COMMUNITY RESOURCE FOR CURATING THE VAST WEALTH OF MICROBIOLOGY-RELATED LITERATURE THROUGH THE POWER OF CROWDSOURCING

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Background

There are currently over 24 million citations in PubMed, including many related to microbiology research. Searching this vast resource does not always give desirable or complete results due to several factors. Ideally, every citation should include a complete set of keywords that describe the original article in detail; however, this would require countless hours of manual curation.

Objectives

Our goal is to make manual curation 'fun', social, and self-correcting, thus enriching resources like PubMed so that users are able to extract more valuable and relevant results. We developed a web-based open-access tool for manual curation of PubMed articles, and other media types, using a crowdsourcing approach. We encourage the use of ontology terms and support them as auto-suggest keyword terms, but we do not restrict users to these so as not to impose any limitations on the annotation types.
Non-English annotation is also supported.

Methods

We constructed a cross-browser and platform-independent application using a NoSQL database. Users perform searches, mark articles for review, load PDFs into the viewer, select annotations (values) within the text, and add appropriate keywords (keys). Article-specific comments can be made, key-value pairs can be edited and rated, live chats between users can be conducted, annotations can be added via Twitter, etc.

Conclusions

The more annotations that accumulate in the database the more reliable the results. We implemented a REST API to make the annotations easily accessible to the research community. We hope this will become the default resource for community-based curation of all online microbiology-related and scientific literature.
THE RISK OF SERIOUS INFECTIONS WITH THE USE OF RITUXIMAB IN RHEUMATOID ARTHRITIS. RESULTS FROM A META-ANALYSIS OF RANDOMISED CLINICAL TRIALS.

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Background

The association between serious infections (SINFs) and the use of rituximab in patients with non-Hodgkin’s lymphoma has been clearly confirmed; but, uncertainty remains about the association of SINFs with rituximab in rheumatoid arthritis (RA).

Objectives

Our study, a systematic review and meta-analysis (SR&MA), aimed to provide reliable assessment of the risk of SINFs and rituximab in RA (RTX-RA).

Methods

This SR&MA was registered with the PROSPERO database (CRD42014015655) as protocol for a complete evaluation of the safety profile of RTX-RA. The search strategy involved randomised clinical trials using rituximab in RA, published from January 1990 to December 2014 in Medline, EMBASE and Cochrane Library databases. The outcomes evaluated were: the number of adverse events and SINFs reported as outcomes of interest. Odds ratio (OR) analysis, 95% confidence intervals (CI95%) and p values (chi-squared) were calculated; heterogeneity was assessed using the I² test. Sub-analysis by doses (500 vs 1000 mg) and type of infection were performed.

Results: Eight publications (3,272 subjects) were selected for review and 5 publications (2,249 subjects) were included in the meta-analysis; the number of events of SINFs in rituximab+ methotrexate (RTX-MTX) group vs control (placebo+ methotrexate) were 35/1,465 vs 27/784. We found no evidence of association between RTX-MTX and SINFs (OR 0.64; CI95% 0.39-1.04; p=0.74; I²=0.000). Similarly, our sub-analysis by RTX doses (500 vs 1000 mg) and type of infection, showed no evidence of association.
Conclusions

Our results suggest no evidence of association between RTX-MTX in RA and SINFs. Further studies are needed to confirm our findings.
Background

“The purpose of notification [of infectious diseases] is to enable the prompt investigation, risk assessment and response to cases...that present a significant risk to human health. Notification has the secondary benefit of providing data for use in…epidemiological surveillance...”¹ Doctors in England have a duty to notify diseases as a legislative requirement.

Objectives
To establish whether general medical doctors in a hospital in London are appropriately reporting notifiable diseases according to 2010 health protection guidance.

Methods

Notifiable diseases managed in adult secondary care were selected: acute meningitis, invasive group A streptococcus, enteric fever, meningococcal septicaemia, malaria.

Adult patients diagnosed with these diseases between May and October 2013 were identified via the microbiology database and clinical coding. The notes were reviewed for documentation of discussion with public health or the notification form, and the time this occurred relative to diagnosis/clinical suspicion.

Conclusions
24 cases of notifiable diseases were identified and of these 17 notes were located (7 malaria, 9 viral meningitis, 1 Salmonella typhi). There was 1 documented notification (6% of 17 notes), with no identifiable date.

Possible causes of the low notification rate include: poor awareness among doctors that it is their responsibility, lack of knowledge which diseases are notifiable, uncertainty which clinician/clinical team has responsibility for notification, and difficulty in locating forms on the hospital intranet.
Measures to improve notification include implementation of electronic notification reminders with results, staff education and more easily accessible information/notification form on intranet.

CHARACTERIZATION OF THE LIPOPOLYSACCHARIDE TRANSPORT MACHINE IN ESCHERICHIA COLI: IN SEARCH OF A FUNCTION FOR THE ELUSIVE COMPONENT LPTC
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Background
The lipopolysaccharide (LPS) transport (Lpt) system is responsible for transferring LPS from the periplasmic surface of the inner membrane (IM) to the outer leaflet of the outer membrane (OM), where it plays a crucial role in OM selective permeability. In *E. coli* seven essential proteins form the Lpt trans-envelope complex: LptBFG form the IM ABC transporter; LptDE form the OM translocon for final LPS delivery; LptC, an IM-anchored protein with a periplasmic domain, interacts with the IM ABC transporter, the periplasmic protein LptA, and LPS. Although essential, LptC can tolerate several mutations and its role in LPS transport is unclear.

Objectives
To clarify the functional role of LptC in the Lpt machine.

Methods
By plasmid shuffling we obtained viable mutants missing *lptC*; genome sequencing of Δ*lptC* mutants revealed single amino acid substitutions at a unique position (*) in the IM component *lptF*; in complementation tests, *lptF* mutants suppress lethality of both Δ*lptC* and *lptC* conditional expression mutants.

Conclusions
Our data show that a specific mutation in LptF can compensate the lack of LptC and suggest that LptC may serve as a chaperon of the Lpt machine assembly and/or activity rather than an essential structural component. Studies are in progress to understand the structure and function of the six-protein Lpt* system.

A NOVEL SCENARIO OF OPRF REGULATION IN PSEUDOMONAS AERUGINOSA
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Background

OprF, the major porin of \textit{Pseudomonas aeruginosa}, has an immunogenic potential and a large set of information regarding its functions, including its role in the outer membrane structure, interaction with lipopolysaccharide and association with virulence and quorum sensing. In the prototype strain PAO1, transcription of \textit{oprF} was reported to be dependent on sigma 70 and the alternative ECF sigma factor SigX.

Objectives

As we have shown that SigX overexpression in PA14 leads to a growth defect, we assessed \textit{oprF} transcription regulation in order to verify if this effect is due to OprF overexpression and to characterize \textit{oprF} regulation in PA14.

Methods

\textit{oprF} promoter activity was determined via \textit{-lacZ} reporter fusions integrated in the chromosome or by relative \textit{oprF} mRNA levels (qRT-PCR) in wild-type PA14, \textit{sigX} null mutant or in PA14 overexpressing \textit{sigX} from an inducible promoter in a multicopy plasmid (ALB04 strain). \textit{oprF} and \textit{sigX} promoters activity was also assessed in the PA14\textit{rpoS} mutant by \textit{β}-galactosidase assays.

Conclusions

Surprisingly, we found that all \textit{oprF-lacZ} promoters activity decreased in ALB04 and the amount of OprF did not increase in a proteomic analysis. The \textit{sigX} null mutant has wild-type levels of \textit{oprF} mRNA, contrasting to other SigX-induced genes. We have also found that transcription of \textit{oprF} occurs mainly via the region containing the putative sigma 70-dependent promoter. Although \textit{oprF} transcription regulation is independent of SigX in PA14, both \textit{oprF} and \textit{sigX} transcription is dependent on the general stress sigma factor RpoS. The data presented here discloses a novel
scenario of *oprF* regulation in *P. aeruginosa*. 
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TRIGGER ENZYME PEPA FROM E. COLI, A TRANSCRIPTIONAL REPRESSOR THAT GENERATES POSITIVE SUPERCOILING
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Background
Hexameric E. coli Leucine-aminopeptidase A (PepA) is a trigger enzyme endowed with catalytic activity and DNA-binding properties prominent in the resolution of ColE1 and pSC101 plasmid multimers and transcriptional regulation of the carAB operon, encoding the unique carbamoylphosphate synthase of E. coli. Previous studies by DNase I footprinting and atomic force microscopy (AFM) had both indicated a pronounced deformation of the carAB control region upon PepA binding, suggestive of DNA wrapping. On the basis of this observation and previous work, PepA was believed to play a major, although merely architectural role in the formation of the synaptic complexes involved in site-specific DNA recombination and in the elaboration of a higher order regulatory protein-carP1 DNA complex.

Objectives
Here we further investigate the molecular mechanism of PepA-mediated transcriptional regulation of the carP1 promoter.

Methods
To this aim we use single-round in vitro transcription and DNA topology assays.

Conclusions
The in vitro transcription assays performed with supercoiled template and purified components demonstrate that PepA is a repressor in its own right, which specifically inhibits transcription initiation at carP1. Furthermore, DNA topology studies performed on artificial DNA mini-circles by means of various topoisomerasers with different substrate specificities demonstrate that PepA binding induces positive supercoiling. Such topological changes may serve as a regulatory mechanism that allows swifter response and multi-layered control of promoter activity in concert with other regulatory components known to be involved in the control of carP1.
OFF THE WALL: FROM FILAMENTOUS GROWTH TO PRIMORDIAL CELLS AND BACK AGAIN

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Background

Objectives

Methods

Conclusions

Streptomyces are filamentous bacteria that grow by apical tip extension. This process is orchestrated by the tropomyosin-like protein DivIVA, which is present at hyphal tips. DivIVA interacts with various proteins, among which the cellulose synthase-like protein CslA. This protein synthesizes a β-(1,4)-glycan, which is thought to protect growing apices that are continuously being remodeled. To obtain further insight in the role of DivIVA and CslA in polar growth and morphogenesis, we have recently generated so-called Streptomyces L-forms that can grow without peptidoglycan. As a consequence, such cells are round and lack any obvious form of polarity. L-form cells have recently been suggested to resemble primordial cell, based on the observation that their growth and proliferation do not require the canonical cytoskeletal or cell division proteins. Instead, their proliferation can merely be explained by physical processes. However, our work on Streptomyces L-forms suggests that these cells require glycans, such as those formed by CslA, for their growth. Such glycans might have served for protection of early life forms, before the modern cell wall was invented. We have recently isolated an L-form mutant strain, which readily switches back and forth between mycelial and L-form growth. This mutant with the capability to re-synthesize peptidoglycan is crucial to understand which genes play an essential role in proliferation of L-forms, but also to unravel the mechanism underlying filamentous growth.
THE ESCHERICHIA COLI MEMBRANE PROTEIN INSERTASE YIDC ASSISTS IN THE BIOGENESIS OF PENICILLIN BINDING PROTEINS

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Background
Membrane proteins need to be properly inserted and folded in the membrane in order to perform a range of activities that are essential for the survival of bacteria. The Sec translocon and the YidC insertase are responsible for the insertion of the majority of proteins into the cytoplasmic membrane. YidC can act in combination with the Sec translocon in the insertion and folding of membrane proteins. However, YidC also functions as an insertase independently of the Sec translocon for so-called YidC-only substrates. In addition, YidC can act as a foldase and promote the proper assembly of membrane protein complexes.

Objectives
Here, we investigate the effect of *Escherichia coli* YidC depletion on the assembly of Penicillin Binding Proteins (PBPs), that are involved in cell wall synthesis.

Methods
Active PBPs were assessed by substrate binding and total protein amount was determined by immunoblotting.

Conclusions
YidC depletion hardly affects the total amount of the specific cell division PBP3 (FtsI) in the membrane, but the amount of active PBP3 is strongly reduced. Similar reductions in the amounts of active PBP2 and PBP4 were observed, while the levels of active PBP1A/1B and PBP5 were essentially similar. PBP1B and PBP3 disappeared from higher Mw bands upon YidC depletion, indicating that YidC might play a role in PBP complex formation. Taken together, our results suggest that the foldase activity of YidC can extend to the periplasmic domains of membrane proteins.
Neisseria gonorrhoeae is an obligate human pathogen causing gonorrhea. It mainly employs homologous recombination to repair double stranded breaks in DNA. The involvement of mismatch repair helicase, UvrD in homologous recombination is debatable till date. Evidences have implied that it could be an anti-recombinase or promote RecFOR pathway. In this study, a detailed biochemical characterization of UvrD from N. gonorrhoeae and its interaction with RecJ exonuclease (NgoRecJ) which is involved in resection of DNA ends during RecFOR pathway of recombination has been carried out.

Objectives

1. To biochemically characterize UvrD from Neisseria gonorrhoeae (NgoUvrD).
2. To study the physical interaction between NgoUvrD and NgoRecJ exonuclease.
3. To elucidate the functional role of this interaction, if any.

Methods

Both the proteins were purified by Ni^{2+}-NTA followed by Heparin-Sepharose chromatography. The unwinding and exonuclease activities were monitored using gel based assays. ATPase assays were carried out using TLC. Protein-protein interaction studies were done using Far-western analysis.

Conclusions

Biochemical analysis suggested that NgoUvrD behaves as a dimer in solution, exhibits 3'-5' polarity on ssDNA and can unwind blunt end duplex DNA as well as different recombination intermediates such as overhang containing DNA and Holliday junction in ATP dependent manner. It was observed that NgoRecJ and NgoUvrD
physically interact with each other. Interestingly, the ATPase activity of NgoUvrD shows 5-fold stimulation in presence of NgoRecJ. This observation for the first time shows that UvrD in complex with RecJ exonuclease might be involved in the initial step of RecFOR pathway, thereby promoting recombination.
Background
Programmed cell death (PCD) in bacteria has long been controversial due to the belief that only multicellular organisms would benefit from this kind of altruistic behavior. However, over the past decade, compelling experimental evidence has established a consensus that such pathways exist in bacteria.

Objectives
Recently, we discovered that expression of a mutant isoform of an essential GTPase, ObgE, causes rapid cell death in *Escherichia coli*. The physiological changes that occur upon expression of this mutant protein (ObgE*) and the genetic basis of this cell death pathway were investigated.

Methods
Besides a clear loss of viability, fluorescence microscopy and FACS analysis revealed that a large array of apoptotic markers – including chromosome condensation, DNA fragmentation, loss of membrane potential and exposure of phosphatidylserine on the cell surface – can be found upon expression of ObgE*, indicating that ObgE* triggers a PCD pathway. Previous reports of bacterial PCD attribute essential roles to either RecA and its function in the SOS response or to the toxic effect of the *mazEF* TA module. However, by analysing viability and physiological changes upon ObgE* expression in *recA* and *mazEF* mutant strains, all currently known bacterial PCD mechanisms could be excluded.

Conclusions
ObgE* triggers a PCD pathway in *E. coli* that differs fundamentally from all other previously described bacterial PCD pathways. Further research into the genetic and molecular basis of this new PCD mechanism may prove useful in unraveling the function and mechanisms of genetically encoded cell death pathways in bacteria.
Background

Lipopolysaccharide (LPS) is the major outer leaflet constituent of the Gram-negative bacteria outer membrane (OM). In *E. coli* LptA protein is a periplasmic component of the LPS transporter (Lpt) made of seven components (LptABCDEF), which ferries LPS from the inner membrane (IM) to OM. LptA interacts with LptC and chaperons LPS through the periplasm. The crystal structure of LptA has been solved and some residues involved in binding LPS and other Lpt proteins have been identified.

Objectives

1. To characterize LptA structure-function.
2. To identify interactions between genes/proteins implicated in OM functionality.

Methods

1. We generated by site-directed mutagenesis *lptA41*, a quadruple mutant in four conserved amino acids potentially involved in LPS or LptC binding; the mutant exhibited increased sensitivity to hydrophobic compounds (Shc phenotype). This suggests that *lptA41* impairs LPS transport thus leading to OM permeability defects.

2. We selected suppressors of *lptA41* Shc phenotype and sequenced the genomes of two mutants (Sup102 and Sup103) to identify the suppressing mutations.

Conclusions

Sup102 harbors a small in-frame deletion in *vacJ*, which codes for a putative OM lipoprotein involved in maintaining OM asymmetry. Sup103 harbors an additional amino-acid substitution (M112I) in LptA41 (*lptA42* allele) and a nonsense mutation in *mdoH*, a gene encoding a glycosyltransferase involved in periplasmic membrane-derived oligosaccharides synthesis.
These results reveal two different strategies adopted by the cell to overcome OM defects caused by defective LPS transport.
INVESTIGATIONS ON THE BIOLOGICAL SIGNIFICANCE OF BURKHOLDERIA CENOCEPACIA J2315 HFQ2 PROTEIN ABILITY TO BIND DNA

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Background

Accumulating evidence has shown that Hfq is a RNA chaperone involved in post-transcriptional regulation, mediating the interaction of small RNAs with their target mRNAs. However, besides its regulatory role in the translation of bacterial mRNA, in Escherichia coli, Hfq is also a DNA-binding protein and is associated with nucleoid DNA. Bcc bacteria are among the few prokaryotes that encode in their genomes two distinct and functional Hfq-like proteins, the 79 aa Hfq and the 188 aa Hfq2. Both proteins bind RNA, however bioinformatics predictions suggested that Hfq2 is able to bind DNA by means of its C-terminal domain.

Objectives

The present study aims to unveil the biological significance of Hfq2 C-terminus predicted ability to bind to DNA.

Methods

A DNA binding domain was bioinformatically predicted for Hfq2 and DNA binding assays were performed using purified His-tagged Hfq or Hfq2 and the araC promoter of pMLBAD plasmid. Electrophoretic Mobility Shift Assays indicates that Hfq2 protein, but not Hfq, is able to bind this DNA fragment. Hfq2 derivatives with complete, partial and absent C-terminus extension were produced to confirm if this region confers the ability to bind DNA. To identify the DNA sequences to which Hfq2 specifically binds, a ChIP-seq strategy is currently being performed, using B. cenocepacia J2315 fragmented total DNA.

Conclusions

Contrasting with Hfq, a putative DNA binding domain was identified in Hfq2 and our results showed that this protein is able to bind DNA. Ongoing work is being performed to identify the DNA sequences and the specificity of DNA-Hfq2 interactions.
Background

*Xanthomonas citri* (Xac) is the causal agent of citrus-canker, a disease that affects citrus plants worldwide, leading to low productivity, culminating in considerable economic losses to orange growers. There is no treatment for citrus-canker, where the eradication of plants is the only reliable method to prevent the spread of Xac to areas considered free of the pathogen. Recently, we reported on the use of new cell-division inhibitors as an alternative to prevent Xac growth, and plant colonization (Silva et al., 2013 *J. Bacteriol.* **195**:85). To further extend the characterization of cell division in Xac, we started the investigation of Xac *minC*.

Objectives

To study *minC* encoded by Xac and its function in site division selection at cell division

Methods

The *minC* gene was deleted using allele exchange. The knocked-out strain was complemented with a replicative vector carrying *minC* under the control of the arabinose promoter. Pathogenicity tests were carried out by infiltration of sweet orange leaves (*Citrus sinensis* (L.) Osbeck). Cell morphology was evaluated using DIC microscopy.

Conclusions

Xac deleted for *minC* exhibited the classic delta-*minC* phenotypes: production of minicells, cell filamentation, and asymmetric/misplaced division constrictions along the rods. Surprisingly, Xac delta-*minC* produced branches that resembled those observed for *Escherichia coli* deleted for PBP5 and other low-molecular-weight penicillin-binding proteins. The delta-*minC* with *minC* supplied *in trans* restituted the wild-type division phenotype. Irrespective of the presence of *minC*, Xac was competent to colonize the host citrus and produce disease symptoms. Altogether, data shows that *minC* is involved in cell division in Xac.
Background

The CsrA protein (Carbon Storage Regulator A) is a global post-transcriptional regulator controlling carbon fluxes and group behaviors in bacteria. Our preliminary SEM analyses revealed that an E. coli mutant deleted for the csrA gene displays a strongly altered morphology. Cells are of small size as compared to the wild-type cells and present an irregular surface and blebs.

Objectives

Cell envelope is the first line target for physicochemical injuries arising from the environment. This complex structure also supports fundamental processes such as energy production and nutrient acquisition. To preserve envelope homeostasis bacteria have evolved intricate stress response pathways. The objective is to evaluate whether envelope stress responses are induced in ΔcsrA mutant and if it is the case, what are the underlying molecular mechanisms.

Methods

Expression of effectors belonging to the 5 well-described envelope stress response pathways (bae, psp, cpx, sigmaE, rcs) were analyzed by RT-qPCR. Envelope integrity was evaluated by measuring sensitivity to specific compounds.

Conclusions

We have shown that in the ΔcsrA mutant, the Psp and Rcs pathways are constitutively induced. Rcs pathway monitors outer membrane integrity while Psp that of the inner membrane. This mutant also shows a high sensitivity to deoxycholate and vancomycin, indicating an increased permeability of the outer membrane. In addition, the ΔcsrA mutant accumulates high ROS concentration, which might participate to psp activation. ROS accumulation might thus represent the link between metabolism and envelope defects. This hypothesis is currently under investigation.
IDENTIFICATION OF THE MULTI-COMPONENTS DNA UPTAKE SYSTEM OF NATURALLY TRANSFORMABLE LEGIONELLA PNEUMOPHILA USING TRANSCRIPTOMIC ANALYSIS.

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Background

Natural transformation is a major mechanism of horizontal gene transfer that is already well-studied in historical Gram-positive models whereas knowledge on Gram-negative bacteria remains sparse. *Legionella pneumophila* is a Gram-negative bacterium that develops, under specific growth conditions or stress, competence for natural transformation, a genetically programmed state allowing bacteria to take up free exogenous DNA and integrate it in their genome. The *L. pneumophila* genome shows a large repertoire of virulence-associated eukaryotic-like genes. Natural transformation offers a plausible route for acquisition of foreign genes contributing to the emergence of this pulmonary pathogen.

Objectives

The DNA uptake system allowing *L. pneumophila* to take up DNA and recombine it into its chromosome remains unknown. The aim of our study was to identify this system and determine its contribution to the infectious process.

Methods

We used a transcriptomic analysis to identify genes up-regulated in an hypercompetent mutant of *L. pneumophila*. We performed a systematic targeted mutagenesis of these up-regulated genes to test their involvement in transformability and DNA uptake. We used immunofluorescence microscopy to visualize components of the DNA uptake system.

Conclusions
Our work allowed us to identify the main components of the *Legionella* DNA uptake system which involves a type IV transformation pilus dedicated to natural transformation. We propose a model of the DNA uptake system that unexpectedly relies on the actin-like protein MreB. These progresses pave the way for a more detailed analysis of the DNA uptake system dynamics and more broadly for a better understanding of Gram-negative bacteria transformation systems.
THE MOLECULAR BASIS OF REGULATION OF TNRA DNA-BINDING ACTIVITY IN BACILLUS SUBTILIS

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Background

TnrA is a master transcriptional regulator of nitrogen metabolism in *Bacillus subtilis*. Its activity was suggested to be regulated by binding to the feedback-inhibited glutamine synthetase (GS). Recently we showed that TnrA also binds to the PII-like GlnK protein.

Objectives

Understanding of TnrA activity regulation via interaction with GlnK or GS

Methods

*In vivo* cross-linking, Surface Plasmon Resonance, ITC

Conclusions

We found that *in vivo* TnrA binds both proteins, but in contrast to the suggested mechanism, TnrA binds GS also under nitrogen-poor conditions. SPR analysis demonstrated that GS in the absence of feed-back inhibitors efficiently binds TnrA, but 1 mM glutamine strengthens the binding 2-fold. By contrast, ATP strongly repressed TnrA-GS interaction, whereas 1 mM glutamine completely neutralized this negative effect. In presence of L-methionine sulfoximine (MSX), a transition-state analogue of GS, GS–TnrA interaction was also stimulated. However, in presence of MSX and ATP, where MSX is phosphorylated and irreversibly fixes GS in transition state, GS binding to TnrA was completely abolished. Isothermal titration calorimetry revealed competitive binding of glutamine and ATP to GS. Therefore, we suggest two conformations of GS for TnrA interaction: (1) a TnrA-binding “N-state”, stabilized by glutamine or its analogue MSX and (2), a non-binding “A-state”, which reflects the conformation of the enzyme in the catalytic transition state or bound with ATP in
absence of glutamine. We propose that GS changes between the “N-state” and “A-state”. In vivo, this transition depends on the intracellular glutamine:ATP ratio and thus regulates the amount of active TnrA.
A VIBRIO VUNIFICUS ORTHOLOG OF ESCHERICHIA COLI RRAA CONTRIBUTES TO DIFFERENTIAL RNASE E-MEDIATED mRNA DECAY

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Background
Endoribonuclease E (RNase E) plays an important role in the degradation and processing of RNAs in Escherichia coli. Expression levels and enzymatic activity of RNase E are tightly controlled by self-cleavage of rne mRNA and regulators of RNase activity, RraA and RraB. The marine pathogenic bacterium Vibrio vulnificus also contains homologs of RNase E and RraA, designated as RNase EV, RraAV1, and RraAV2.

Objectives
In this study, we show that RraAV1 actively inhibits the enzymatic activity of RNase EV on a subgroup of substrate RNAs.

Methods
Notably, RNase EV cleavage on rne mRNA encoding RNase EV was greatly affected by RraAV1 in vitro and in vivo, contributing to autoregulation of both expression and activity of RNase EV.

Conclusions
Our findings suggest that RraAV1 plays an active role in differential RNase E-mediated mRNA decay.
THE STUDY OF BIOGENESIS FEATURES OF OUTER MEMBRANE VESICLES PRODUCED BY LYSOBACTER SP. XL1

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Background

Gram-negative bacteria produce outer membrane vesicles (OMVs) containing biologically active proteins. Despite of extensive information about OMVs biogenesis available, this process is still not enough clear. Gram-negative bacterium Lysobacter sp. XL1 forms OMVs containing bacteriolytic endopeptidase L5, – one of five bacteriolytic enzymes secreted by this microorganism. Knowledge about OMVs biogenesis by bacteria of Lysobacter genus is rather restricted.

Objectives

The objective of our work was the study of factors causing biogenesis of OMVs produced by Lysobacter sp. XL1.

Methods

Methods were used: fractionation of OMVs using sucrose density gradient centrifugation, electronic microscopy including immunocytochemistry with the protein A-gold, SDS-PAGE electrophoresis, Western blotting assay, protein and 2-keto-3-deoxyoctonate assays, thin-layer chromatography, determination of lytic OMVs action.

Conclusions

Enzyme L5 was found to be localized inside of the lightest OMVs fraction which differed in protein composition from other fractions. The L5 protein was revealed at certain loci of bacterial outer membrane (OM). OMVs were produced from these loci. Thereby, secreting enzyme L5 took part in biogenesis of OMVs. OM was shown to contain cardiolipin, phosphatidylethanolamine, phosphatidylglycerol. OMVs contained the only one major phospholipid, – cardiolipin. Thus, OMVs of Lysobacter sp. XL1
were formed from loci enriched with cardiolipin. It has been offered the model of vesicles biogenesis produced by *Lysobacter* sp. XL1. Besides, OMVs containing protein L5 were shown to lyze broad range of opportunistic and pathogenic bacteria including multidrug-resistant strains. The data obtained are the basis for development of artificial vesicular structures – liposomes – containing bacteriolytic enzymes of *Lysobacter* sp. XL1 as a new effective antimicrobial preparation.
Background

Despite its essential role as a trace element, copper becomes toxic at higher concentrations, prompting bacteria to develop a tight regulation of copper homeostasis in order to survive.

Objectives

The alphaproteobacterium Caulobacter crescentus lives in poor and contaminated environments and gives rise to two distinct cell types upon cell division: a motile swarmer cell and a sessile stalked cell. The swarmer cell is thought to seek optimal environments in order to differentiate into a stalked cell, which will in turn replicate its DNA and divide. This dimorphic cell cycle likely provides a better adaptation to various stresses such as an increase of copper concentration.

Methods

We found that the swarmer and the stalked cells respond differently to a toxic copper concentration. Copper slows down swarmer to stalked cell transition and the further initiation of DNA replication, whereas copper-treated stalked cells undergoes a normal DNA replication. Accordingly, copper concentration in swarmer cells is 3 fold higher than in the stalked cells. We showed that copper homeostasis in the stalked cells is ensured by the conserved PcoABCc system, which is also able to sustain copper detoxification in the swarmer cells when constitutively expressed in trans, thereby restoring a proper swarmer to stalked cell transition.

Conclusions

Both C. crescentus cell types regulate copper homeostasis differently in order to progress throughout the cell cycle.
AN RNASE G-MEDIATED PATHWAY FOR PATHOGENICITY IN SALMONELLA TYPHIMURIUM

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Background
Endoribonuclease G (RNase G) is involved in rRNA processing and degradation of a subgroup of mRNAs in *Escherichia coli*. However, little is known about physiological role of this enzyme.

Objectives
We observed RNase G expression-dependent alterations in the survival rate of *Salmonella* Typhimurium cells in both macrophages and mice. These alterations were strongly associated with expression levels of *rbs* mRNA encoding ribose transport system, which was identified as a substrate of RNase G.

Methods
Analysis of polycistronic *rbs* mRNA identified two RNase G cleavage sites in the 5'-untranslated region of *rbsA*. The induced *rbs* expression during macrophage infection coincided with decreased rates of bacterial infection and survival.

Conclusions
Our study shows that RNase G mediates a previously uncharacterized pathway that involves ribose transport system as a key factor for the survival and virulence of *S. Typhimurium* in host cells.
IDENTIFICATION AND CHARACTERIZATION OF A CORYNEBACTERIUM GLUTAMICUM MUTANT IMPAIRED IN THE BIOSYNTHESIS OF PEPTIDOGLYCAN

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Background

Bacteria belonging to Corynebacteriales, an order of actinomycetes group that includes corynebacteria, mycobacteria, nocardia and rhodococci possess an atypic, complex multilayered envelope. This envelop is composed of a heteropolymer of peptidoglycan (PG) and arabinogalactan (AG) covalently associated to an outer membrane mostly composed of mycolic acids. We explored the biogenesis of Corynebacteriales cell envelope by using a genome-wide transposon mutagenesis approach targeting Corynebacterium glutamicum. For this purpose, we developed an effective immunological screen that allows us to rapidly identify bacteria exhibiting an altered cell envelope.

Objectives

We wanted to identify missing actors of the Corynebacteriales envelope biosynthesis pathways. One interesting mutant of our library was chosen and characterized in this study.

Methods

We identified the gene inactivated by the transposon and constructed a strain in which this gene was deleted. We performed analyses of the main cell wall compounds of this mutant strain, i.e. PG, AG and mycolic acids. A physiological study was also conducted and in particular a screen for the sensitivity of this mutant to various antibiotics.

Conclusions

Our analyses revealed that this mutant was impaired in the PG biosynthesis pathway and more specifically in a modification of this polymer that also occurs in mycobacteria species and in other Corynebacterium pathogens species such as Corynebacterium jeikeium. Lack of this modification led to morphologically-altered
cells with high susceptibility to lysozyme and to antibiotics of the β-lactam family.
Background

Beneficial interactions between gut microbiota and the host have been revealed but little is known at the molecular level. To identify bacterial factors that account for these symbiotic interactions, we recently carried out reverse genetics of *Lactobacillus casei* which allowed identification of 47 key genes for *L. casei* establishment in the gut (Licandro-Seraut et al. 2014. PNAS).

Objectives

Now, we aim to decipher the functions encoded by these key genes in the gut context.

Methods

Some of the *L. casei* mutants have been investigated individually with regard to their morphology, metabolism, gene regulation...

Conclusions

A mutant for the predicted cysteine synthase was investigated to demonstrate the metabolic role of the cysteine synthase. Also, it was less resistant than the wild type to several stresses. Three mutants for three genes in an operon encoding a two-component system and a penicillin-binding protein were characterized. Analysis of primary data suggests that these genes are involved in the cell surface modulation and particularly in the regulation of peptidoglycan hydrolases which are required for the bacteria protection in the intestinal environment.
Background: Deinococcus radiodurans (D. radiodurans) is a poly-extremophilic organism, capable of tolerating a wide variety of different stresses such as gamma/UV radiation, desiccation, and oxidative stress. It is known that PprI (DR0167) is a global regulator, which is essential for its extreme resistance, and cold shock protein homologue PprM (DR0907) is presumably controlled by PprI. PprI mutation decreases the catalase activity of D. radiodurans, but, the effect of PprM on catalase has not been studied.

Objectives: We investigated the role of PprM, which is involved PprI-mediated signal transduction, in catalase gene expression.

Methods: First, we performed an in-gel catalase activity assay to observe activity of three catalases (DR1998, DRA0146, and DRA0259) independently in wild type, pprM, pprI, and pprI/pprM double mutant strains. We also constructed catalase mutants of D. radiodurans to identify the catalase that is affected by PprM and purified catalases to check the activity in vitro. The mRNA and protein levels of catalase genes were monitored by using real-time PCR and western blot assays in various mutant strains.

Conclusions: Among three catalases, we confirmed that DRA0146 does not have catalase activity. Deletion of pprM decreased catalase activity and protein level of DR1998, but not its mRNA level. We could not observe an additional reduction of the DR1998 protein in the pprI/pprM double mutants compared to pprI or pprM single mutants. These suggest that PprI can affect DR1998 via PprM, and PprM may exert its effect on DR1998 at the post-transcriptional level.
Background

*Acinetobacter baumannii* is an emerging pathogen involved in severe infections and outbreaks mainly occurring in clinical settings. Multi-drug resistance and ability to form biofilm could play a role in its persistence in hospital settings.

The present study analyzes an *A. baumannii* clinical strain, belonging to the SMAL clone, isolated in an Italian hospital.

Objectives

The aim of this work is to study the effect of glucose, at a concentration similar to human blood, on production of biofilm determinants in *A. baumannii*.

Methods

Genome sequence and transcriptome analysis were performed by using deep sequencing and RNA-seq techniques (Illumina).

Transcription of selected genes were measured by using transcriptional fusions with the reporter gene *lacZ*.

Exopolysaccharide (EPS) production was analysed by EDTA extraction and phenol-sulfuric acid quantification.

Lipopolysaccharide (LPS) production was measured using tricine SDS-PAGE.

Cytokine production was tested by ELISA analysis.

Conclusions
Genome sequence of the analysed strain showed identity with the sequence type 78, epidemic in several Mediterranean countries.

Growth in the presence of glucose showed higher EPS production; consistently, transcriptome analysis suggested a deep modification of gene expression in presence of glucose. In particular, several genes involved in EPS and LPS biosynthesis and modification were up-regulated in presence of glucose. Remarkably, *A. baumannii* grown in presence of glucose appears to release LPS, which in turn stimulates cytokine production in macrophages.

Our results suggest that glucose at physiological concentrations might act as a trigger for LPS production and virulence in *A. baumannii*. 
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ACTION OF MOLECULAR CHAPERONES DEDICATED TO THE MATURATION OF REDUCTIVE DEHALOGENASES
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Background
Reductive dehalogenases (RDases) are key enzymes in organohalide respiration (OHR) as they catalyze the reduction of halogenated compounds such as tetrachloroethene (PCE), a major environmental pollutant. RDases contain a corrinoid and two iron-sulfur cofactors and require cofactor assembly and maturation prior to transport across the cytoplasmic membrane via the Twin-arginine translocation (Tat) pathway. Recently, a new dedicated Tat molecular chaperone, PceT, has been identified that seems to act in the maturation of the PCE RDase (PceA) of Dehalobacter restrictus and members of Desulfitobacterium hafniense.

Objectives
This work intends to study the molecular action and specificity of PceT and other members of the RdhT chaperone family on the maturation of RDases.

Methods
Heterologous co-expression of the pceA and pceT genes will be investigated in E. coli in various growth and induction conditions in order to evaluate the effect of PceT on the production, stability and cofactor load of PceA. Additional general molecular chaperones will be also considered here. A detail characterization of the protein-protein interaction between PceT and PceA will be obtained by a combination of in vitro biochemical approaches. Site-directed mutagenesis of conserved residues of RdhT proteins should allow identifying key amino acids in the recognition of, i.e. action on, PceA.

Conclusions
Preliminary data already suggested that PceT mainly recognizes the Tat signal peptide of PceA, therefore resembling the paradigmatic Tat specific chaperones (TorD, NapD,...) as they play a major role in quality control of their cognate redox enzyme allowing sufficient time for cofactor assembly and folding.
IDENTIFICATION OF A PUTATIVE CHROMOSOMAL REPLICATION ORIGIN FROM BDELOVIBRIO BACTERIOVORUS AND ITS INTERACTION WITH THE INITIATOR PROTEIN DNAA
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Background
Bdellovibrio bacteriovorus is a small Gram-negative, obligate predatory bacterium that attacks and invades other Gram-negative bacteria, including pathogens such as Helicobacter pylori or Pseudomonas aeruginosa. Its life cycle consists of two stages – non-replicative attack phase, wherein predator searches for prey and replicative growth phase, wherein it actively divides in host periplasm. Initiation is the first and strictly regulated step of bacterial chromosome replication, which leads to duplication of the genetic material in bacterial cells prior to their division.

Objectives
Our aim was to identify and characterize the key elements of initiation of chromosome replication in B. bacteriovorus: origin of chromosomal replication (oriC) and initiator protein.

Methods
Using in silico analysis, we identified the oriC region, which is located downstream of the dnaA gene; the B. bacteriovorus oriC (BdoriC) contains eight putative DnaA boxes. Comprehensive in vitro studies using EMSA, DMS footprinting and SPR revealed that the DnaA protein specifically binds all eight DnaA boxes. By P1 nuclease assay we localized the DNA unwinding elements (DUE) where DNA replication starts. In addition, we compared the architecture of the DnaA–oriC complexes (orisomes) in homologous (oriC and DnaA from B. bacteriovorus) and heterologous (BdoriC and DnaA from prey, E. coli or P. aeruginosa) systems. Interestingly, we demonstrated that DnaA proteins from preys (relatively distantly related from B. bacteriovorus) not only specifically bind BdoriC, but also unwind DNA at the DUE.

Conclusions
To conclude, we identified the oriC of B. bacteriovorus and characterize in details its interaction with the replication initiator protein.
Background

*Brucella*, an ALPHA2-proteobacteria, is the etiological agent of brucellosis that generates a worldwide zoonosis. It can infect human and will lead, if not treated, to a chronic infection with severe complications.

To proliferate, bacteria need to get carbon sources from their environment and the phosphotransferase system (PTS) contributed to this goal. It displays regulating functions in carbon metabolism and forms a phospho-relay, ending with the entry and concomitant phosphorylation of a sugar.

*Brucella abortus* possesses a paralogous system called Nitrogen PTS (PTS\textsuperscript{Ntr}), acting the same way except that no sugar entry occurs. Starting from the phosphoenolpyruvate, the phosphoryl group is successively transferred on histidine residues of Enzyme I (EI\textsuperscript{Ntr}) (ptsP gene), then to the NPR (ptsO) protein and finally to EIIA\textsuperscript{Ntr} (ptsN) or EIIA\textsuperscript{MAN} (ptsM).

Objectives

We were interested in the identification of a link between central metabolism and the *B. abortus* PTS\textsuperscript{Ntr}.

Methods

We have generated the knockout mutant of those 4 protagonists and studied their bacterial growth behavior on media containing different single carbon source. We found that *B. abortus* was mainly able to grow on sugar entering the pentose phosphate pathway. Interestingly, compared with the wild type strain, the ptsP (EI\textsuperscript{Ntr}), ptsO (NPR) and ptsN (EIIA\textsuperscript{MAN}) mutants shows a growth defect on those sugars while the ptsM (EIIA\textsuperscript{Ntr}) mutant growths better. Moreover, point mutation study on NPR protein shows that phosphorylation state of the histidine residue seems to be the main actor controlling growth behavior.

Conclusions

It suggests a role of the *Brucella* PTS\textsuperscript{Ntr} in the regulation of central metabolism.
ENRICHMENT FOR G1-ARRESTED DAUGHTER CELLS OF BRUCELLA ABORTUS

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**Background:** In HeLa cells and RAW264.7 macrophages, the facultative intracellular pathogen *B. abortus* blocks its growth and its cell cycle at the G1 stage during the first hours of infection (M. Deghelt *et al*.). G1 bacteria are proposed to have an increased ability to invade these host cells. Therefore, it would be interesting to prepare a bacterial population enriched with G1-arrested daughter cells to investigate *B. abortus* cell cycle in culture and in interaction with the host cells.

**Objectives:** Searching the best method for synchronizing the cell cycle of *B. abortus* at the G1 stage. Then, testing G1 bacteria for their potential invasion of host cells.

**Methods:** Nutrient downshift method was used to produce G1-arrested cells of *Sinorhizobium meliloti* (De Nisco *et al*.). Our results indicate that this is not an effective way to synchronize G1-arrested daughter cells of *B. abortus*. However, culture of *B. abortus* in rich medium until the early stationary phase resulted in a reproducible enrichment in G1 bacteria (about 70%), as assessed by flow cytometry. Another method called “baby machine”, that immobilize a mixed population of bacteria labelled with NHS-biotin (*N*-hydroxysuccinimido-biotin) by streptavidin-coated magnetic beads, was set up to recover only G1 bacteria. We expected that the unipolar growth will be resumed on the beads in rich medium, allowing the enrichment of daughter cells at the G1 stage of the cell cycle.

**Conclusions:** Culture of *B. abortus* in rich medium yielded a partial and transient enrichment of G1 bacteria. It seems that the unipolar nature of growth is no more detectable when bacteria are labelled with NHS-biotin. Other labelled compounds for “baby machine” method are currently tested and analysed to achieve G1 bacteria of *B. abortus*. 
CHARACTERIZATION OF LMO1521 AND LMO2591 MUREIN HYDROLASES FROM LISTERIA MONOCYTOGENES
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Background

*L. monocytogenes* is a gram-positive, foodborne pathogen causing listeriosis with a high mortality rate (1). This species is widespread in nature which is connected with a high number of surface proteins. Very important class of these proteins are murein hydrolases (autolysins), involved in several crucial processes, including: cell growth, turnover of cell wall components, cell separation and division, biofilm formation, protein secretion, autolytic activity of some antibiotics and pathogenicity (2). This work was supported by a grant from the National Center of Science 2013/09/B/NZ6/00710

Objectives

Determination of the activity and substrate specificity of two autolysins from *L. monocytogenes*: Lmo1521 and Lmo2591.

Methods

Lmo1521 and Lmo2591 proteins with C-terminal hexa-His-Tags were expressed in *E. coli* BL21 using expression vector pET-28a (Novagen). These proteins were purified on Ni-NTA Agarose column (Qiagen), and the isolation of the correct proteins was verified by immunoblotting. The effect of overexpression of the proteins on host cells morphology (*E. coli* BL21) was tested using scanning microscopy. To investigate activity we have performed spectrophotometric and zymographic assay (using different purified bacterial cell walls).

Conclusions

Bioinformatic analysis showed the presence of a N-acetylMuramoyl-L-alanine domain in Lmo1521, N - acetylMuramidase domain in Lmo2591 and GW-motif in both. Our results indicate muralytic activity of the studied proteins against different bacterial cell walls. We have also determined the substrate specificity of these two autolysins.
THE EFFECT OF RSD, THE ANTI-SIGMA FACTOR OF SIGMA 70, ON BIOFILM FORMATION AND MOTILITY IN ESCHERICHIA COLI

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Background

In bacteria, σ subunit of RNA polymerase (RNAP) directs transcription initiation. The regulation of σ activity is important for fine tuning of gene expression. σ activity is determined by their cellular level, affinity for core RNAP, and interactions with regulatory proteins. In Escherichia coli, housekeeping σ factor, σ⁷₀, has the highest affinity for core RNAP and is the most abundant σ factor. Rsd, regulator of sigma D, binds specifically to σ⁷₀ and it has been known as an anti-σ factor of σ⁷₀.

Objectives

Even though Rsd is known as an anti-σ⁷₀ factor, no specific phenotype has been associated with deficiency or overexpression of Rsd to date. In this study, we found new phenotypes of the rsd mutant.

Methods

Cell-to-cell autoaggregation assay
Biofilm formation assay
Semi-solid agar assay for assessment of motility

Conclusions

An rsd-deficient mutant cell sank much faster than wild type. In spite of its increased cell aggregation, biofilm formation decreased in the rsd mutant. We found a protein of ~ 43 kDa whose expression was significantly higher in the rsd mutant compared to wild-type. Peptide mass fingerprinting revealed that this enriched protein is antigen 43 (Ag43) encoded by agn43, whose transcription is σ⁷₀-dependent. Also, one protein band increased in wild-type and Rsd-overexpressing strains compared to the rsd mutant. Mass spectrometry identified this protein band as the flagellin protein FliC. Based on these results, we propose that Rsd decreases the transcriptional level of agn43 through the regulation of σ⁷₀ activity and consequently it influences the biofilm formation and motility.
Background

Horizontal Gene Transfer (HGT) is an evolutive mechanism by which entire genes are transferred among bacterial cells, thus enabling an immediate adaptation to new environmental conditions. HGT has obvious implications for human health as it is used by pathogenic microorganisms – even among different species– during the spread of virulence factor and antibiotic resistance. Three main routes can be distinguished: conjugation (plasmid transfer through the direct interaction of two bacterial cells), transduction (bacteriophage-mediated DNA transfer) and transformation (uptake of naked DNA from the environment). The term DNA Uptake Pump (DUP) refers to the translocating machinery used by bacteria to incorporate naked DNA from the environment to the cytoplasm during bacterial transformation.

Objectives

The specific aim of this project is to determine the structure of the DNA uptake pump responsible for bacterial transformation in gram-positive bacteria using X-ray crystallography. This machinery is mainly formed by a DNA receptor, a transmembrane channel that mediates DNA translocation across the cytoplasmic membrane and an ATPase which pulls DNA into the cytoplasm at the expense of ATP.

Methods

We adopted a high-throughput approach to achieve our goals. We designed 96 constructs from five different species with several solubility-enhancing fusion partners and affinity tags. The constructs can be divided into three groups: putative soluble constructs, fragments containing trans-membrane helices and full-length integral membrane proteins.

Conclusions

We already have cloned the target genes and have tested them for soluble expression in E. coli. Large scale purification and preliminary crystallization trials are in progress for the soluble constructs.
Background

Why bacteria have evolved and maintained their specific shapes is one central question in bacterial cell biology. Rod-shaped bacteria cells are remarkable in keeping their geometry and shape. How this shape is regulated is still mostly uncharted territory.

Objectives

We are setting out to understand how and if rod-shaped bacteria are maintaining their rod shape when subjected to mechanical deformation. We explore how mechanical force changes the bacteria morphology and the consequences for bacteria after the mechanical force is released. The behaviour of proteins, that are involved in maintaining rod-shape, e.g. MreB, Mbl, is analyzed microscopically to understand if mechanical deformation can influence protein localization.

Methods

With the help of soft lithography we are applying microfabrication tools to create microenvironments to manipulate the shape of bacteria. To analyse the shape morphology and protein localization we are using different types of microscopy and automated image analysis tools.

Conclusions

Rod-shaped bacteria are maintaining their rod shape in response to mechanical deformations. Cells return to their original shape with a fast rate in cell wall recovery. It appears that the rod shape is a strong evolutionary feature and tightly controlled.
GLYCAN FORAGING SYSTEMS SIGN THE ADAPTATION OF CAPNOCYTOPHAGA CANIMORSUS TO THE DOG’S MOUTH.
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Background

*Capnocytophaga canimorsus* (Cc) are Gram-negative bacteria that are part of the normal flora of dogs’ mouth and can cause rare but severe infections in humans which have been in contact with a dog.

Cc grow in co-culture with eukariotic cells by harvesting surface glycans via the PUL5 encoded Gpd complex. PUL5 mutant bacteria show a reduced growth and display coccoid forms, which strongly resemble *E. coli* mutant bacteria that are unable to synthesize aminosugars, and are deficient in peptidoglycan synthesis.

We thus speculated that Cc’s arrest of growth and cell rounding would be due to starvation of aminosugars.

Objectives

Here we address the question why Cc rely on host aminosugars and how they retrieve them from their niche, the dog’s mouth.

Methods

Cc strain 5 (Cc5) genome analysis revealed that Cc5 could not synthesize N-acetylglucosamine (GlcNAc) because of the lack of two enzymes which convert glucosamine into GlcNAc. Heterologous expression of these enzymes as well as supplementation with GlcNAc completely restored the growth and abolished the formation of coccoid forms.

Conclusions

We show that *C. canimorsus* cannot synthesize GlcNAc and thus rely on exogenous GlcNAc which they retrieve by foraging glycans from mucin and N-linked
glycoproteins through two different apparatuses, Muc and Gpd, both related to the *Bacteroides* starch utilization system (Sus).

We propose that the GlcNAc auxotrophy reflects the adaptation of *C. canimorsus* to its host and ecological niche, the dog's mouth.
STREPTOMYCES PEUCETIUS VAR. CAESIUS AND ITS GLUCOKINASES: WHAT THEIR PHYSIOLOGICAL RELEVANCE IS?

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Background

Streptomyces peucetius var. caesius, a relevant industrial strain overproduces the antitumor, doxorubicin. As reported for other secondary metabolites, doxorubicin production is also repressed by glucose. In streptomycetes, the glucokinase (ATPGlk), participates in the process of carbon catabolite repression (CCR). However, the mechanism by which this enzyme exerts its regulatory role, has not been elucidated. In this bacterium, we have reported the presence of an additional glucokinase (PPGlk), which uses polyphosphate as its phosphate donor group. Contrary to what is observed for other Streptomyces such as S. peucetius, S. coelicolor, S. lividans and S. thermocarboxydus, in S. peucetius var. caesius, PPGlk activity is higher compared to that of ATPGlk. A similar situation has been reported for a chlortetracycline overproducer strain of Streptomyces aureofaciens. So far, the implications of the presence of two glucokinases in development and physiology of the genus Streptomyces are unknown.

Objectives

To evaluate the role of both glucokinases in the physiology of S. peucetius var. caesius, their participation in the CCR, morphological development and synthesis of anthracyclines.

Methods

ATPGlk::aac(3)IIV and PPGlk::aadA mutants were obtained by replacing the genes for an apramycin and a streptomycin resistance cassette (aadA), respectively. Strains were grown in different carbon sources, and their ATPGlk, PPGlk, b-galactosidase and glycerol kinase activities were determined. Total anthracyclines and morphological differentiation (microscopy) were also evaluated.

Conclusions

The ATPglk::aac(3)IV mutant showed inability to grow in high glucose concentrations and exhibited deregulation in CCR. In regard to the parent strain, the mutant growth and production of anthracyclines, was delayed on solid medium.
REGULATION OF DIFFERENTIATION AND ANTIBIOTIC PRODUCTION IN STREPTOMYCES GRISEUS

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Background

The genus *Streptomyces* comprises soil-dwelling, filamentous bacteria having great biotechnological significance since these bacteria produce 75% of the known antibiotics. Autoregulatory molecules play a key role in controlling antibiotic production and morphological differentiation. In *Streptomyces griseus* A-factor (2-isocapryloyl-3R-hydroxymethyl-γ-butyrolactone) proved to be significant as the A-factor negative (AFN) mutant had a non-differentiating phenotype.

Objectives

Previous studies showed that the A-factor biosynthetic *afsA* gene was functional and transcribed in the AFN strain. Our aim is to understand the background of the AFN phenotype.

Methods

In this study the production of AfsA protein, aerial mycelium, spores, extracellular protease and antibiotics was followed on rich and minimal medium. To detect the presence of AfsA by Western blotting we produced the AfsA as a GST-fusion protein in *Escherichia coli*. The purified AfsA was used for immunization of rabbits.

Conclusions

The AFN strain did not form aerial mycelium on rich medium due to the lack of A-factor but spores were formed from the substrate mycelium. Aerial mycelium emergence and sporulation were observed on minimal medium in AFN although the production of spores, extracellular protease and antibiotics were decreased compared to the control strain. The AfsA protein was detected in the AFN strain. These data confirm the hypothesis that the presence of AfsA is not enough for normal A-factor production and A-factor is essential for the aerial mycelium but not for spore formation on rich medium. Moreover our data support the existence of a crosstalk between the A-factor mediated and the starvation induced pathway.
Background

The biogenesis of bacterial cell-wall polymers (e.g., peptidoglycan, lipopolysaccharides, teichoic acids) requires undecaprenyl-phosphate (C_{55}-P). The glycan units are linked to this lipid at the inner face of the membrane. Thereafter, the membrane intermediate is translocated in order to transfer the glycan unit to the periplasmic side, where the glycan moiety is transferred to the growing polymer. The process releases undecaprenyl-pyrophosphate (C_{55}-PP). C_{55}-P originates from the dephosphorylation of C_{55}-PP, itself generated by de novo synthesis and recycling.

Objectives

Identification and biochemical characterisation of bacterial C_{55}-P phosphatase.

Methods


Conclusions

Two families of membrane proteins exhibit C_{55}-PP phosphatase activity: BacA and members of the PAP2 super-family. *Escherichia coli* possesses one BacA and three PAP2 proteins (PgpB, YbjG and LpxT), raising the question of the significance of such a multiplicity. LpxT catalyses the transfer of the C_{55}-PP phosphate group onto the lipid A moiety of the lipopolysaccharides (LPS), yielding C_{55}-P and lipid A-1diP. The role of this LPS modification is under investigation (e.g., antibiotic resistance, ion homeostasis, innate immunity). This raises a tantalising hypothesis that the other C_{55}-PP phosphatases could also exhibit such a phospho-transferase activity with different acceptor molecules. Our research project is aimed at the complete functional and structural characterisation of these multiple membrane phosphatases.
Background
Bacterial cell division is carried out at mid-cell position by a group of cell division proteins referred to as the divisome. For the Gram-negative model bacterium *Escherichia coli*, ten of these proteins (FtsZ, -A, -K, -B, -L, -N, -Q, -I, -W and ZipA) are considered as the core players of division. Thus far, interaction networks between individual divisome proteins have been investigated extensively with use of various biochemical and genetic techniques and many interactions between cell division proteins have been found. Still, no oligomeric interactions have been demonstrated that show that the divisome is present as a large multi-protein machinery.

Objectives
To detect whether or not a complete ‘divisome’ assembly is present in the cell.

Methods
Clear Native gel Electrophoresis on mildly solubilized *E. coli* cells, followed by 2D Electrophoresis and Western Blotting.

Conclusions
We describe the finding of a large 1 MDa cell division protein complex in *E. coli*, that includes at least 8 essential division proteins; FtsZ, ZipA, FtsK, FtsQ, FtsB, FtsL, ans FtsN. The complex is present in cultures of rapidly dividing cells, but not when cells are not dividing. Also, we found that a slight overexpression of *ftsQ D237N* (encoding interaction-impaired FtsQ), prevents formation of this 1 MDa complex. Combined, our findings indicate that a large protein complex containing cell division proteins indeed exists.

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Background

Campylobacter jejuni is an asaccharolytic human pathogenic Gram-negative bacterium that utilizes amino acids as carbon and energy source and colonizes the gastrointestinal tracts of warm-blooded animals. Some strains of C. jejuni possesses the enzyme gamma-glutamyltranspeptidase (GGT), which enables growth on glutathione and glutamine by generation of glutamate in the periplasm and contributes to persistent colonization of the avian gut.

Objectives

We have shown that the RacRS two-component system is involved in cytoplasmic glutamate anabolism by regulating the gltBD genes, here we investigated whether the RacRS system also regulates the periplasmic glutamate anabolism by regulating the ggt gene.

Methods

Our results show that RacRS regulates the expression of ggt under low oxygen conditions. Under these conditions, higher transcription levels of the ggt gene and enhanced GGT activity are observed in the wt compared to the racR mutant strain. By using different ggt truncated promoter elements in EMSA and luciferase reporter assays we show that RacR binds directly on a specific location of the ggt promoter. Furthermore we show that under high oxygen conditions ggt expression peaks around end-log phase and GGT activity is highest in stationary phase and is not dependent on RacR. GGT expression and activity is repressed by addition of glutamine to the medium or glutamine catabolic products.

Conclusions

In conclusion we show that the C. jejuni GGT activity is dependent on multiple factors and one of them is the RacRS two-component system.
Background
Antimicrobial peptides (AMPs) are natural antibiotics widespread throughout the animal kingdom, from bacteria to mammals. They are important components of both innate and adaptative immunity, providing protection against a broad-spectrum of pathogens, such as viruses, bacteria, fungi, and parasites.

Objectives
Investigate the effect of two human AMPs (HBD-3 and LL-37) on *Legionella pneumophila*

Methods
In this study, we investigated the action of synthetic LL-37 and HBD-3 on both extracellular and intracellular lifestyle of *L. pneumophila*.

Conclusions
We showed that both peptides exhibit a phase dependent bactericidal effect on extracellular *L. pneumophila*. We also observed that LL-37 and HBD3 inhibit intracellular replication of *L. pneumophila* in macrophages and pneumocytes. We showed by colony counting assays that the adherence and internalization of *L. pneumophila* was not affected in presence of LL-37, but was stimulated with HBD-3, suggesting that this two peptides restrain the bacterial replication by different mechanism of action.

Human AMPs LL-37 and HBD-3 seems to be involved in innate immunity against *L. pneumophila* by acting on the extracellular bacteria and on its intracellular replication.
Background
Membrane proteins perform vital cellular functions like respiration, signaling and nutrient uptake. For proper functioning, conformational dynamics, complex formation and ability to diffuse in the membrane are vital parameters. Despite a lot of work on model membranes, little is known about lateral mobility of proteins in bacteria.

Objectives
To investigate how molecular crowding, protein size and membrane fluidity affect the mobility of membrane proteins in living E. coli bacteria
Methods
Here we use single-molecule wide-field epi-fluorescence microscopy to track the
lateral mobility of seven integral membrane proteins of different size fused to green fluorescent protein in living *E. coli*. We apply a novel method, IPODD to extract accurate diffusion coefficients from the 2-D projected diffusion trajectories along the 3-D curved bacterial membrane.

**Conclusions**

The diffusion coefficients we find are significantly lower than those reported in *in vitro* studies of isolated membrane proteins in GUVs. Our results indicate that molecular crowding in the plasma membrane of *E. coli* substantially slows down trans-membrane protein diffusion. Surprisingly, all seven proteins studied diffuse heterogeneously: they all show a faster and a slower moving component. Our interpretation of the heterogeneity is that it reflects heterogeneity in membrane composition. To further support this interpretation, we stained *E. coli* inner membrane with a lipid-mimicking dye Dil-C12 and tracked its lateral mobility. Strikingly, Dil-C12 diffuses heterogeneously yet again reflecting the dynamic heterogeneous nature of the plasma membrane. Therefore, from these observations, we propose that the plasma membrane of *E. coli* might contain micro-domains with different fluidity that could play key roles in specific localization of proteins.
Bacterial spores

WHEN MICROBIAL SURVIVAL STRATEGIES NEVER STOP TO WONDER:
SPORULATION OUTSIDE FIRMICUTES

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Background
Spore or spore-like structures are only found in four bacterial phyla: Actinobacteria, Cyanobacteria, Proteobacteria and Firmicutes. These structures provide resistance to adverse conditions. The ability to form spores is not, however, a widely spread characteristic and it is restricted to only some orders within those phyla. For example amongst Proteobacteria, solely δ-proteobacteria can produce spore-like fruiting bodies, or so we knew. A novel γ-proteobacterium, Serratia ureilytica str. Lr5/4, was found to produce spores that not only resemble structurally to those produced by endospore-forming Firmicutes, but also provide heat-resistance.

Objectives
The aim of this study is to describe this novel strain and its Firmicute-like spores and to reveal the molecular pathway of this sporulation procedure in comparison to those that are already known.

Methods
Physiological, biochemical, carbon source assimilation and antibiotic resistance tests were performed. Morphology of vegetative cells and spores was described by phase contrast microscopy, SEM, and TEM. Moreover, spores of Lr5/4 were revived after heat-shock tests and shown to contain dipicolinic acid (DPA). These two characteristics were so far unique to the heat-resistant endospores found in Firmicutes. Sequencing and annotation of its full genome has been performed in order to reveal the relationship of spore formation in Lr5/4 to other known sporulation pathways.

Conclusions
It has been previously proposed that the properties of spore formation in non-sporulating species were due to molecular gene transfer. However, the present study rejects this scenario and demonstrates a novel mechanism for the formation of the described spores of S. ureilytica.
**FEMS-1489**

**Bacterial spores**

**BACILLUS SUBTILIS BIOFILMS INCREASE GUT COLONIZATION AND LIFESPAN IN THE ANIMAL MODEL CAENORHABDITIS ELEGANS**

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**Background**

Biofilm proficiency has a great impact in bacteria-host interactions. *Caenorhabditis elegans* and the spore-forming bacteria *Bacillus subtilis* are both common soil inhabitants which are used as models of lifespan studies. However, the role of *in vivo* biofilm development during gut colonization and its effects on animal lifespan are poorly documented.

**Objectives**

Here, we investigated the ability of *B. subtilis* to form a biofilm in the intestine of *C. elegans* and its effects on gut colonization and lifespan of the nematode.

**Methods**

The results showed that wild-type NCIB3610 strain was able to colonize and persist in the gut of *C. elegans* more efficiently than the laboratory strain JH642. The ability to make a biofilm was essential for the observed behavior because FICT-labelled NCIB3610-derived biofilm-mutant cells, but not FICT-wild-type cells, lost the capacity to persist and colonize the nematode gut. *In situ fluorescence microscopy* and beta-galactosidase expression, driven from the promoter of surfactin harbored by *B. subtilis* engineered cells and used to feed the worm confirm the higher ability of NCIB3610, instead of JH642 cells, to colonize the gut of *C. elegans*. The ability to make a persistent biofilm correlated well with a positive lifespan effect of the bacterium on the nematode.

**Conclusions**

*B. subtilis* spores are able to germinate, growth, make biofilms and persist in the intestine of *C. elegans*. In addition, we provide evidences showing that the intestinal life cycle of *B. subtilis* making a biofilm is beneficial and required for the increase for the lifespan of *C. elegans*. 
Background

The North-Western part of Argentina is particularly rich in wetlands located in the Puna at an altitude between 3,600 and 4,600 m above sea level. Here, incidence of high levels of UV radiation and contamination with high contents of toxic metals, particularly arsenic (As) is common. We hypothesize that one strategy to contribute to the remediation of these area could be the use of *Bacillus* spp.

Objectives

We collected soil and water samples from different parts of the Puna and isolated native spore-forming Bacilli. The novel isolates were examined at different growth temperatures and osmolarities on their ability to grow and make biofilms in the presence of toxic concentrations of As and high UV radiation.

Methods

The Bacilli communities were characterized by pyrosequencing 16S rRNA gene amplicons derived from specific PCR oligonucleotides. The Andean *Bacillus* isolates displayed a greater capacity to make persistant biofilms, colonize surfaces (swarming motility) and tolerance to high temperatures, salinity and UV-B / UV-C radiations at levels that were lethal to common laboratory strains.

Conclusions

The present results allow the potential biotechnological application of the isolated *Bacillus* strains with bioremediation potential of pristine environments contaminated with metals.
Bacterial spores

NOVEL GENES INFLUENCING THE GERMINATION RATE OF SPORES OF BACILLUS SUBTILIS FOOD ISOLATES

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Background

Bacillus subtilis responds to starvation with formation of endospores. Spores can easily contaminate food materials and their resistance impedes their removal from food products. In response to various triggers, spores reinitiate vegetative growth via germination. Vegetative cells can subsequently cause food spoilage. Accurate prediction of germination behavior is difficult as differences in germination responses are observed even among various strains of one species. Thus, data obtained for the model strain, B. subtilis 168, cannot be directly applied to food-spoiling strains.

Objectives

The purpose of this study was to elucidate germination behavior of spores of B. subtilis strains that cause food spoilage and to couple the observed germination phenotypes with the strains’ genomic contents.

Methods

Genomes of thirteen B. subtilis industrial isolates were sequenced. Germination of their spores was induced under various conditions and analyzed via absorbance measurements and phase-contrast microscopy. The spore germination responses of different strains were linked with their genomic content using gene-trait matching software.

Conclusions

Strains were divided into two phenotypic groups based on the ability of their spores to germinate, in particular their germination rates. Slower germination correlated with the presence of specific genes in industrial isolates, which are absent in the laboratory strain B. subtilis 168. Insertion of these genes into B. subtilis 168 decreased the rate of germination of its spores. Thus, new genes influencing B. subtilis germination responses were identified. The function of these genes is currently being elucidated. These genes can be used in the future as biomarkers indicating slow germination.
Background

Background: Spores of *Bacillus subtilis* have been used extensively as biological indicators for industrial purposes such as sterilization or decontamination. For several years a number of activities in the field of sterilization of heat sensitive materials by means of non-thermal plasmas have been known. Plasma sterilization methods are characterized by the use of gas or gas mixtures that are partially excited by an applied electric field. Plasma discharges contain a high degree of UV/VUV-radiation, as well as charged particles and free radicals, which exert detrimental effects on microorganisms by damaging genetic material, outer cell layers and proteins.

Objectives

Objectives: We are interested in understanding the protective attributes and molecular mechanisms involved in the *B. subtilis* spore resistance to plasma sterilization.

Methods

Methods: In a systematic manner different *B. subtilis* spores varying in their protection (e.g., coat, crust, DPA, SASP formation) and DNA repair capabilities were studied on their impact towards plasma sterilization.

Conclusions

Conclusions: We will present our recent findings on the protective attributes and molecular mechanisms involved in the spore resistance to plasma sterilization. The multi-layered proteinaceous spore coat, being the first barrier to environmental influences, was shown to be a major factor contributing to spore resistance towards plasma treatment. During spore revitalization, DNA damage accumulated during the dormant spore stage is the subject of a variety of different repair systems. Deficiencies in repair genes of non-homologous end-joining, spore photoproduct
lyase, or nucleotide excision repair led to a significant decrease in spore resistance to low pressure plasma sterilization.
Bacterial spores

SPORULATION TEMPERATURE HAS LIMITED EFFECT ON THE SPORE PROTEOME OF BACILLUS WEIHENSTEPHANENSIS.

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Background

Bacillus weihenstephanensis is a spore forming food pathogen known for its ability to sporulate, germinate, grow and produce toxins at lower temperatures than its close relatives. As such, it presents an increased risk to food safety where standard procedures rely on cold-inactivation of microbes. Properties of the infective form Bacilli, the spore, are known to vary with the conditions under which the spores were produced. For instance, lower sporulation temperature has been indicated to result in a lower wet heat resistance. This resistance is in part attributed to the spore coat layers, proteinaceous layers surrounding the core which contains the bacterial DNA.

Objectives

The aim of this study was to determine the proteomic basis for the heat resistant phenotype. As no specific singular protein can be indicated to confer heat resistance, this supposedly acquired trait can only be appreciated by analysis of the spore proteome as a whole.

Methods

Analysis of the proteome of spores of B. weihenstephanensis strain WSBC10204 produced at either 12°C or 30°C by mass spectrometry provided insight into variations in the protein content of the spore coat at different sporulation temperatures.

Conclusions

Interestingly, only minor differences were observed, suggesting wet heat resistance does not depend on the identity of the proteins making up the coat layers. As wet heat resistance was indeed lower for the spores produced at lower temperatures, low temperature might instead affect the rate of spore maturation instead, a process where spore coat proteins are cross-linked after they have been deposited onto the spore.
Background
Akinetes are resting cells of species members of the Nostocales and Stigonematales orders of cyanobacteria. These are spore-like, non-motile cells that differentiate from vegetative cells and serve a perennating role. During their differentiation, akinetes vary their metabolic activities and cellular pools.

Objectives
Here we follow carbon assimilation in the cyanobacterium *Aphanizomeno ovalisporum* and accumulation of cyanophycin during akinetes differentiation and maturation.

Methods
Photosynthetic assimilation of $\text{H}^{13}\text{CO}_3^-$ was studied by NanoSIMS technology and variations in abundance and distribution of cyanophycin globules were followed by transmission electron microscopy and histochemistry techniques.

Conclusions
NanoSIMS results clearly indicate that during their differentiation, akinetes maintain metabolic activity and assimilate inorganic carbon via photosynthesis. Towards advanced stages of differentiation and maturation, the metabolic activity of akinetes reduced and many of the free akinetes lost their photosynthetic capacity as indicated by changes in $^{13}\text{C}/^{12}\text{C}$ ratio. Nevertheless, a small group of free (mature akinetes) still maintains their photosynthetic capacity. Structural changes during akinetes differentiation observed by transmission electron microscopy and histochemistry techniques, included the accumulation of cyanophycin bodies in akinetes. The rate of cyanophycin accumulation and carbon allocation into this protein storage is currently being investigated in a complementary NanoSIMS analysis.
Background

In response to nutrient limitation Gram positive organisms like \textit{Bacillus subtilis} form dormant spores. These cellular entities are survival capsules, resistant to chemical and environmental assaults. They pose challenges to the food and medical sectors. Upon contact with germinants spores return to vegetative life through a process called ‘germination and outgrowth’. The vegetative cells may cause food spoilage and food borne diseases.

Objectives

To perform a detailed quantitative time-resolved proteomics study of (dormant)spores and their secretome during germination and outgrowth

Methods

Using a quantitative proteomics approach a study of the time resolved break down of spore proteins during early stages of germination was initiated. Spores were germinated in minimal medium supplemented with a germinant mixture (L-Asparagine, D-glucose D-fructose and Potassium Chloride). The spore layers and the secretome were analyzed separately by Ion Trap LC-MS/MS analysis

Conclusions

During germination the action of proteases, lytic enzymes and peptidases in the spore results in proteolysis, cortex hydrolysis and break down of spore dormancy. Partially digested proteins as well as intact proteins are released from the spore into the medium. Study of these proteins by our method allowed us to understand molecular details of the germination process. Current analyses are aimed at correlating the germinating spore proteome to its transcriptome under control as well as thermal and organic acid stress conditions (Keijser et al. 2007 and Ter Beek et al., our unpublished observations). We aim at deconvoluting heterogeneous germination and outgrowth profiles using our live imaging technology (Pandey et al., 2013).
Background

Undomesticated spore formers isolated from the environment or from processing facilities commonly show higher diversity, resistance and more heterogeneous behaviour compared to widely used reference strains domesticated in laboratories and that may have lost relevant traits. Analysis of such undomesticated isolates therefore provides valuable insights in sporulation capacity, spore resistance, germination and outgrowth efficiency that determine survival and fitness in different environmental niches including food and food processing environments.

Objectives

In our project we aim to obtain insight in the diversity among pathogenic Bacillus cereus strains with focus on spore properties. To this end, B. cereus reference strains ATCC14579 and ATCC10987 were analysed and compared to behaviour of strains isolated from food and food production facilities.

Methods

Growth, sporulation and germination capacity of 20 B. cereus strains isolated from different food products and two reference strains was characterized under different conditions. Spore stress resistance and germination characteristics were assessed upon exposure to disinfectants, heat and mild processing technologies. Behaviour of untreated and treated spores was analysed both at population level as well as at the level of individual spores by using Flow cytometry (FCM) in combination with sorting (FACS).

Conclusions

The B. cereus strains displayed a highly diverse response with respect to growth, sporulation, spore resistance and germination capacity. Additionally, spore population behaviour showed substantial differences with some strains displaying a
homogenous and other a heterogeneous performance. We have demonstrated the relevance of including environmental isolates in the studying behaviour of pathogenic spore former *B. cereus* along the different steps of life cycle.
Background

*Bacillus licheniformis*, *B. amyloliquefaciens* and *B. thermoamylovorans* can cause significant non-sterility issues in high heat treated foods due to survival of heat resistant spores that can subsequently germinate followed by outgrowth during the shelf life.

Objectives

To gain improved control of spores of these species in food manufacturing, a genetic basis for spore heat resistance and germination efficiency was investigated by combining spore phenotypes and genome information of individual strains.

Methods

The heat inactivation kinetics of spores of different isolates were determined. In addition, the genomes of over 20 strains within these species were sequenced to investigate a genetic basis for different spore heat resistances.

For *B. licheniformis* (10 strains) and *B. amyloliquefaciens* (9 strains) significant differences in heat resistance of spores were found for different isolates. Strains producing heat resistant spores contained a subset of genes belonging to a cluster that confers high heat resistance in *B. subtilis*.

For spores of *B. thermoamylovorans* (4 strains) spore heat resistance, nutrient germination, non-nutrient germination, and detailed spore counts were determined. Non-nutrient germination using calcium-dipicolinic acid induced good germination of spores of all strains. Poor germination on regular rich cultivation media resulted in gross underestimation of the viable spores truly present.
Conclusions

The above findings shed light on different phenomena that can contribute to spore survival. Such information is important to devise appropriate strategies to enumerate spores in food ingredients and to inactivate spores, leading to improved control of heat resistant spores in foods.
Background
Streptomyces are multicellular bacteria with a complex life cycle. During the reproductive phase aerial hyphae transition into chains of hundred or more spores via a highly orchestrated cell division process. Coordination of sporulation is regulated by the SALP proteins (SsgA-like proteins). SsgA forms an array of foci along the hypha and provides an anchor for SsgB to multimerize on the cell membrane, after which SsgB recruits FtsZ and stimulates FtsZ polymer formation.

Objectives
SsgB is a highly conserved protein that forms trimers. The C-terminal α3 helix is important for trimerization and interacts with α1 and α2 of the neighboring SsgB monomers. To elucidate how SsgB interacts with SsgA and FtsZ, we have created an SsgB mutant library.

Methods
These ssgB variants were introduced in the ssgB mutant strain, after which strains were automatically scored for restored sporulation via a scanner based imaging approach. Over 500 variant SsgB a single amino acid change and these were further analysed.

Conclusions
All sporulating samples were examined with transmission electron microscopy (TEM), which revealed effects varying from changes in spore wall thickness, size distribution, DNA segregation to affecting spore shape. In contrast wild type spores are similar sized, have condensed DNA in the center of the spores and have a thick spore wall.

We have shown that single amino acid changes in SsgB can facilitate many division related phenotypes and ultimately dictate septum orientation resulting in longitudinal division. The latest results in elucidating the function of individual amino acids will be presented.
FEMS-0486
Biofilms

EVALUATION OF TWO SCREENING METHODS FOR DETECTION OF BIOFILM FORMATION AND ITS EFFECT ON ANTIBIOTIC SUSCEPTIBILITY AMONG STAPHYLOCOCCI SPECIES
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Background

Biofilm formation is a hallmark characteristic of staphylococcal infections as it allows attachment to solid surfaces, exchange of genes that can result in more virulent strains, production of high concentration of toxins, evasion of immune defenses and antimicrobial resistance.

Objectives

This study was conducted to evaluate two different screening methods for detection of Biofilm formation and its effect on antibiotic susceptibility among the clinical isolates of different Staphylococci species.

Methods

Seventy five isolates of staphylococci were tested by modified tissue culture plate method (MTCP), congo red agar (CRA) method and tube method (TM) for their ability to form biofilm. Antibiotic susceptibility testing was done for thirty isolates, 15 biofilm-producers and 15 biofilm nonproducers, using broth microdilution assay.

Conclusions

The rate of detection of biofilm formation among the staphylococcal isolates by the MTCP method, the CRA method and the TM were 48%, 38.7% and 25.3% respectively. Comparing to the results of MTCP method as a gold standard, the sensitivities of the CRA method and tube method were 80.6% and 52.8% respectively. Both showed 100% specificity. Regarding the antibiotic susceptibility of the biofilm producers and non-producers, statistically significant differences were detected for oxacillin, ceftriaxone and augmentin susceptibility (P<0.02). All the
isolates were sensitive to vancomycin. The MTCP takes 3 days and many steps. The TM is observer dependent. Congo red agar method can be used as a screening method for detection of biofilm formation among staphylococcal isolates as it is cheap, rapid with no subjective errors and requires less expertise.
FEMS-1037
Biofilms

INFRARED SPECTROSCOPIC ANALYSIS OF XYLELLA FASTIDIOSA BIOFILMS
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Background
Xylella fastidiosa (Xf) affects plant xylem vessels, causing diseases such as Pierce’s disease. As abundant plants of economic relevance may be affected by Xf, it is essential understanding the biofilm formation behavior of Xf, whose genome has been sequenced partly by our collaborators for developing appropriate countermeasures.[1]

Objectives
The appearance of Xf infected olive trees in Italy is of substantial concern due to the first appearance of this species within Europe. Considering the role of biofilms within many human diseases, the investigation of the adsorption behavior of bacteria along with the involved biomolecules is evident.[2][3] Particularly interesting is the formation
of disulfide bonds appearing essential during biofilm formation.[4]

Methods
This study focuses on the analysis of biomolecules involved in Xf biofilm formation via vibrational spectroscopy. In a unique instrumental combination, infrared attenuated total reflection (IR-ATR) spectroscopy and atomic force microscopy (AFM) were applied to characterize the growth media and biofilms associated with Xf.

Conclusions
Experimental and analytical procedures will be discussed providing first insight on the involved molecular processes during biofilm formation and maturation.

SUPPLEMENTARY MICROBIOLOGICAL ANALYSIS OF FILLING MATERIALS REVEALS NEW INSIGHT INTO THE ECOLOGY OF PERSISTENT ENDODONTIC INFECTIONS
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Background

Persistent root canal infections correlate with endodontic treatment failures. Microorganisms have been found in 35%-100% of previously treated root canals. The analysis of the microbiota of endodontic infections is prerequisite for improvement of treatment methods of infected root canals.

Objectives

1. The microbiological analysis of infected root-filled teeth with periapical lesions using culture-dependent and -independent approaches

2. The analysis of the microorganisms adhered to the retrieved endodontic filling materials (EFM) and the comparison with the results gained from the root canal samples alone.

Methods

Twenty patients were enrolled in this study and samples from the corresponding twenty root-filled teeth were taken according to standard protocols. In addition to these samples, the EFM were also analyzed microbiologically using culture and culture-independent methods and transmission electron microscopy. The culture-independent technique was conducted by cloning and sequencing of 16S rRNA amplicons.

Conclusions

Results: Bacteria were revealed in 15 root canal samples and in 18 EFM samples at a concentration ranging from 10^3 to 10^7 cfu/ml. 56 different species were found in association with the root canal filling materials. In contrary, only 47 species could be identified in the root canal samples. 20 species were detected solely in the EFM samples including Aggregatibacter actinomycetemcomitans and Eubacterium nodatum. The most abundant species belonged to the phyla Firmicutes, Actinobacteria and Proteobacteria.
Conclusions: The analysis of microorganisms associated with root-canal filling materials in addition to the root canal samples taken after revision reveals a better insight into endodontic infections and should not be neglected.
Background
The human nasopharynx is colonized by different species of the genus Neisseria, including commensals, such as N. lactamica, but also an important pathogen, i.e. N. meningitidis. In situ, these bacteria are organized in micro-colonies, structures that resemble biofilms and that may represent a defense mechanism for the bacteria to survive in a hostile environment. In vivo, these species must interact and compete for the same niche.

Objectives
Here, we designed novel constructs to generate green and red fluorescent Neisseria strains to facilitate the discrimination between different microorganisms by microscopy in several assays.

Methods
These constructs were stably integrated in a highly conserved region on the chromosome of both species, as evidenced PCR assays, and allowed for constitutive expression of the fluorescent proteins. We next optimized biofilm formation assays and studied the organization of biofilms of independent and mixed Neisseria strains.

Conclusions
Our results showed that indeed biofilms of monocultures of N. meningitidis and N. lactamica are organized as micro-colonies with intervenient spaces. However, the organization of these spaces and the distribution of the biomass varied between strains of the same species. Mixed biofilm assays revealed that both species can differently interact, altering the organization of the biofilms. This work is the first report of the interaction between both species and allows for speculation about the process of human colonization.
CHANGES IN SEWER BIOFILM MICROBIAL COMMUNITIES RELATED TO DOWNSTREAM NITRATE DOSAGE

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Background

Nitrate (NO₃⁻) is an effective and widely used chemical in sewer systems to reduce biogenic sulfide (H₂S) and methane (CH₄) produced in anaerobic rising main pipes. However, the main limitation of this approach is that anoxic conditions must be continuously kept through the whole pipe, otherwise H₂S-producing biofilms prevail.

Objectives

The aim of this study is to determine the effects of the Downstream Nitrate Dosage strategy (DND) on anaerobic sewer biofilms with regards to sulfide oxidation and, for the first time, on methane production.

Methods

Effectiveness of the strategy was assessed on H₂S and CH₄ abatement on the effluent of specially designed laboratory-scale systems that mimics a real sewer biofilms. A combination of process (normal functioning monitoring and batch tests) and molecular (by 454-pyrosequencing) methods were used to investigate the impacts and microbial activities related to the nitrate addition.

Conclusions

Results showed a complete abatement of H₂S generated, due to the growth of a Sulfide-Oxidizing Nitrate-Reducing population. Methane was reduced to 50% while nitrate was added, due to the CH₄ oxidation in the anoxic conditions established at the end of the pipe. An increase of microorganisms of the genera Simplicispira, Comamonas, Azonexus and Thauera was detected during nitrate addition. Regarding anoxic methane oxidation, only one Operational Taxonomic Unit (OTU) was identified, which is likely related with this metabolism. Both sulfidogenic and methanogenic activities resumed upon cessation of NO₃⁻ dosage. Obtained results are relevant for the optimal management of nitrate dosage strategies in sewer systems.
Background

Transport of wastewater along sewer pipes facilitates the formation of microbial biofilms that grow attached to their inner surfaces. Anoxic conditions in sewers favour the production of both sulfide (H$_2$S) and methane (CH$_4$) as end products of anaerobic microbial metabolisms, i.e. sulfate-reduction and methanogenesis, respectively. The build-up of H$_2$S and CH$_4$ in sewerage causes different detrimental effects such as odour, corrosion and toxicity.

Objectives

The aim of this study was to investigate the initial stages of development of microbial biofilms in sewer systems with a special focus on the interactions between sulfate-reducing bacteria (SRB) and methanogenic archaea (MA).

Methods

The work was carried out using a laboratory sewer pilot plant fed with wastewater that mimic the functioning of a real anaerobic pressured sewer. Biological activities and phylogenetic community composition were investigated during sewer colonisation using a combination of molecular techniques (DGGE, qPCR and 16S rRNA tag sequencing) and process data (H$_2$S and CH$_4$ production).

Conclusions

Results showed that the SRB community was established and active after two weeks of biofilm development, causing substantial sulfide accumulation in the pilot plant. In turn, growth of MA and methane production were low during initial stages of biofilm colonisation but readily increased over time after the replacement of initial MA species derived from human faeces (*Methanobrevibacter* spp.) by other MA representatives more adapted to sewer conditions (*Methanoseta* spp.). Altogether, our results pointed to different dynamics and activity of key community members from sewer biofilms, greatly affecting the production kinetics of H$_2$S and CH$_4$ along time.
Background
To better understand the molecular events that occur during *Vibrio cholerae* biofilm formation *in vivo*, we did a previous proteomic analysis of planktonic and biofilm cells, after 14, 24 and 48h of growth under conditions that mimic the intraintestinal environment, namely, pH 7.4 at 37°C, Pi limitation and the presence of the bile salt, sodium deoxicholate (DOC). The groups of differential proteins identified indicated that free cells were at stationary phase, whereas, those in biofilms were at exponential culture phase, after 48h growth. Moreover, in planktonic cells at all times, alkaline phosphatase was produced, suggesting Pho regulon activity, which was not observed in the biofilms cells.

Objectives
To perform a lipidomic analysis to explore differences in lipid profiles between planktonic and biofilm cells of *V. cholerae*.

Methods
Cells were grown overnight in MGLP (MOPS, salts, glucose, pH 7.4 with KH₂PO₄ at 0.65μM-low phosphate) without (free cells) or with 0.2% DOC (biofilm) for 14-48hs, at 37°C, without agitation. The lipids were extracted according to Bligh and Dyer 1959 and analyzed by Q-TOF LC/MS.

Conclusions
In the 14h planktonic cells, the great majority of phospholipids was replaced by ornithine containing lipids (OL). Between 24 and 48h, OLs were found in these cells, but in smaller amounts. In biofilm cells, phosphatidylethanolamine (PE) was the major lipid at all times, whereas OLs were detected in small amounts. These data suggest, for the first time, the involvement of PhoB regulated genes in the expression regulation of OLs and PE in *V. cholerae*. 
ADHESION OF ENTEROCOCCUS FAECIUM ON PLASTIC AND STAINLESS STEEL AND ITS ANTI-ADHESIVE POTENTIAL TOWARDS PATHOGENS

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Background
Bacteria have the ability to attach to surfaces commonly found in the food processing environment, such as polystyrene and stainless steel and to become more resistant to environmental stresses and sanitizers. So the search for new fight strategies is needed

Objectives
In this study, a strain of Enterococcus (En.) faecium, originally isolated from a milking machine inner-surface, was studied for its biofilm formation potential on plastic and stainless steel supports and its antibacterial and anti-adhesive potential against four pathogens (Escherichia coli ATCC 25922, Staphylococcus aureus ATCC 25923, Pseudomonas aeruginosa ATCC 27853 and Listeria innocua CLIP 74915).

Methods
The adhesion and the anti-adhesive potential of this strain were studied (at 25 and 37°C) using polystyrene microtiter plates and stainless steel 304 L coupons after their surface characterization by contact angle method.

Conclusions
The results revealed that the strain was a strong biofilm producer on polystyrene and stainless steel either at 25 and 37°C with the best adherence level observed at 25°C. En. faecium cells and culture supernatant showed a significant (p<0.05) inhibition potential of the pathogens most probably due to bacteriocins synthesis.
ARCHAEOLOGICAL BIOFILMS: TOLERANCE OF THE THERMOACIDOPHILIC ARCHAEOLE SULFOLOBUS ACIDOCALDARIUS TO 1-BUTANOL EXPOSURE

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Background

Archaeal biofilms are interesting for biotechnological applications like whole cell biocatalysis, because they promise a higher tolerance towards toxic compounds, which is a characteristic property known for biofilms. In this study the effect of the organic solvent 1-butanol on biofilms of the thermoacidophilic, aerobic crenarchaeon Sulfolobus acidocaldarius with growth at 78 °C and pH 3 was investigated. S. acidocaldarius is adapted to extreme environments, genetically tractable and able to form biofilms.

Objectives

The aim of this study was to characterize the tolerance of S. acidocaldarius biofilms to 1-butanol exposure. The effect to 1-butanol on biofilm formation as well as on established biofilms was investigated.

Methods

Submerged biofilms were grown (4 d, 78 °C) in 96-well microtiter plates, Petri dishes or µ-dishes on either polystyrene or glass surfaces. 1-butanol was present during biofilm formation or added to already established biofilms. Analysis of biofilms was performed by microscopic methods (confocal laser scanning microscopy, epifluorescence microscopy, atomic force microscopy, environmental scanning electron microscopy), and microbiological and biochemical methods (determination of total cell counts, viable counts and aerobic respiration activity). Extracellular polymeric substances were isolated and quantified by colorimetric assays.

Conclusions

S. acidocaldarius tolerates 1-butanol concentrations up to 1.5 % [v/v] as validated by viability assays. 1-butanol exposure led to stress responses like aggregation of cells, a change of carbohydrate distribution and composition as well as carbohydrate and
protein amounts in the biofilm matrix. Butanol-exposed biofilm cells revealed alterations of shape and loss of cell appendages.
Background

*Pseudomonas aeruginosa* is the most common Gram-negative bacteria causing fatal nosocomial infections in hospitalized patients especially with the immunocompromised subjects. Bacteria can resist a wide variety of currently available antibiotics. One of resistant mechanisms is by forming the biofilm in infected host preventing drugs to access to the respective targets of the bacterial cell. Novel strategy to overcome this antibiotic resistant mechanism is to use the combination of antibiotics with inhibitors of biofilm formation.

Objectives

The objective in this study is to investigate the effect of mulberry-leaf, fruit and goji berry on biofilm formation from *P. aeruginosa*.

Methods

The result was shown by specific biofilm formation (SBF) index and the ethanolic extract (256 mg/ml) and the ether extract (32 mg/ml) of mulberry-leaf presented strong activity (SBF index > 1.1) to reduce biofilm in 44.0 % and 44.8 %, respectively. The plant extract with anti-biofilm activity was tested further in combination with gentamicin (0.125 to 1024 µg/dl) by Checkerboard method indicated that there was no synergistic efficacy. The results of MBEC and CLSM revealed that the ethanolic extract (32 mg/ml) was effective on *P. aeruginosa* biofilm.

Conclusions

The extract of mulberry-leaf could be an alternative thai herbs for biofilm formation inhibiting the most common drug resistant bacteria.
Background

In natural and man-made ecosystems, anammox bacteria demonstrate a strong tendency for attached growth and biofilms formation.

Objectives

We studied biofilms structure and formation by microbial community with dominant group of anammox bacteria, enriched in anaerobic vertical upflow bioreactor with nitrogen load 5 g N/l per day and upflow vertical concentration gradient of nitrogen substrates and pH.

Methods

Biofilms structure and microbial community composition were studied via microscopy (light, electron, confocal and atomic force) and molecular genetics methods (sequencing and FISH). Biofilm formation de novo was studied on microscopic slides submerged in the upper part of the bioreactor.

Conclusions

Several groups of microorganisms are involved in biofilm formation. Anammox bacteria belong to three different species. Their satellites are: trichal forms (presumably Chloroflexi), coccoid and rod-shaped cells (likely different species of nitrifier and denitrifier communities involved in nitrogen removal and trace amounts of oxygen consumption). Chloroflexi community includes 3 phylotypes of Anaerolineae and Dehalococcoidia strains responsible for biofilm spatial structure formation and trace amounts of organics elimination.

Primary biofilms are discovered on day 10-12. Subsequent growth of biofilms appears due to increasing the number of cells involved in biofilms and extracellular polymeric matrix accumulation. Mature biofilms (80-90 days) include cells of the same morphotypes as mature biofilms inside the reactor: clustered cocci with typical
anammox ultrastructure, interstitial rods and remarkably long trichal cells (more than 100 µm long). Surface microrelief images of such biofilms were obtained via atomic force microscopy.
ASSESSMENT OF BIOFILM DESTABILIZATION AND REMOVAL BY LAUNDRY AGENTS

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Background

The formation of biofilms is a well known phenomenon in a variety of technical and natural environments such as rivers, water systems or washing machines. They can harbor a potential risk to human health serving as habitat for pathogens. Furthermore, biofilm growth within washing machines leads to biocorrosion and unpleasant odours. Therefore, the removal or prevention of biofilm growth is of great interest.

The relevance of biofilm EPS in terms of bacterial adhesion to surfaces has been indicated in several studies. Therefore, EPS can be one working point for laundry agents for the destabilization and detachment of biofilms.

Objectives

The objective of this investigation was to test the efficacy of different laundry agents on biofilms.

Methods

Biofilms grown in rinse water were treated with cleaning bases in a flushing chamber. Several concentration and fluxes of cleaning bases were tested. The attack of the cleaning agents on EPS components was analyzed by confocal laser microscopy after simultaneous staining of bacteria, proteins and polysaccharides.

Conclusions

Our findings showed that cleaning agents did not contribute an attack on specific polymer-groups of the EPS. The selection of the detergent as well as the applied shear forces were of rather great impact. Laundry agents containing oxidative bleach were much more effective than agents containing low or non bleach. CLSM images revealed the compact biofilm structure changed into a small-sized structured biofilm. Worst results were obtained by using liquid detergents. Furthermore, shear forces were of greater impact than the flushing duration and supported the effects of cleaning agents.
RESPONSE OF SESSILE CELLS OF CANDIDA SPECIES TO OXIDATIVE STRESS

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Background

The biofilms of Candida species are associated with high indices of hospital morbidity and mortality [1,2]. Sessile cells are highly resistant to antifungals and to the host mechanisms of defense [1,3-5]. Though the ability of some Candida species to form biofilms on various medical implants has been evaluated as well as their response to oxidative stress [4,6], it is however important to investigate how the reactive oxygen species impacts on sessile cells of different Candida species.

Objectives

To evaluate the response of oxidative stress on biofilm formation by C. albicans, C. glabrata, C. krusei and C. parapsilosis.

Methods

Sessile and control cells of Candida species were exposed to increasing concentrations of H₂O₂. Biofilms were observed by scanning electron microscopy and quantified by the XTT assay.

Conclusions

To our knowledge, this is the first report describing the effect of oxidative stress on biofilm formation by four Candida species in vitro. Our results suggest that candidiasis in vivo is a multifactorial and complex process where the human immune system and the adaptability of the pathogen should be considered altogether to provide an effective treatment of the patient.
Background

The *fap* operon is present in the majority of *Pseudomonas* species and encodes for the Fap functional amyloid system, which is associated with cell aggregation and enhanced biofilm formation. Six genes make up the operon.

Objectives

However, it is so far unknown how the *fap* operon is regulated.

Methods

In this study, a biotinylated dsDNA probe was constructed in order to isolate potential DNA binding molecules, which specifically associate with the promoter region of the *fap* operon of the amyloid expressing *Pseudomonas* sp. UK4 and potential transcription factors were identified using LC-MS/MS taking advantage of a closed genome sequence. Two global regulators (MvaU and MvaT) were found to interact specifically, with the *fap* promoter. Specific binding of the alternative sigma factor (RpoN) was also observed. The latter observation is interesting as a gene encoding an RpoN activator protein is located next to the *fap* operon. Therefore, we created overexpression and deletion mutants of *Pseudomonas* sp. UK4 with the RpoN activator protein and the two general transcription regulators MvaU and MvaT.

Conclusions

A hydrolysis probe based qPCR assay was developed in order to determine the effect of all these gene regulatory players on the *fap* operon. Consequently, the effect of elevated and decreased levels of c-di-GMP was also studied and experiments will show how these regulators affect transcription of the *fap* operon. This study contributes with an increased understanding of the transcriptional machinery that
control Fap-mediated biofilm formation. In the future, this regulatory system could be an important target in antibiofilm strategies.
EFFECT OF DENTAL BIOMATERIALS ON STREPTOCOCCUS MITIS/HUMAN GINGIVAL FIBROBLASTS CO-CULTURE
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Background

The biocompatibility of oral biomaterials depends not only on the biomaterial properties, but also on the host and oral indigenous microbiota response.

Objectives

A co-culture model of \textit{Streptococcus mitis}/Human Gingival Fibroblast (HGF) was set-up to evaluate the biocompatibility of: 2-hydroxyethylmethacrylate (HEMA), triethyleneglycoldimethacrylate (TEGDMA) and new synthesized bioactive lactose-substituted chitosan-silver-nanoparticles (Chitlac-nAg) in colloidal solution and as thermosets coating.

Methods

\textit{S. mitis} was co-cultured with HGFs with the above mentioned materials in presence of saliva in terms of bacterial adhesion and aggregation, HGF cell toxicity, expression of apoptosis-associated proteins and intracellular signaling.

Conclusions

HEMA treatment showed an increased aggregation and adhesion of \textit{S. mitis} on HGFs, mediated by the PKCa/integrin b1 signalling system. HEMA treatment decreased viable eukaryotic cell that was balanced by the presence of bacteria and saliva.

Concerning TEGDMA, \textit{S. mitis} growth was not decreased and large bacterial aggregates were present on HGFs. Moreover, \textit{S. mitis} and saliva reduced the HGFs oxidative stress and apoptosis induced by TEGDMA treatment.

Chitlac-nAg colloidal solution, in the HGF/\textit{S. mitis} co-culture, induced less numerous viable bacteria adhering to the HGFs, in respect to the control. For coated thermosets, our results showed that the Chitlac-nAg coated thermosets cytotoxicity exerted on HGFs was absent in the co-culture model, where HGFs adhere and migrate on biomaterial.

Taken together, our results shed new light on the interaction of dental biomaterials/\textit{Streptococci}/HGFs occurring in oral environment, underling the
importance of the co-culture model, resulting closer to the *in vivo* situation, to obtain a more realistic outcome.
IMPACT OF BIOFILM GROWTH PHASE, DIVERSITY AND EPS QUALITY ON THE REMOVAL EFFICIENCIES OF BIODISPERSANTS USED FOR REDUCING BIOFILM ADHESION IN COOLING TOWERS

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Background

Biocides are commonly used to control unwanted biofilm development in cooling tower. The more environmentally friendly biodispersants can also be used, but little information is available on the efficiency of their application, likely affected by the biofilm structure, composition and growth phase.

Objectives

The objective of this work was to study the structure and diversity of biofilms developed from makeup water used in a full scale industrial cooling tower. The efficacy of the biodispersant was further evaluated on biofilms at different growth phase.

Methods

Biofilms were grown on glass slides, in a batch system, using as source community the cooling tower makeup water under four different nutrient conditions. The taxonomic bacteria affiliation was investigated by Fluorescence In Situ Hybridization (FISH) and diversity index was calculated. EPS were detected in situ by epifluorescence microscopy after staining with fluorochromes. A biodispersant commonly utilized in full scale cooling towers was then used and removal efficiencies at different stage of development were evaluated.

Conclusions

FISH allowed to identify about 90% of total bacteria, mainly belonging to Proteobacteria. Starting from the source community, biofilms showed an initial increase in biodiversity likely due to the functional niche diversification. Then, diversity decreased, mainly due to the adhesion of eukaryotes and to the concurrent reduction in bacteria taxon richness. The removal efficiencies of biodispersant decreased over time likely due to the higher biofilm stability at the late growth stage, where an increase of α and β glucans, mainly found surrounding eucaryotic and
alphaproteobacterial cells, was observed.
ΔPPIB ENHANCED MOTILITY AND BIOFILM FORMATION ABILITY IS POSSIBLY MEDIATED THROUGH CHANGES IN THE PPIB INTERACTIONS WITH SOME OF ITS PREY PROTEINS

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Background

E. coli ppiB is a peptidyl-prolyl cis/trans isomerase with chaperone activity. ΔppiB strain shows enhanced swimming and swarming motility as well as biofilm formation ability.

Objectives

We hypothesize that the effects of ppiB on such cellular behaviors are likely accomplished through its protein interactions. Using a candidate approach we aim to identify multicopy suppressors of the ΔppiB phenotypes and to characterize protein interactions with putative ppiB prey proteins.

Methods

Based on data provided by IntAct database we considered available ppiB prey proteins as putative interacting partners. To address whether these proteins could be direct effectors inhibiting motility or biofilm formation we over-expressed each of them in the ΔppiB cells and checked for restoration of the corresponding phenotypes. Further, we examined possible protein interactions with ppiB under native or denaturing prey conditions. To explore the potential involvement of the PPIase active site on the binding of ppiB to these proteins, we determined the inhibitory effect of each of them on the PPIase activity of ppiB.

Conclusions

Both the hyper-motility and enhanced biofilm formation ability of ΔppiB strain was reversed by over-expression of certain prey proteins albeit different in each condition. With very few exceptions, we were not able to detect stable protein complexes, probably an indication for weaker transient interactions. However, under denaturing prey conditions, more interactions were revealed suggesting that ppiB is able to recognize denatured preys. A decrease in the ppiB PPIase activity, in the presence of many of them, further supports many of the putative associations.
EFFECT OF SHEAR STRESS ON PSEUDOMONAS AERUGINOSA ISOLATED FROM THE CYSTIC FIBROSIS LUNG

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Background
Chronic Pseudomonas aeruginosa lung infections are the main cause of morbidity in cystic fibrosis (CF) patients. Previously, we have sequenced the genome of the transmissible Belgian epidemic strain CF_PA39. This CF-adapted strain has been present in the UZ Brussel hospital environment for at least ten years. Furthermore, earlier experiments showed that culturing P. aeruginosa PAO1 in a low fluid shear environment, obtained by means of the Rotating Wall Vessel (RWV) technology, leads to the formation of a biofilm phenotype comparable to that observed in the CF lung.

Objectives
To study the effect of fluid shear on biofilm formation by an adapted P. aeruginosa CF strain in artificial sputum medium (ASM) at the genotypic, transcriptomic, and phenotypic level.

Methods
In this study, an RWV experiment was performed that closely resembled the in vivo situation by inoculating this adapted CF isolate in ASM in the RWV either with or without two glass beads, simulating high and low fluid shear conditions, respectively. Scanning electron microscopy (SEM) was utilized to determine biofilm formation in both conditions, while RNAseq and qPCR was used to study gene expression in both conditions.

Conclusions
Increasing fluid shear in the RWV model disrupted biofilm formation of an adapted P. aeruginosa CF isolate.
In accordance with the biofilm disruption, several genes involved in denitrification, tryptophane synthesis, choline metabolism, and alginate biosynthesis were down-regulated in the high fluid shear condition, resembling the planktonic stage of growth. Furthermore, we identified small RNAs that are differentially expressed.
ZINC OXIDE NANOROD PHOTOCATALYTIC COATINGS FOR BIOFILM PREVENTION
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Background
It has been shown that zinc oxide (ZnO) nanostructures can photocatalytically inhibit growth of fresh water bacterial and fungal strains under solar irradiation.

Objectives
The objective of this study was to investigate the prevention of formation of marine biofilms by ZnO nanorod coatings in the laboratory and out-door experiments.

Methods
ZnO nanorod coatings were fabricated on microscope glass substrates by a simple hydrothermal technique using equimolar solutions of 10 mM zinc nitrate and hexamethylenetetramine. In laboratory conditions, upon 5 h of white light irradiation (light intensity= 100 Klx from a tungsten-halogen lamp), nanorod coatings significantly reduced the growth of marine bacterium Acinetobacter sp. and inhibited biofilm formation as compared to coatings kept in the dark (not exposed to light). In the continuous 7 days out-door experiment conducted in an aquaria (volume = 70 L) with sea water collected from Oman sea (Muscat, 23°34′55″ N, 58°36′27″ E), the nanorod coatings significantly reduced density of bacteria in comparison to the control (no coatings). In the absence of sunlight, test and control slides were equally colonized by bacteria. De novo sequencing of bacterial biofilms on MiSeq system® demonstrated that different communities were formed in the presence and absence of light on nanorod coatings.

Conclusions
Our study suggests that ZnO nanorod coatings effectively prevent biofilm formation and can be used as a novel green antifouling technology.
Bacterial Alpha-Amylase Enzyme with Inhibition Effect on Biofilm Formation in Clinical Isolates of Acinetobacter baumannii

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Background
Acinetobacter baumannii is a pathogen with a high quality to form biofilm which can be isolated from different environmental sources. In addition, pathogenicity and biofilm formation associated with infectious agent.

Objectives
In this study, we investigated that degradation of biofilm formation by Acinetobacter baumannii is determined with amylase enzyme from Bacillus amyloliquefaciens.

Methods
Bacillus amyloliquefaciens is amylase enzyme producer which was obtained from Hacettepe University Department of Biology/Biotechnology. The biofilm forming ability in clinical isolates of Acinetobacter baumannii was determined by both microscopic and spectrophotometric analyses with using 24-well polystyrene plates.

Conclusions
According to results, amylase which obtained from Bacillus amyloliquefaciens was observed to inhibit biofilm formation in Acinetobacter baumannii. In continuous, amylase enzyme prevent to increase Acinetobacter baumannii cell population. As well as, in most of pathogen bacteria like Acinetobacter baumannii, amylase enzyme is considered to degradation of biofilm formation. In a result, amylase enzyme can be used as a drug supplement.
EFFECT OF SUBINHIBITORY CONCENTRATION OF SILVER NANOPARTICLES ON ADHESION OF GRAM-POSITIVE AND GRAM-NEGATIVE BACTERIA ONTO POLYSTYRENE SURFACES

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Background
In biofilms, bacterial cells adhere irreversibly to a solid surface by extracellular polymeric substances. This way of life provides several advantages over the planktonic mode, as responses to environmental stress.

Objectives
The objective was determined the effect of subinhibitory concentration of silver nanoparticles (Ag-NPS) and silver nitrate (AgNO₃) on adhesion of gram-positive and gram-negative bacteria.

Methods
Ag-NPs were synthesized by reduction of AgNO₃ with sodium citrate. Bacterial strains used were gram-positive Staphylococcus aureus ATCC 6538, Bacillus cereus, isolated from milk cooling tank and Enterococcus faecalis ATCC 51299 and gram-negative Escherichia coli ATCC 11229, Salmonella enterica serovar Typhimurium ATCC 13076 and Pseudomonas aeruginosa ATCC 15442. The minimum inhibitory concentration was determined by macrodilution technique according CLSI, 2003. Müller-Hinton broth supplemented with subinhibitory concentration of Ag-NPs or AgNO₃ were transferred to 96-wells polystyrene microtiter plates, and inoculated with 5.0 x 10⁵ cfu/ml of cell suspension. After 48 h at 37°C, the optical density was determined at 600 nm and supernatant was discarded. The surface-attached cells were stained with crystal violet for 30 min and washed three times with water. The absorbance at 590 nm was determined by addition of ethanol.

Conclusions
Adhesion was estimated by calculating the ratio between the absorbance of violet crystal and the optical density of cells. Subinhibitory concentrations of Ag-NPs and AgNO₃ increased the adhesion of the gram-positive bacteria, probably, as response to stress. The adhesion of E. coli and S. Typhimurium was not affected by presence of Ag-NPs or AgNO₃. However, P. aeruginosa adhesion decreased in the presence of Ag-NPs.
Candida glabrata biofilms response to amphotericin B

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Background
Candida species are responsible for recurrent human infections, mostly in immunocompromised patients, due to their high vulnerability. Candida glabrata has been showing to have a major role in these infections being the second most prevalent species involved in human fungemia. Amphotericin B (AmB), a common antifungal drug, is a hospital-environment exclusive polyene, normally being efficient when used to fight candidiasis.

Objectives
The main goal of this work was to infer about the influence of AmB in Candida glabrata biofilms formation and its effect on matrix composition and ERG genes expression.

Methods
Candida glabrata biofilms were formed in the presence of AmB and analyzed by dry weight. Moreover, ERG genes expression was evaluated by qRT-PCR and matrix was analyzed in terms of composition in carbohydrates, proteins, beta-glucans and a new finding: ergosterol.

Conclusions
In addition to an inefficient reduction of the C. glabrata biofilms, this work showed that ERG genes seem to be less involved than the matrix composition in C. glabrata biofilms response to AmB. Specifically, C. glabrata biofilms matrices respond with an increase of carbohydrates, particularly beta-1,3 glucans, and with a decrease of total proteins. The ergosterol values did not expressively changed in the presence of AmB.

The present work support the theory of multifaceted mechanisms developed by C. glabrata biofilms as response to the presence of AmB.
FLOW CELL ANALYSIS OF THE PROCESS OF BACTERIAL CELL ADHESION MEDIATED BY THE ADHESIVE NANOFIBER PROTEIN ATAA UNDER SHEAR STRESS

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Background

Elucidation of bacterial adhesion is important for preventing detrimental biofilm formation and for applying it to microbial immobilization. An environmental strain, Acinetobacter sp. Tol 5 shows nonspecific, remarkably high adhesiveness to solid surfaces through AtaA (Acinetobacter trimeric autotransporter adhesin) nanofibers.

Objectives

We aim to examine the effects of flow rate on the adhesion and autoagglutination of resting Tol 5 cells to reveal adhesion property mediated by AtaA.

Methods

Bacterial adhesion was analyzed through using a flow cell system and the shear stress of the flow was controlled by flow rates and the viscosity of cell suspension supplied with PEG-400. The biomass of attached cells was quantitatively measured by staining with crystal violet. The thickness of the cell clump adhered on the surface was measured by confocal laser scanning microscopy.

Conclusions

Higher shear stress in the flow cell decreased bacterial adhesion and the thickness of cell clump formed on the flow cell surface. By contrast, the bacteria adhesion to well-plates was increased by mild agitation compared with that in a static condition due to enhanced autoagglutination of the cells. These results suggest that there is the optimum flow rate for cell adhesion in the balance between enhancement of autoagglutination due to the increase in the collision probability of bacterial cells and detachment of cells and/or inhibition of cell-cell or cell-surface interaction by shear stress.
HRPB DEAD-BOX RNA HELICASE IS IMPORTANT FOR MOTILITY, BIOFILM FORMATION AND CANKER DEVELOPMENT IN XANTHOMONAS CITRI SUBSP. CITRI

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Background

DEAD-box RNA helicases are proteins highly conserved, present in all eukaryotic cells and in many bacteria. DEAD-box RNA helicases are involved in various processes of RNA metabolism and play important roles, e.g., translation initiation, ribosome biogenesis and RNA decay. Several bacterial DEAD-box proteins have been studied extensively due to their importance in *E. coli*, *Bacillus subtilis* and *Staphylococcus*. Interestingly, in *Staphylococcus aureus*, the DEAD-box protein CshA was involved in biofilm formation via modulation of *agr* mRNA stability. However, the function of DEAD-box RNA helicases remains unexplored in relation to adhesion, biofilm formation or pathogenicity in *Xanthomonas citri*.

Objectives

To characterize the function of DEAD-box RNA helicase (XAC0293/HrpB) in relation to motility, biofilm formation and citrus canker development in *Xanthomonas citri* subsp. *citri*.

Methods

We conducted assays for analysis of sliding motility, biofilm formation on biotic surface and pathogenicity of the wild type and the *hrpB* mutant of *X. citri* subsp. *citri*. Expression of different genes between the wild type and mutant strains was determined by quantitative RT-PCR.

Conclusions

Deletion of the *hrpB* gene reduced the expression of type IV pili genes, which are required for motility, biofilm development and adherence. Our data show that HrpB regulates the mobility and adherence of the *X. citri* in host. HrpB hereby is the first DEAD-box RNA helicase in *Xanthomonas citri* that is implicated in the regulation of
pili genes and that it is involved in motility, biofilm formation and citrus canker development.
COMPARATIVE GENOMICS OF IRON TRANSPORTING SYSTEMS AND FUNCTIONAL ANALYSIS IN BACILLUS CEREUS STRAINS FOR GROWTH AND BIOFILM FORMATION
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Background
Human pathogens such as Bacillus cereus are in constant competition for iron with other microbes and their host. In most environments, including food and mammal hosts, iron is bound to complex compounds and is not readily available. However, pathogenic bacteria developed mechanisms to scavenge iron from a range of sources.

Objectives
The objective of this study was to investigate the use of different complex and non-complex iron sources by Bacillus cereus reference strains ATCC14579 and ATCC 10987, and 20 undomesticated food isolates, for which growth phenotypes were linked to genotypes.

Methods
Genome sequences were analysed for the presence of putative iron transport systems. Growth and biofilm formation were monitored in LB with and without iron scavenger Bibyridine and different iron sources. Transcriptome studies in B. cereus ATCC10987 revealed upregulation of many putative iron transport systems upon depletion of iron.

Conclusions
All 22 B. cereus strains could effectively use Fe citrate and FeCl₃ for growth, and formation of air-liquid interface biofilms was promoted. Hemoglobin and Hemin were used by all, except one strain lacking functional petrobactin and IIsA systems. Interestingly, Hb and Hemin triggered a submerged type biofilm formation by several strains. Ferritin and transferrin could be used for growth only by three and six strains respectively, but biofilm formation was inhibited. Lactoferrin did not restore the growth, however it supported submerged biofilm formation by several strains. Knowledge of iron transport systems and their functioning can serve as an indicator for bacterial fitness in different environments including usage of host-derived iron sources.
MICROSCOPIC ANALYSIS OF THE PROCESS OF BACTERIAL CELL ADHESION AND AUTOAGGLUTINATION MEDIATED BY THE ADHESIVE NANOFIBER PROTEIN ATAA
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Background

Elucidation of bacterial adhesion is important for preventing detrimental biofilm formation and for applying it to microbial immobilization. An environmental strain, Acinetobacter sp. Tol 5 shows nonspecific, remarkably high adhesiveness to solid surfaces through AtaA (Acinetobacter trimeric autotransporter adhesin) nanofibers.

Objectives

We aim to reveal the mechanism of cell adhesion mediated by AtaA and describe the cell adhesion process in a microscopic level.

Methods

The time profile of cell adhesion was analyzed through confocal laser scanning microscopy (CLSM) and flow cell analysis. The mechanism of autoagglutination was described by clustering process of colloidal particles by measuring the surface potential of Tol 5 cells with and without AtaA nanofibers.

Conclusions

By the CLSM, Tol 5 wild-type (WT) but not ΔataA mutant was revealed to form a thick cell layer (> 30 μm) with void structure on a glass surface. The process of the cell clumping was described by DLVO theory and CCA model, which were developed as colloidal aggregation models. The autoagglutination of Tol 5 cells was realized by Brownian motion and the collision of AtaA fibers, but not that of cell bodies. By flow cell analyses, it was demonstrated that autoagglutinated WT cells attached well to a glass surface, whereas its single cells hardly attached. These results suggest that autoagglutination greatly contributes to cell adhesion due to enlarged particle size.
Background
Candida albicans is a major fungal pathogen of humans. It is a commensal on the skin and mucosal surfaces, but can cause invasive and systemic infections particularly in the immunocompromised patients. C. albicans can easily form biofilm on supporting surfaces, such as mucosal surfaces and indwelling medical devices. Biofilms enhance C. albicans resistance to antifungals and may help cells to escape from the host immune systems.

Objectives
The identification and study of transcription factors related to biofilm formation can provide important insights into molecular mechanisms controlling biofilm formation.

Methods
The systems biology approach is a novel trend in biological research that focuses on the complex interactions among genes, proteins, and intracellular metabolites. Using systems biological approach, we identified several transcription factors that may involve in biofilm formation. MSS11-deleted mutant showed a defect in forming a mature biofilm and partially attenuates the virulence of C. albicans in a mouse model of infection. In contrast, deletion of the gene encoding another transcription factor enhanced cell adhesion and biofilm formation compared to wild type. Interestingly, this mutant also showed an increase in the expression of adhesin genes.

Conclusions
Taken together, our results support applying the systems biology approaches to study various aspects of fungal biofilm formation and pathogenesis.
Background
Francisella novicida forms a robust biofilm on most surfaces, whereas F. tularensis subsp. tularensis and subsp. holarctica form little or only a poor biofilm. F. novicida colonies are also dark red on Congo Red agar, whereas F. tularensis colonies are light salmon. However, colonies of F. tularensis lipopolysaccharide O-antigen mutants are also dark red in color. We hypothesized that the O-antigen, or other surface components, of F. tularensis interferes with biofilm formation.

Objectives
Our objectives were to determine if mutants of F. tularensis lacking O-antigen and/or a capsule-like complex (CLC) were more capable of forming a robust biofilm than parent strains.

Methods
F. novicida, F. tularensis (types A and B), and mutants were grown in polystyrene wells or on glass coverslips, and biofilms examined by crystal violet staining, confocal laser scanning microscopy, and scanning electron microscopy. Biofilm-associated exopolysaccharide (EPS) was isolated by enzyme digestion and phenol extraction, and composition determined by gas chromatography-mass spectrometry.

Conclusions
O-antigen mutants of F. tularensis made a more robust biofilm than parent strains within 10 days incubation. However, only a mutant lacking both O-antigen and CLC attached better and made significantly more biofilm than the parent after 15 days incubation. Some F. novicida O-antigen mutants also formed significantly more biofilm than the parent. Optimum biofilm formation was dependent upon the growth medium. A novel EPS was isolated and identified as glucan from the F. tularensis biofilm. We conclude that the O-antigen and CLC interfere with F. tularensis biofilm formation, likely through interfering with attachment.
Background

The development of reliable models of biofilm growth in biofilters at the industrial scale, suffers from a lack of 3D measurements at the scale of the biofilter’s constituents. Currently, only three experimental studies, using Synchrotron X-ray Microtomography, proposed protocols based on the use of different contrast agents to image such complex media at the pore scale.

Objectives

The aims of the present study, based on the adaptation of these protocols to laboratory X-ray microtomograph, are threefold:

(i) the 3D visualization and quantification of the interface between the biofilm and the bulk fluid. This will give us access to the shape, the spatial distribution and volume of the biofilm at the pore scale;

(ii) the numerical evaluation of physical properties such as permeability or diffusivity arising in macroscopic models using up-scaling methods;

(iii) the evolution of the aforementioned biofilm characteristics with time and according to the operating conditions (flow rate, nutrient conditions.)

Methods

Thus, the *Pseudomonas putida* biofilms were grown on glass beads in small biofiltration columns. A new protocol permitting to avoid the use of contrast agent was proposed and 3D X-ray images of the biofilms grown under various operating conditions were obtained (i,iii).

Conclusions
The 3D microstructural properties were quantified using appropriated image processing tools (i) and the physical properties were estimated using numerical tools (ii). The representativity of the imaged volume was checked for both structural and physical properties giving access to some of the missing elements to build the macroscopic model.
ANTI-BIOFILM FORMING EFFECT OF ESSENTIAL OILS ON LISTERIA MONOCYTOGENES IN MONO- AND MIXED CULTURES

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Background

Listeria monocytogenes can be found frequently in mono- or mixed culture biofilms. These make sanitization difficult and can lead to cross-contamination and food safety problems. Beside heat treatment, preservatives can be used to eliminate pathogens but sometimes they are not effective and can produce unpleasant by-products. Application of natural antimicrobial agents like essential oils (EOs) could be a potential solution.

Objectives

Our aim was to investigate the anti-biofilm forming effect of selected EOs and their major components on L. monocytogenes biofilms and on mixed-cultures of L. monocytogenes and E. coli.

Methods

MIC values were determined by micro-dilution method. Biofilm formation was conducted in microtiter plates; after 4 h adhesion time, fresh medium containing EOs or components was added in MIC/2 concentration. Biofilm formation was monitored by crystal violet staining. For mixed cultures L. monocytogenes and E. coli were mixed in 1:1 ratio and concentration intervals between the MIC values of the two bacteria were used.

Conclusions

In case of monocultures, cinnamon and cinnamaldehyde have the best anti-biofilm formation effect. For mixed cultures, the effect of the EOs was concentration dependent: α-pinene and linalool showed better anti-biofilm forming effect than the parent essential oil.
CHARACTERIZATION OF NaCl STRESS-INDUCIBLE PALMELLOIDS AND UNDERSTANDING THE ROLE OF A STRESS-RESPONSIVE GENE, BOLA FROM THE GREEN CHLOROPHYTE CHLAMYDOMONAS REINHARDTII.

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Background
Chlamydomonas reinhardtii, a unicellular green alga, is a preferred model organism for studying stress-induced responses. Depending on the stress dose and time of exposure, the flagella of this organism are paralyzed / lost and later cells manifest ‘palmelloids’ or apoptose. Palmelloids, akin to bacterial biofilms, are a stress-responsive temporary, dormant ‘colonial’ stage exhibiting conserved physiological changes.

Objectives
The current study deals with characterization of palmelloids formed by C. reinhardtii in response to NaCl stress and explores the role of a conserved stress-responsive gene family, C. reinhardtii bolA in biofilm / palmellloid formation.

Methods
A detailed morphological and biochemical analysis of NaCl stress inducible palmelloids was performed. Drawing an analogy, it is known that the morphogene, bolA, significantly facilitates the formation of biofilms and induces morphological changes in response to stress in Escherichia coli. Chlamydomonas reinhardtii genome harbors five bolA-like genes. The ORF with highest homology to algal systems (CrbolA-4) was cloned and the protein over-expressed in E. coli.

Conclusions
Palmelloids rapidly dissociated upon de-stress suggesting the reversibility of this phenomenon. The cells in palmelloids displayed conserved physiological features such as clustering of cells in a common envelope, decreased viability, accumulation of lipid and starch granules and presence of extracellular polysaccharides. An iTRAQ analysis of the spent medium from palmellloid-containing cells suggests a strong involvement of cell wall proteins. Further heterologous over-expression of CrbolA-4 in E. coli did not affect its growth; but, induced biofilm formation and changed its morphology, indicating functional conservancy. Its role in the NaCl-induced palmelloidy remains to be explored.
Background
Ornithine lipids (OLs) are interesting bacterial lipids that are widely found in outer membrane of many Gram-negative bacteria, but not detected in Eukarya and Archaea. *Pseudomonas aeruginosa* has *olsBA* operon encoding acyltransferases that functions the OL biosynthesis. OlsBA works in two steps for the OL biosynthesis, in which OlsB transfers an acyl group to ornithine to make lyso-ornithine lipid and OlsA converts the lyso-ornithine lipid into ornithine lipid by another acyl-group transfer.

Objectives
Acyltransferase genes of *P. aeruginosa* were originally screened for the effect on the virulence and biofilm formation of *P. aeruginosa*. The *olsBA* operon was found to have significant pleiotropic effects on the virulence related-phenotypes of *P. aeruginosa*. In this study, we addressed how OlsBA modulates the virulence and biofilm formation of *P. aeruginosa*.

Methods
1) The virulence was investigated by using two different host models, *Tenebrio molitor*, an insect and *Caenorhabditis elegans*, a nematode. 2) The effect of the *olsBA* overexpression on biofilm formation in *P. aeruginosa* was analyzed in flow cell system. 3) The host response to OLs was monitored through the expressions of COX-2 and iNOS using Western blot and real-time PCR analyses.

Conclusions
We found that the overexpression of *olsBA* operon modulated some virulence related-phenotypes of *P. aeruginosa* by reducing the quorum sensing response. In addition, the overproduced OLs directly alleviate the virulence of *P. aeruginosa*, enhances biofilm formation, and modulates the host physiology.
THE NITROGEN DIOXIDE INCREASES PSEUDOMONAS FLUORESCENS BIOFILM FORMATION: IDENTIFICATION OF THE BACTERIAL RESPONSE TO AIR POLLUTANT

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Background
Nitrogen dioxide is an air pollutant of increasing interest in biology. Exposure of animals to NO₂ results in several toxic effects, mostly lung injury.

Objectives
Aware of the effect of NO₂ on pseudomonal biofilm formation, we looked for the mechanisms of bacterial response that could explain their resistance to NO₂.

Methods
The confocal microscopy was used for biofilm studying, completed by motility assays. In a second time, in silico and in vitro genomics tools were used to narrow our research scope. Additionally, in order to characterize the mechanism of P. fluorescens resistance to NO₂ pollution these data were completed with proteomics and lipidomics studies. For this purpose, the MALDI-TOF MS Imaging was coupled to HPTLC and compiled with the traditional GC-MS.

Conclusions
When the NO₂ increases P. fluorescens biofilm formation, the bacterial motility decreases. In coherence, the level of cyclic di-GMP evolves in NO₂ exposed cells. The lipidic study shows no drastic change in membrane charge and its composition in phospholipids and fatty acids. This suggests that NO₂ free radical could freely pass through membrane. In contrast, NO₂ promotes an extensive modification in protein production, notably the over-production of the proteins of stress response, involved in oxidative stress tolerance and iron transport/metabolism. Thus both iron up-regulation and C-di-GMP level could be signals for biofilm development. This knowledge should probably offer therapeutical solutions in antibacterial treatment.
Biofilms

OXYGEN LIMITATION TRIGGERS PELLICLE FORMATION IN BURKHOLDERIA GLUMAE

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Background

Bacteria adapt to unfavorable environmental conditions for survival. Burkholderia glumae, the causal agent of rice panicle blight, absolutely requires oxygen for respiration. For aerobes such as B. glumae, oxygen limitation is a serious problem for survival. Many bacteria including B. glumae possess genes encoding proteins with a PAS/PAC domain whose functions sense changing environments and manage gene regulation accordingly. Oxygen availability in bacteria is related to biofilm or pellicle formation. Cyclic-di-GMP synthesized by proteins carrying a GGDEF domain is a messenger for the pellicle formation.

Objectives

We aimed to investigate how B. glumae adapts to oxygen availability.

Methods

Overnight culture of B. glumae grown in LB broth was serially diluted with the same medium to give approximately 1×10⁷ cells/ml. A portion of 200 µl was placed in 96 well cell culture plates and incubated at 37°C without shaking. Growth was monitored from day 0 to day 7 of the incubation period. Morphologically altered colonies were isolated, and a mutation responsible for colony variation was identified by whole genome re-sequencing.

Conclusions

Mutations in the bpaA (B. glumae pH associated protein A) gene encoding a protein possessing PAS/PAC and phosphatase domains conferred wrinkly colony types. A mutation in bpaA resulted in elevated expression of cellulose biosynthesis genes responsible for pellicle formation. Thus, mutations in bpaA and pellicle formation in oxygen limited conditions suggest how B. glumae has evolved to survive under unfavorable conditions.
ROLE OF EACH EPM GENE ON THE FORMATION OF A NOVEL BIOFILM-SCAFFOLD EXTRACELLULAR POLYMER FROM PSEUDOMONAS

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Background

Pseudomonas alkylphenolia KL28 forms unique three-dimensional biofilm bodies depending on the environmental stimuli. It forms highly branched mushroom-like aerial architecture during vapor p-cresol utilization and circular floating pellicles on standing LB medium. These indicate that P. alkylphenolia is a good bacterial sample for studying bacterial community formation and cell-cell interactions. An epm gene cluster consisting of eleven genes showing deduced amino acid sequence similarities to those encoded on alg genes has been identified to be responsible for formation of the special biofilm structures.

Objectives

In this study, the role of each epm gene was studied in relation to the biofilm formation and polymer processing.

Methods

For this purpose, mutants specifically deleted in epm genes from an epm-overproducing spontaneous mutant SG1-WC1-10' were constructed. With those mutants, changes in surface-related phenotypes and polymer production were examined.

Conclusions

It was found that epm genes except epmL are required to form wrinkle colonies and mature biofilms including aerial architecture and circular floating bodies. Mutants in epmI, epmJ, epmX (acetylation, deduced from alg genes), epmG (epimerization), epmE, epmK (transporter) were still positive to polyuronic acid production. In addition, some of these epm genes cannot be substituted by alg genes. Thus, these results indicate that regardless of the similarities between Alg and Epm proteins the structure of Epm polymer derived from an epm gene cluster is different from alginate and the process of Epm polymer biosynthesis is different from that of alginate in Pseudomonas.
FEMS-0902
Biofilms

INDOLE EFFECT ON ANTHRANILATE DEGRADATION IN PSEUDOMONAS AERUGINOSA
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Background

Anthranilate and indole are alternative degradation products of tryptophan in nature. Pseudomonas aeruginosa produces anthranilate from tryptophan degradation. Previous studies showed that anthranilate and indole has an opposite effect on P. aeruginosa biofilm formation. While indole enhances the biofilm formation of P. aeruginosa, anthranilate was able to disintegrate the mushroom structure of biofilm.

Objectives

We addressed how indole could enhance the biofilm formation of P. aeruginosa in relation to anthranilate degradation.

Methods

P. aeruginosa biofilm formation was analyzed in flow-cell system. High Performance Liquid Chromatography was used to measure the level of anthranilate in P. aeruginosa culture supernatant. The effect of indole on AntR activity was analyzed by reporter fusion in vivo and gel shift assay in vitro.

Conclusions

In P. aeruginosa, the transcription of antABC operon encoding anthranilate dioxygenase complex that functions to degrade anthranilate, was activated by the indole treatment. Although indole alone failed to activate AntR, co-addition of indole with anthranilate boosted the activation of AntR by anthranilate. As consequence, the anthranilate level in P. aeruginosa culture supernatant decreased. Since anthranilate has a biofilm-disintegrating effect, we suggest that indole may enhance the biofilm formation of P. aeruginosa by reducing anthranilate level through the modulation of AntR activity.
Background
Trimeric autotransporter adhesins (TAAs) comprise a widespread family of adhesive molecules in Gram-negative bacteria, many of which are important virulence factors [1]. The prototypical trimeric autotransporter is the Yersinia adhesin YadA from *Yersinia enterocolitica*, which mediates attachment to collagen and other extracellular matrix (ECM) molecules, promotes serum resistance and mediates autoagglutination.

Objectives
The autotransport process depends on a conserved translocation domain and associated factors. Inhibiting this process is a possible antimicrobial strategy, as no homologous systems exist in Eukaryotes, and as many of the TAAs are important or even essential in host colonization by a broad range of Gram-negative pathogens.

Methods
We recently solved the structure of the *Yersinia* YadA translocation domain (using solid-state NMR [2]) and of a chaperone-like protein involved in translocation of *Salmonella* SadA (using x-ray crystallography [3]). Conserved residues thought to be important for translocation are targeted by mutagenesis. Adhesion assays and antibody-based assays such as FACS are used to quantify translocation.

Conclusions
A better understanding of the translocation process on the molecular level is essential for efficient inhibition of the surface display of these important pathogenicity factors. The methods developed to quantify surface localization can be scaled up for systematic screens for antimicrobial substances.

DISSECTING METHANO- AND METHYLOTROPHIC BIOFILM FUNCTIONING IN A IODINE-RICH SUBSURFACE SPRING CAVERN

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Background

Massive microbial biofilms have recently been discovered in a subsurface spring cavern fed by iodine-rich deep formation water with high thermogenic methane loading. The biomass produced by the microbes and exopolysaccharide slime completely covers the walls and ceilings of the cave, the latter bearing snottites of up to 10 cm length.

Objectives

However, the nature of these unique biofilms as well as their ecophysiology are not yet understood.

Methods

Our primary taxonomic characterization of the biofilms revealed a surprisingly diverse polymicrobial community assembly, substantiating a complex network of bacteria and archaea interacting within the sticky matrix. Distinct methanotrophic and methylotrophic populations within the Alpha-, Beta- and Gammaproteobacteria dominated the biofilms. Methylophilaceae and Methyllococcaceae were abundant on the walls and especially in the cavern water, but not so in ceiling biofilms. Here, ribosomal and functional gene analysis hinted at dominating methylotrophic and potentially also iodine-cycling populations, thus supporting a hypothesized cycling of iodomethane in this system. Geochemical gradients in the cavern were clearly reflected within community assembly, as well as elemental and stable isotope composition of the biofilms. These findings were further supported by distinct microaerophilic oxidation rate measurements for methano- and methylotroph substrates in spatially resolved biofilm samples. Ongoing work involves the elucidation of ecological niche partitioning between strict methanotrophs and potential methyl halide-oxidising populations in this unique habitat.

Conclusions
In conclusion, primary insights into a dominantly chemolithoautotrophic natural biofilm system are revealed, thriving on deep subsurface energy inputs but only a few meters under our feet.
BACKGROUND

Streptococcus pneumoniae forms biofilm to persist in the human nasopharynx. Biofilms are often associated with pneumococcal pneumonia and otitis media. β-lactam agents remain the first line antibiotic for the treatment of S. pneumoniae infections. Subinhibitory concentrations of antibiotics have been reported to enhance biofilm formation.

OBJECTIVES

This study aims to evaluate the effect of subinhibitory concentrations of a third generation cephalosporin, cefotaxime (CTX) on biofilm formation in S. pneumoniae.

METHODS

The effect of CTX (at 1/2, 1/4 and 1/16 MIC) were tested against biofilm formation in R6 and four clinical isolates in 24-well microtiter plates. Crystal violet (CV) staining and colony forming unit (CFU) were used to quantify biofilm biomass. Similar experiments were carried out with erythromycin and ciprofloxacin.

CV staining results showed that 1/2 MIC CTX resulted in 52.3%, 64.9%, 68.2%, 69.5% and 84.5% (all p values <0.01) reduction in biomass of biofilm for R6 and the clinical strains tested. 1/4 MIC CTX led to 13.2% to 57.5% (p value <0.01) reduction of biofilm while the inhibitory effect of 1/16 MIC CTX on biofilm formation were insignificant (<20% inhibition). Living bacterial cells of R6 and two cefotaxime resistant strains recovered from biofilms unexposed to CTX were 1.5×10^8, 3.6×10^4 and 1.3×10^6 CFU/well, respectively and decreased to 4.7×10^7, 53 and 43 CFU/well, respectively when exposed to 1/2 MIC CTX. Subinhibitory concentrations of non-β-lactam antibiotics showed no significant influence on biofilm formation.

CONCLUSIONS

Subinhibitory concentrations of CTX significantly inhibited biofilm formation.
CHARACTERIZATION OF A NAPHTHALENE-DEGRADING MULTI-SPECIES BIOFILM ISOLATED FROM OIL-CONTAMINATED GROUND WATER

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Background
Environmental microorganisms exist predominantly as biofilms and gain high tolerance to environmental stresses. Biofilm systems are especially suitable for the treatment of recalcitrant compounds as polycyclic aromatic hydrocarbon (PAH) because of their high microbial biomass and ability to immobilize compounds. To our knowledge, reports on naphthalene degradation by biofilm-forming bacteria are limited. In this study we isolated multi-species biofilm degrading naphthalene from oil-contaminated ground water (oil refinery, Murcia, Spain).

Objectives
The aims of this work were to characterize the biofilm microbial community and the biofilm structure and to isolate the bacteria involved in the biofilm formation and degradation of naphthalene.

Methods
The microbial community composition was characterized by culture dependent and molecular methods. For a qualitative understanding of biofilm structure and composition we used Scanning Electron Microscope (SEM). We also designed degenerated probes to detect and amplify naphthalene dioxygenase nahA gene homologues from environmental DNA and bacterial isolates.

Conclusions
Molecular analyses showed that the initial community was dominated by Betaproteobacteria (79% Rhodoferax, 6% Azovibrio). After enrichment on naphthalene minimal medium we found Betaproteobacteria (54% Variovorax) and Alphaproteobacteria (43% of a Xanthobacteraceae strain) as dominant bacteria. We isolated members of Microbacterium, Starkeya, Rhizobium, Brevundimonas, Variovorax, Pseudomonas, Pseudoxhanthomonas, Epilithonimonas and Aquabacter. Some of the isolates degraded naphthalene in pure culture and some other were able to produce biofilm. Homologues of nahA genes could be retrieved from the enrichment cultures and from some isolates.
NITRIC OXIDE (NO) DONORS TO DISPERSE BIOFILMS OF INDUSTRIAL SIGNIFICANCE FORMED BY SALMONELLA ENTERICA, PATHOGENIC ESCHERICHIA COLI AND LISTERIA SP.

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Background

Biofilms formed in post-harvest production facilities are recalcitrant reservoirs of pathogens, which are difficult to control. Pathogens in biofilms are resistant to common disinfectants and their mechanical removal is only partially effective. Therefore, novel approaches for controlling biofilms are needed. Recent discoveries of the function of nitric oxide in dispersing preformed biofilms offer an opportunity to test the feasibility of using this gas in industrial applications.

Objectives

Determine the effect of several nitric oxide donors on biofilm of Salmonella enterica, Escherichia coli and Listeria innocua preformed on surfaces of industrial interest.

Methods

Biofilms were pre-formed in appropriate media for 24 hours or 1 week in 96-well plastic plates (polypropylene, polystyrene). After incubation, medium was removed and nitric oxide donors were resuspended in Phosphate Saline Buffer (PBS) and added to the wells using different concentrations. Biofilms were exposed to Molsidomine and MAHMA-NONOate donors from 2 hours to 6 hours at 22°C and 4°C to mimic the post-harvest environments. Dispersal was measured by staining the remaining biofilms using crystal violet or using a GFP labeled Salmonella strain. The donors were also tested in association with a cellulose nanocrystals hydrogel (CNC).

Conclusions

Molsidomine and MAHMA-NONOate were able to disperse at least 50% of the biofilms preformed by human pathogens, Salmonella enterica 14028, Escherichia coli O157 (EHEC). The association of the two nitric oxide donors with cellulose nanocrystals was also effective in dispersing Salmonella preformed biofilms on polypropylene up to 15% of the total biomass. Our results show that nitric oxide donors expand the toolset of proactive solutions for removing industrial biofilms.
Effect of pomegranate peel extract on biofilm and planktonic cells of Candida albicans

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Background: Pomegranate peel is used by many people against diarrhea, ulcers, periodontal disease, stomatitis and pharyngitis. Candida albicans can infect any part of the body (gum, tongue, toes...) with tissue invasion thus inducing candidiasis. Candida infections are difficult to manage due to their persistent nature and the biofilm growth of this yeast serves as an increasing source of clinical infections.

Objectives: This study investigates the antioxidant activity of acetone peel extract of sour pomegranate peel named Quares (QE) and its effect against biofilm and planktonic cells of Candida albicans.

Methods: Acetone 50% (v/v) was used for phenolic compounds extraction. The antifungal activity is evaluated using three strains of: CA1, CA2 and CA3. Various concentrations have been used to determine the MIC. Preliminary screening of activity: The agar disc diffusion method was conducted. The concentrations tested are 10mg/disc to 0.5mg/disc. Two models were used for the biofilm formation inhibition of Candida albicans on micropaque (Hamada et al., 1978) and on hydroxyapatite discs (Guggenheim, 2001). The samples were then viewed under a scanning electron microscope.

Results and discussion: The extract from the variety QE showed a high content of total polyphenols. The inhibition percent of H2O2 is 96.32 ± 0.56 %. The inhibition of Candida albicans biofilm formation on hydroxyapatite test showed strong inhibitory action against the strains in this order CA1 > CA3 > CA2.

Conclusion: This study reports the high content of phenolic compounds of pomegranate peel extract, antioxidant activity and Candida albicans biofilm inhibition.
IDENTIFICATION OF CANDIDA SPECIES ISOLATED FROM MEDICAL DEVICES CONNECTED MEDICAL ICU PATIENT AND EVALUATION OF THEIR BIOFILM FORMATION AND DRUG SUSCEPTIBILITY

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Background: Nowadays, mortality and morbidity due to infections caused by biofilm on medical devices such as catheter and implants are increasing and Candida has become one of the most common causes of nosocomial infections. One of the most important characteristics of biofilms is broad-spectrum resistance to antimicrobial drugs. However, too much using of this medicine can lead to the resistance of Candida species to fluconazole.

Method: A total of 60 samples of medical devices attached to patients in ICU were investigated. Yeast colonies isolated from medical devices using routine laboratory procedures were identified. The MIC of fluconazole for all samples was carried out using Broth Microdilution according to CLSI. To assess biofilm formation by MTT, the experiment was carried out in a 96-cell microplate. Over half of the isolates of Candida had the ability to form biofilms, and the ability of biofilm formation was read by ELISA reader at a wavelength of 570 nm.

Results: Out of 60 samples, 48 isolates of Candida with an abundance of Candida albicans (42%), C. glabrata (27%), C. kruise (17%) and C. tropicalis (14%) were identified. Among the isolates, 9 were susceptible to fluconazole (19%), 10 cases were dose dependent (21%) and 29 were resistant to fluconazole (60%). Candida isolates had the ability to form biofilms. C. albicans and C. tropicalis had the maximum and minimum power of biofilm formation respectively.

Conclusions: The results showed that Candida was resistant to fluconazole. All resistant isolates had the ability to form biofilms.
MICROFLUIDIC MODEL FOR THE INVESTIGATION OF PSEUDOMONAS AERUGINOSA BIOFILMS
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Background

Pseudomonas aeruginosa is an opportunistic pathogen due to its capacity to form recalcitrant biofilm structures, while cohabiting with other harmful/pathogenic species and harboring the capability to release toxins that cause tissue necrosis. P. aeruginosa has been implicated in a number of microbially-induced or aggravated diseases, including cystic fibrosis and wound infections (e.g. burns, bites). Very little is known about the complex interactions that occur within polymicrobial communities and few tools exist for studying these interactions.

Objectives

In this study, we report on the development of a microfluidic model that mimics the relevant physiological properties of the wound microenvironment while incorporating materials present in the human extracellular matrix/wound environment.

Methods

We have validated the robustness of our model comparing traditional GFP-tagging to new fluorescent staining techniques to visualize/resolve individual species within a polymicrobial habitat. We have also demonstrated that chemotactic stimuli may be incorporated into our model through specialized ports in our chamber; we can readily monitor changes in motility in response to the introduction of amino acids, such as arginine and glutamine.
Conclusions

We show that our model can be used to investigate the spatio-temporal mechanobiological structures of the wound environment, which will significantly contribute to our understanding of the development and progression of polymicrobial biofilm infections and its interactions with the host environment. Through the implementation of microfluidic platforms, we pose complex and relevant questions that may enhance our understanding of microbial interactions, all with the goal of
identifying new and specific targets for more effective antimicrobial therapies.
Background

Listeria monocytogenes is a foodborne human pathogen which is commonly found in the environment and is capable of withstanding environmental threats by adhering to surfaces to form a biofilm. Biofilm formation is believed to be a major factor for cross-contamination in food processing premises and therefore, elucidating the molecular mechanism of biofilm formation in L. monocytogenes can contribute to secure food safety.

Objectives

To identify genes involved in biofilm formation of L. monocytogenes 15G01

Methods

A library of 6,500 mutants was constructed via transposon mutagenesis of L. monocytogenes 15G01, a persistent strain in New Zealand seafood processing premises, with the Himar1 mariner-based transposon. This library was screened for altered biofilm formation using a microtiter plate assay, using conditions defined as optimum through preliminary studies. Quantification of biofilm mass was undertaken by optical density measurement after staining with crystal violet. Mutants of interest were selected through statistical analysis (two-sample t-test, p≤0.05). The insertion site of the transposon was identified through semi-arbitrary PCR followed by DNA sequencing or by genome sequencing, and the inactivated loci analysed using BLAST.

Conclusions
Sequencing results revealed genes previously known to be involved in biofilm formation by *Listeria monocytogenes* and/or other bacterial species, which corroborates the method of transposon mutagenesis for functional analysis of biofilm formation. Furthermore, in this study, new genes influencing biofilm formation (either positively or negatively) have been identified. Further functional analysis of these genes is ongoing to elucidate their roles in biofilm formation.
HYPOCHLORITE-INDUCED OXIDATIVE STRESS STIMULATES C-DI-GMP SYNTHESIS AND BIOFILM FORMATION IN PSEUDOMONAS AERUGINOSA

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Background

The opportunistic human pathogen \textit{Pseudomonas aeruginosa} is able to adapt to a variety of often harmful environmental conditions due to different survival strategies including the formation of resistant biofilms.

Objectives

In this study, we investigated the adaptation of \textit{P. aeruginosa} towards hypochlorite (HOCl), a strong oxidant used by human neutrophils to kill invading bacteria and which can be found in chronically inflamed host tissue, e.g. in the lungs of CF patients

Methods

In static biofilm assays, we observed a significant increase of up to 3-fold in initial cell attachment in the presence of sub-lethal hypochlorite concentrations. Transcriptome analyses revealed a substantial upregulation of genes involved in oxidative stress response as well as biofilm formation in the presence of hypochlorite. Among others, we identified a 26-fold upregulation of ORF PA3177 coding for a putative diguanylate-cyclase (DGC), which catalyzes the synthesis of the second messenger c-di-GMP, and therefore influences motility, biofilm formation and persistence in \textit{P. aeruginosa}. Subsequent LC-MS/MS analyses revealed a strong increase in c-di-GMP levels suggesting a key role of this second messenger in hypochlorite induced adhesion and biofilm development. The DGC PA3177 was further characterized in more detail demonstrating its involvement in motility, biofilm formation, antimicrobial resistance and persistence in \textit{P. aeruginosa}. Using a subset of different mutant strains, we were able to show that both the pel and psl exopolysaccharides are effectors in the PA3177-dependent c-di-GMP network.
Conclusions
Our results demonstrate that host-derived antimicrobials are sensed by invading bacteria and exert a huge impact on bacterial pathogenesis including biofilm formation.
DISTINCT SAGA FROM HOSPITAL-ASSOCIATED CLADE A1 ENTEROCOCCUS FAECIUM STRAINS CONTRIBUTES TO BIOFILM FORMATION


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Background

*Enterococcus faecium* is an important nosocomial pathogen causing biofilm-mediated infections. Elucidating *E. faecium* biofilm pathogenesis is pivotal for development of new strategies to treat these infections. In several bacteria extracellular DNA (eDNA) and proteins act as matrix components contributing to biofilm development.

Objectives

In this study, we investigated biofilm formation capacity and the role of eDNA and secreted proteins in 83 *E. faecium* strains with different phylogenetic origin that clustered in clade A1 and clade B.

Methods

Although there was no significant difference in biofilm formation between *E. faecium* strains from both clades, addition of DNase I or proteinase K to biofilms demonstrated that eDNA is essential for biofilm formation in most *E. faecium* strains, while proteolysis primarily impacted on biofilm formation of hospital-associated *E. faecium* strains that cluster in clade A1. Secreted antigen A (SagA) was the most abundant protein in biofilms from clade A1 and B *E. faecium* strains, although localization differed between the two groups.

Conclusions

*sagA* is present in all sequenced *E. faecium* strains, with a consistent difference in the repeat region between the clades, which correlated with proteinase K susceptibility in biofilms. This indicates an association between the SagA repeat profile and the localization and contribution of SagA in *E. faecium* biofilms.
Background

The worldwide occurrence and recent rapid spread of multiresistant strains of *Klebsiella pneumoniae* is of great concern and has generated an urgent need to identify new drug targets. Bacterial adhesion and biofilm formation are important for the establishment of *K. pneumoniae* infections. Elucidation of mechanism of biofilm formation by pathogens is the first step towards the development of novel approaches for the treatment and prevention of biofilm related infections.

Objectives

The goal of this study was to gain additional insights into biofilm formation in *K. pneumoniae* MTCC 3384 by using mini-Tn5 transposon mutagenesis.
Disruption of Colanic acid synthesis in *K. pneumoniae* enhances biofilm formation

**Quantitative analysis of biofilm formation by Crystal violet (CV) staining**

**Colony morphologies of the strains**
Methods

In this study, mini-Tn5 transposon insertion mutants were constructed and screened for their ability to form biofilms on abiotic surfaces by crystal violet staining. It was observed that disruption of wcaJ conferred high biofilm forming ability as compared to the parent strain. WcaJ is the initiating enzyme of colanic acid synthesis which loads the first sugar (glucose-1-P) on the lipid carrier undecaprenyl diphosphate. Its absence rendered the mutant defective in colanic acid, resulting in non-mucoid colonies. From the Calcofluor (binds cellulose) binding studies, it was evident that ∆wcaJ mutant does not produce much exopolysaccharide, thereby facilitating cell-cell interactions leading to the formation of closely packed structures. In addition, complementation of wcaJ in trans fully restored the wild type phenotype that confirms the observed effect of wcaJ on biofilm formation of K. pneumoniae.

Conclusions
Colanic acid expression in *K. pneumoniae* inhibits its cellular attachment to the substratum and biofilm formation, contradicting earlier studies that polysaccharides enhance biofilms in related species.
SALT STRESS INDUCES BIOFILM FORMATION IN CLOSTRIDIUM LJUNGDAHLII.

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Background

*Clostridium ljungdahlii* is a homoacetogen capable of producing organic commodities from carbon dioxide using electrical energy provided by an electrode, i.e. microbial electrosynthesis. The efficiency of this process likely depends on the biofilm formation on the electrode. So far, however, only limited attachment to electrodes has been obtained with *C. ljungdahlii*, while biofilm formation by *C. ljungdahlii* has not yet been reported.

Objectives

The presented work investigated whether adapted growth conditions can induce *C. ljungdahlii* biofilms and characterized the obtained attachment.

Methods

*C. ljungdahlii* was grown in 6-well plates using a tryptone containing medium. Different stress conditions (salt, antibiotic, temperature) were tested and biofilm formation was quantified using a crystal violet assay. The addition of NaCl (200 mM) most significantly induced biofilm formation, i.e. after 3 days the attachment was two order of magnitudes higher than in the control. Confocal laser scanning microscopy showed viable, 45 ± 5 µm thick biofilms, while without the addition of salt only a single cell layer (5 µm) was observed. Furthermore, a 15 ± 5 µm thick biofilm was obtained on a vertically placed piece of graphite, simulating an electrode, by adding NaCl to the medium. RNA sequence data of *C. ljungdahlii* grown with and without the NaCl addition are currently being analyzed to explore the genes involved in biofilm formation.

Conclusions

This work is the first to demonstrate biofilms in *C. ljungdahlii* and suggests that salt-induced biofilms of *C. ljungdahlii* should be tested for their microbial electrosynthesis potential.
CHARACTERIZATION OF XANA MUTANT OF THE XANTHOMONAS CITRI SUBSP. CITRI
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Background

*Xanthomonas citri* subsp. *citri* is the causal agent of citrus canker disease. This bacteria is able to form biofilm in vitro and on leave surface. The ability to form biofilm is important to citrus canker development, because it implicates to survival of bacteria on leaf surface of host plants, when it is sprayed on the plant. Cells in biofilm are embedded in a matrix composed for extracellular polysaccharide (EPS) and other proteins. In *Xanthomonas campestris*, the gene *xanA* is required for the synthesis of the exopolysaccharide xanthan, but participation of *xanA* in biofilm formation process was not evaluated in *Xanthomonas citri*.

Objectives

To characterize the function of *xanA* (XAC3579) in relation to adhesion, biofilm formation, motility, and epiphytic behavior of *X. citri* on leaves.

Methods

We realized assays for analysis of biofilm formation and adhesion in abiotic and biotic surface, EPS quantification, sliding and swimming motility to compare the mutant XAC3579 tn5 and *Xanthomonas citri* wild type.

Conclusions

The reduction of xanthan produced reduced the adherence and also the swimming and sliding motility. The presence of the xanthan was required for mature biofilm development on sweet orange leaf surfaces. This reduction can decrease the *Xcc* pathogenicity.
PROTEOMICS ANALYSIS IDENTIFIES PROTEINS ASSOCIATED WITH CURCUMIN-TREATED HELICOBACTER PYLORI BIOFILM

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Background
Helicobacter pylori is an important human pathogen, which is implicated as etiologic agent of peptic ulcer, chronic gastritis and gastric cancer. Recently, H. pylori is shown to have ability to form biofilm both in vitro and in vivo. Biofilm formation enables the bacterium to survive in adverse environments, to persist in patients causing chronic infection and confers enhanced resistance to antibiotics. Curcumin (diferuloylmethane), a natural compound found in turmeric, has been demonstrated for broad chemotherapeutic properties. We have previously reported that curcumin inhibits biofilm formation of H. pylori but its impact at a molecular level is unclear.

Objectives
This study was established to identify proteins involved in anti-biofilm activity of curcumin in H. pylori biofilm using proteomics analysis.

Methods
Biofilm of H. pylori ATCC43504 was established by pellicle assay. Two-dimentional gel electrophoresis (2-DIGE) and matrix-assisted laser desorption/ionization-time-of-flight (MALDI-TOF) were performed in order to investigate the difference in protein profile expression between curcumin-treated and -untreated H. pylori biofilms.

Conclusions
There were total 28 expressed proteins identified here. Eighteen proteins were regulated in both curcumin-treated and -untreated H. pylori biofilms. Proteins with decreased expression following exposure to 1/4 MIC of curcumin were associated with carbohydrate and nitrogen metabolisms, chemotaxis and motility, and electron transport. However, several chaperon proteins were up-regulated in response to a presence of curcumin. These data reveal a probable mechanism of curcumin that inhibit biofilm formation of H. pylori. Further studies need to be investigated in order to develop curcumin as a potential alternative medicine for treatment of H. pylori infections.
NOVEL METHODS FOR THE ASSESSMENT OF MARINE ANTI-BIOFILM TECHNOLOGIES.
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Background

Marine biofilms, mainly comprised of bacteria and diatoms, are now recognized to be a significant issue for a wide range of engineered structures such as ship hulls, energy systems, sensors, and oil/gas pipe lines. Biofilms constitute a major component of the overall marine growth on ship hulls and may lead to a 14\% increase in ship fuel costs and to an up to 18\% fuel consumption penalties. The scientific community is now placing significant effort towards the creation of environmentally benign surfaces with anti-biofilm capacity. However, there is significant gap in screening assays that test the bacterial/surface interface during initial attachment for anti-biofilm technologies while dynamic testing is often overlooked.

Objectives

In this work a range of bioassays have been developed to directly test bacterial attachment and biofilm growth on experimental anti-biofilm
surfaces, under both static and hydrodynamic conditions.

Methods

Following screening of several compounds for anti-biofilm efficacy, juglone (MIC at 5ppm) was chosen to act as model inhibitory agent, which was then successfully incorporated into a coating system and illustrated good anti-biofilm capacity. Novel microfluidic devices were developed for the assessment of bacterial attachments and biofilm formation in the presence and absence of juglone under hydrodynamic conditions. Juglone inhibited biofilm formation and following nucleic acid staining, toxicity against *Cobetia marina* was evident. This was not clear during the static assays.

Conclusions

The developed bioassays illustrated promising reproducibility and greater relevance for anti-biofilm technologies. New microfluidic approaches have been employed to reveal differences in results when comparing static vs. dynamic testing.
The impact of carbon source on biofilm formation, antigenicity and adhesion properties of the probiotic \textit{Lactobacillus rhamnosus} GG

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Background. Biofilm formation is the preferred growth mode and survival strategy of several bacteria for improving adaptation and viability under hostile conditions. For mucosal and health-promoting bacteria the biofilm formation is likely to play an important role in colonization and immunostimulation. We have previously shown that the probiotic paradigm \textit{Lactobacillus rhamnosus} GG displays a high number of adhesive and immunostimulatory moonlighting proteins at the cell surface, and that surface-bound proteins are important mediators of biofilm formation of this strain.

Objectives. The aim of this study was to explore the effect of fourteen different fermentable carbohydrates on the biofilm formation of \textit{L. rhamnosus} GG, and to uncover their impact on the surface protein/antigen composition and the adhesive properties of the strain during biofilm and planktonic growth.

Methods. Biofilm formation was assessed using the standardized polystyrene microtiter plate assay, and the viability and structure of selected biofilms were evaluated with LIVE/DEAD staining coupled with confocal microscopy analyses. Surface-associated proteins and antigens were identified and analyzed using in-house proteome and immunoproteome methods. Selected biofilm and planktonic cultures were further tested for their adhesion activities in vitro.

Conclusions. This study indicated that carbohydrate source plays a significant role in the protein-mediated biofilm formation and the antigenicity of the biofilm cells. We also show that moonlighting proteins are the major protein components of the biofilm matrix, and that certain carbohydrates affected the adhesion properties of biofilm cells.
Background

Biofilms are communities of microorganisms which live in a self-produced matrix of extracellular polymeric substances. Their infections are hard to treat due to their resistance to immune defense and antibiotics. Therefore it is important to develop tools to investigate biofilms for therapy improvement or to find new antimicrobial substances.

Objectives

The aim of this work is to establish a method for the fully automated large-scale screening of biofilms which could be used for examining the ability of biofilm formation of bacteria under different culture conditions and most importantly for determining the effects of different antibiotics and antimicrobial substances.

Methods

We enhanced our previous published VideoScan technology, which is based on fully automated fluorescence microscopy to perform high-throughput screening of biofilms. VideoScan enables the analysis of multiplex assays such as microbead or cell-based assays [Rödiger et al. 2013; Frömmel et al. 2013]. We use a 96 well plate format for the formation of biofilms, which are stained with Live/Dead staining followed by a VideoScan analysis. This analysis represents a two-step evaluation realized by our software and image processing. In the first step overview pictures of biofilms are taken and in a second step, a fine-grained analysis of the biofilm, bacteria are counted in different z-stacks of the biofilm.

Conclusions

With our VideoScan technology it is possible to study biofilms in a fully automated large-scale screening. In a next step a large panel of commensal and pathogenic E. coli strains will be screened for biofilm formation under different growth conditions and presence of biofilm-associated genes.
Background

The development of alternative strategy to conventional antibiotics in the struggle against pathogens and antibiotic resistance is a topical issue. The interference with the adhesion, the first stage of pathogenic process, can modulate virulence mechanisms like colonization, invasion of host tissues and biofilm formation. The curcumin can inhibit the adhesion of both Gram positive and Gram negative pathogenic strains and different mechanisms can be involved (inhibition of sortase A, Quorum–sensing interference, binding to curli, etc.).

Objectives

To obtain novel agents against pathogenic bacteria, targeting virulence mechanisms and biofilm formation (anti-virulence agents).

Methods

In this study, we synthesized a group of molecules derivatives of curcumin, and the inhibition of biofilm formation was evaluated at a screening concentration of 7.5 μg/ml against reference staphylococcal strains \textit{S}.\textsl{aureus} ATCC 29213, 25923, 6238, \textit{S}.\textsl{epidermidis} RP62A, and Gram negative reference strains \textit{E}.\textsl{coli} ATCC 10536 and \textit{P}.\textsl{aeruginosa} ATCC 15442. The activity as sortase A inhibitors was also screened in a high throughput assay by using the standard Dabcyl-QALPETGEE-Edans fluorescence resonance energy transfer (FRET)-peptide substrate for measurement of enzyme activity.

Conclusions

Some curcumin derived compounds resulted effective at lower concentration to prevent biofilm formation than the curcumin. The discovery of novel agents that target virulence mechanisms and biofilm formation offers new potential therapeutic strategies to treat chronic bacterial infections. They can act either alone or in combination with current antibiotics and could significantly impact on overcoming the problem of antibiotic resistance, which is recognized by WHO as one of the most important global challenges of our time.
UNRAVELING EROSION DYNAMICS: MICROBIAL STABILIZATION OF FINE SEDIMENTS

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Background
During the formation of aquatic biofilms microbes produce extracellular polymeric substances (EPSs) inter alia to attach to the sediment. Thus, sediment grains can be glued together and thereby are granted a significantly increased resistance versus erosive forces - a phenomenon called biostabilization.

Objectives
This process constitutes an essential ecosystem function (e.g. detainment of contaminated fine sediments in rivers) but is currently barely addresses in lotic waters. In our current DFG project engineers and biologists work together to elucidate the fundamental principles of biostabilization.

Methods
Straight flume mesocosm experiments were run at different seasons and under varied boundary conditions to investigate the effect of biotic and abiotic factors upon biofilm development and resulting biostabilization. Besides biofilms’ stabilization capacity (adhesiveness and erosion resistance) biochemical and biological features (e.g. microbial biomass, EPS production and microbial community composition) were investigated.

Conclusions
A general temporal increasing stabilizing effect could be detected - one of the first scientific proofs for this process in lotic waters. Furthermore, a strong effect of season and environment upon biofilm development and stabilizing capacity was detected, e.g. a significant maximum during spring but a minimum under minimal illumination. Besides the possible role of EPS protein content as proxy for biofilm stability the importance of successional processes were revealed: especially mutual adaption of algae and bacteria (e.g. expressed by the development of dynamics or functional organization within the bacterial community) is apparently a driving factor.
Background

Recent studies show that extracellular DNA is a major component of the EPS (extracellular polymeric substances) in biofilms and it was found to have widespread importance in biofilm formation. However, detailed knowledge about how it promotes biofilm formation and how it influences biofilm morphology is just starting to emerge (1).

The γ-Proteobacterium F8 was isolated from the South Saskatchewan River in Canada. It was found to produce huge amounts of extracellular DNA forming a filamentous network when grown on agar plates containing FBM medium (2, 3).

Objectives

The aim of this study was to examine this phenomenon for the first time under continuous flow conditions in a biofilm reactor.

Methods

F8 was grown under controlled conditions in a continuous flow biofilm reactor and eDNA-production as well as biofilm morphology was monitored over time. Cells and EPS components were visualized by differential staining with various fluorescent dyes and confocal laser scanning microscopy (CLSM).

Conclusions

F8 cells attached to glass slides and showed biofilm formation from day 1 on. Filamentous eDNA could be detected by staining with Syto 9 and Propidium Iodide, but not with DAPI. Results so far indicate that eDNA is emerging from living cells.
FEMS-1461
Biofilms

PSEUDOMONAS AERUGINOSA THAT COLONIZED BURN PATIENTS HAS HIGH CAPACITY TO FORM BIOFILM
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Background
In burn patients, \textit{Pseudomonas aeruginosa} can lead to a great variety of systemic infections with higher mortality rate. In addition, treatment of \textit{P. aeruginosa} infections is a serious medical challenge because these bacteria are associated to high resistance to antibiotics and ability to form biofilms, becoming a serious problem in burn wound center (BWC).

Objectives
The aim of this study was to investigate the antibiotic resistance and biofilm formation from 35 \textit{P. aeruginosa} collected from wound burn patients and on the surface of the bath tank where balneotherapy was performed.

Methods
The antibiotic resistance was carried out by disk diffusion method, following CLSI guidelines and the evaluation of biofilm formation was achieved through microtiter plate assay incubated for 24h. Genotypic characteristics of these strains were determined by Pulsed-field gel electrophoresis (PFGE).

Conclusions
It was observed that the clone A, the most prevalent and presents in wound patients and on the surface of the bath tank, had a high capacity to form biofilm. These strains also showed resistance to drugs as ceftazidime, aztreonam, gentamicin, ciprofloxacin and even meropenem. We believed that this clone which is more able to accumulate drug resistance mechanisms and to form biofilm has greater conditions to adapt and to persist in the BWC.
MICRO-DIVERSITY OF ANAMMOX BACTERIA IN NITRITATION-ANAMMOX BIOFILMS
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Background
An emerging technology for nitrogen removal in wastewater is the Anammox process. However, questions about the stability of the process remain. It is generally believed that a higher diversity can increase ecosystem stability. Coexistence of two anammox species in a Moving Bed Biofilm Reactor (MBBR) was observed in a previous study (Persson et al. 2014).

Objectives
We studied if coexistence of different anammox populations is common in nitritation-anammox MBBRs.

![Figure 1: Different anammox populations (detected by different FISH anammox probes) coexisting in biofilms from two different MBBRs. Scale bar 20μm. A) Red: AMX820 probe, Yellow: Ban162 and AMX820. B) Red: Bfu613, Green: Ban162.](image)

Methods
Anammox micro-diversity was assessed with automated ribosomal intergenic spacer analysis (ARISA) using a nested PCR approach, and fluorescence in situ hybridization (FISH). Four pilot MBBRs at two different pilot wastewater treatment plants in Sweden with different running conditions and one full-scale integrated fixed-film activated sludge reactor (IFAS) were studied.
Conclusions
Preliminary ARISA results suggest that nitritation-anammox MBBRs usually harbor more than one anammox population and FISH from two MBBRs confirmed this (Figure 1). Possible explanations include presence of biofilm microenvironments and temporal variations in environmental conditions such as influent composition. However, one MBBR operated at the challenging conditions of low nitrogen concentrations and at low temperature (13°C) was dominated by a single species.

References
FUNCTIONALIZATION OF PDMS WITH CELLOBIOSE DEHYDROGENASE YIELDS ANTIBIOFILM SURFACE

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Background

Patients with medical indwelling devices (e.g. urinary catheters) are prone to acquiring infections caused by biofilm forming pathogens colonizing catheter surfaces. Catheter associated urinary tract infections (CAUTI) are among the most frequent ones. One of the polymers widely used for making urinary catheters is polydimethylsiloxane (PDMS) due to its favorable biocompatibility properties.

Objectives

In order to decrease the risk of infection, an antimicrobial enzyme, namely cellobiose dehydrogenase (CDH) was successfully grafted onto PDMS surface.

Methods

The system is based on the ability of CDH to use oxygen as electron acceptor and different oligosaccharides (e.g. cellobiose) as electron donors to produce H₂O₂. Several approaches of immobilizing CDH on PDMS surface were exploited including surface activation using oxygen plasma followed by covalent linkage of CDH. The success of the immobilization process was monitored by analyzing the change in the functional groups on the surfaces by FTIR measurements as well as measuring the ability of grafted CDH to produce H₂O₂. Antimicrobial activity of the immobilized enzyme was assessed against S. aureus by quantifying the amount of viable cells and total biomass formed on the enzyme treated sheets compared to the control.

Conclusions

CDH was successfully immobilized on the surface of PDMS as evidenced by H₂O₂ production in the presence of cellobiose. The modified surface showed up to 70% reduction of microbial growth as compared to the untreated control. The CDH modified PDMS catheters could help to prevent current problems of microbial colonization and multidrug resistant bacteria associated with catheters.
ENHANCED FLOC FORMING MUTANTS OF RHODOVULUM SULFIDOPHILUM OBTAINED BY UV MUTAGENESIS
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Background
A marine photosynthetic bacterium, *Rhodovulum sulfidophilum*, produces nucleic acids extracellularly¹. Interestingly, the nucleic acids thus produced outside cells are utilized as a sticky glue to make bacterial self-aggregates called “floc” in nutritionally poor medium conditions such as on the sea bottom where the bacterium was originally isolated. However, what gene regulates its flocculation or why this bacterium makes a floc under nutritionally stringent conditions is still unclear.

Objectives

Our objectives are to establish UV mutagenesis in *Rhodovulum sulfidophilum* and to obtain UV mutagenic clones showing aberrant flocculation behavior even in rich medium conditions where the bacterium usually doesn’t make an obvious floc.

Methods

*Rhodovulum sulfidophilum* DSM 1374ᵀ was exposed to UVC (254 nm) irradiation and UV mutagenesis was induced. After UVC irradiation, rifampicin-resistant mutants were collected and these flocculation behaviors were examined.

Conclusions

UV mutagenesis in *Rhodovulum sulfidophilum* DSM 1374ᵀ was established and 107 rifampicin-resistant mutants were obtained. Two out of the obtained clones showed strong flocculating behavior even in nutritionally rich medium conditions and these flocculation ratios were approximately 40% and 60%, respectively, whereas that of wild type was ca. 20%.
MUTUAL INFLUENCE OF S. EPIDERMIDIS AND S. AUREUS ON GENE EXPRESSION IN DUAL SPECIES BIOFILMS

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Background

S. aureus and S. epidermidis are two important nosocomial pathogens which can interact in biofilms (e.g. S. epidermidis inhibits S. aureus biofilm formation by producing proteases).

Objectives

To study the mutual effect of S. epidermidis and S. aureus on gene expression in dual species biofilms.

Methods

RNA-Seq was performed on single and dual species biofilms and the results were confirmed by the appropriate phenotypic tests.

Conclusions

In dual species biofilms, S. epidermidis genes encoding resistance to erythromycin, oxacillin and tobramycin were upregulated and when biofilms were treated with these antibiotics, we observed that more S. epidermidis cells survived oxacillin treatment in dual species biofilms than in single species biofilms.

Urease genes of S. aureus were downregulated in dual species biofilms and an urease assay confirmed that there was more urease activity in S. aureus single species biofilms compared to dual species biofilms. Literature data described a link between urease activity and pH and we also observed that the pH of S. aureus single species biofilms was lower than the pH of dual species and S. epidermidis single species biofilms. Metabolic activity results in acid production and RNA-Seq data showed there was less expression, in dual species biofilms, of S. aureus genes encoding proteins involved in metabolic processes. Viability assays showed that there were more metabolically active cells in S. aureus biofilms compared to dual species and S. epidermidis single species biofilms.

Altogether, our results show that S. epidermidis has an inhibitory effect on the metabolic activity of S. aureus in dual species biofilms.
ANTI-BIOFILM EFFECT OF RED RASPBERRY FRUIT JUICE ON PSEUDBOMONAS AERUGINOSA BIOFILM

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Background

Bacterial cells in biofilm are more resistant to wide range of disinfectants than free living cells, and finding an appropriate agent against them is demanding task.

Environment is certainly the source of many products which have anti-biofilm effects, but they have not been proven, yet.

Objectives

To investigate the anti-biofilm effect of 100% pure red raspberry fruit juice on Pseudomonas aeruginosa biofilm formation.

Methods

Minimal inhibitory concentration of red raspberry fruit juice was evaluated by dilution method, and its anti-biofilm effect was tested in microtiter plate using crystal violet staining according to modified technique by Stepanovic.

Conclusions

Out of 30 tested strong-adherent Psudomonas aeruginosa isolates, after treating with red raspberry fruit juice, 19 (73.3%) isolates were poorly adherent, while 8 (26.7%) had no adherence ability.

This study confirmed the great potential of red raspberry fruit juice as anti-biofilm agent for preventing microbial colonization, but it is neccesary to discover the exact biochemically active ingredient against biofilm formation.
Background

Many of the world's most precious artworks are made of stone. Their irreversible deterioration due to biological attack is a worldwide concern. Microorganisms colonize outdoor lithic surfaces and develop into biofilms at the interface solid/air (subaerial biofilms, SABs), which, in turn might cause aesthetic, chemical and physical decay. Although it has been estimated that at least 99% of the world's microbial biomass exists in biofilms, the role and behavior of microorganisms within the biofilm matrix and their complex interactions with the external environment is still unknown.

Objectives

This work provides a pioneering and multidisciplinary research to investigate the behavior of microorganisms within the biofilm matrix for sorting out time-spatial relationships and to elucidate microorganism-EPS, inter-organism, biofilm-atmosphere and biofilm-stone interactions.

Methods

This work spans sophisticated molecular, chemical, physical and data modeling techniques and it is approached from two complementary angles:
1- Lab-scale study to delineate specific transcriptional responses of mono- and multi-species biofilms as well as the biofilm-stone interactions under controlled environmental conditions.
2- Real heritage case studies to investigate the shifts in the microbial community structure and function under different environmental conditions. Through comparing phylogenetic and functional diversity under different environmental scenarios, we
provide evidence that any intuition gained from the lab-scale experiments is relevant to true environmental biofilms.

Conclusions

The findings obtained so far will contribute to better understand the complexity of all the interactions encountered within SAB communities, and how these interactions may influence the biofilm outcome and the biodeterioration of the stone materials under different environmental conditions.
BIOLOGICAL FUNCTIONS AND CELLULAR LOCATION OF COLLAGEN-LIKE PROTEINS IN BACILLUS AMYLOLIQUEFACIENS

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Background

Bacterial collagen-like proteins (CLPs) have been identified in a broad range of pathogen bacteria and it is important to biofilm formation and bacterial adhesion to host cells. Some bacterial CLP-encoding genes (clps) have also been found in non-human pathogenic strains such as B. cereus and B. amyloliquefaciens, which are types of plant-growth promoting rhizobacteria (PGPR).

Objectives

To elucidate the role of CLPs playing in the interaction between PGPR with plant host, the biological functions and cellular location have to explore.

Methods

CLP-encoding genes ClpA, ClpB, ClpC, and ClpD in strain FZB42, were inactivated separately by Site-Directed Mutagenesis. Scanning electron microscopy (SEM) and hydrophobicity value detection were used to assess the morphologic bacterial cell shape and cell surface architecture. Immune gold labeling was used to indicate the location of CLPs on the cells. The bacterial flagella morphology observed by transmission electron microscopy (TEM).

Conclusions

Comparing with wild type strain, clps mutant strains showed differently on phynotypes of bacterial colony shape, cell autoaggregation, biofilm formation, as well as adhesion to surface of abiotic materials or the roots of Arabidopsis thaliana. Immune gold labeling shown CLPs located in the outer layer of the bacterial cell, including the cell wall, outer membrane, flagella, or other associated structures. In addition, the bacterial flagella appeared fewer, scattered and bent for clps mutant strains. Above
results indicate that the bacterial outer layer located CLPs are closely involved in cell shape development, biofilm formation, and bacteria-plant interaction.
SYSTEMS MICROBIOLOGY OF ALGINATE-LIKE EXOPOLYSACCHARIDES BIOSYNTHESIS IN GRANULAR SLUDGE BIOFILMS: A PILOT AND FULL-SCALE STUDY WITH DOMESTIC WASTEWATER

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Background

Environmental biotechnology systems using aerobic granular sludge (AGS) are becoming new standards for intensified wastewater treatment. AGS originates from self-aggregation of activated sludge microorganisms into mobile biofilm structures under operational selection pressures. Extracellular polymeric substances (EPS) such as alginate-like exopolysaccharides (ALE) play an important structural role by supporting microbial encapsulation in a gel matrix.

Objectives

The complex microbiome of AGS was studied using a systems biology approach. Investigations targeted bacterial phylotypes carrying genetic and metabolic potential for ALE biosynthesis. Such correlation analysis will enable to elucidate molecular mechanisms underlying granular biofilm formation.

Methods

A 98-L pilot sequencing-batch reactor was operated for granulation and nitrogen removal from domestic wastewater. Evolution of particle size distribution and EPS polysaccharide and protein fractions were determined by laser diffraction, phenol-sulfuric acid, and folin-phenol methods, respectively. AGS from full-scale system was subjected to physicochemical characterization of ALE. Bacterial populations and genetic signatures related to ALE biosynthesis are currently prospected from pilot and full scales using phylogenetic and metagenomic profiling.

Conclusions
Over 3 months of operation granulation displayed gradual increase in the fraction and mean diameter of granular biofilms from 5 to 60% and 50 to 300 µm, respectively. The polysaccharide-to-protein ratio increased concomitantly from 0.4 (m/m) in activated sludge flocs to 0.8 and 1.0 in nitrifying and denitrifying granules, respectively. Lyophilized granules from full-scale plant contained 15% of ALE, i.e. twice higher than typically measured in flocs. Correlating these physicochemical gradients to phylogenetic structure and genomic signatures will allow for fundamental understanding of the granulation phenomenon and its temporal triggering.
INFLUENCE OF COPPER CHLORIDE AND COPPER SULFATE ON BIOFILM FORMATION OF TWO STAPHYLOCOCCUS AUREUS STRAINS

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Background
Biofilms pose a way to overcome toxic influences like antibiotics and metal ions. As the number of multiresistant bacteria increases (e.g. \textit{Staphylococcus aureus}) steadily, metals (copper, silver) celebrate their renaissance/revival for many purposes in health care facilities. Not much is known about the tolerance against these heavy metals.

Objectives
The known positive effect of the heavy metal tolerance of formed biofilms implicates that there could be a regulatory effect of copper ions on the formation and maturation of biofilms. Previous studies on this matter present inconclusive data, pointing in both directions.

Methods
The influence of different CuCl\textsubscript{2} and CuSO\textsubscript{4} concentrations (0.5 – 5 mM) on the formation of biofilms of two \textit{S. aureus} strains as well as on already formed biofilms was tested in a multi well plate assay. Protein and polysaccharide concentrations were quantified and biofilm growth was measured after 48h and 96h.

Conclusions
If grown in copper containing solutions the both strains used showed a different behavior. One strain was inhibited at higher copper concentrations (> 2.5 mM), the other strain presented an increased EPS production at the same level. Both, CuCl\textsubscript{2} and CuSO\textsubscript{4}, did not show an inhibitory effect on already formed biofilms of both strains. Interestingly lower concentrations (<3 mM) revealed a biofilm promoting effect. Further studies with more strains are needed to be certain which influence copper has on biofilms and their formation as apparently the strains show different behavior.
DEVELOPMENT OF BIOFILM MATRIX AND CELL-CELL COHESION IN RHIZOBIUM LEGUMINOSARUM

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Background

Biofilm formation is important for rhizobia to colonize several environmental niches. Within the microcolonies developed by \textit{Rhizobium leguminosarum}, rhizobial cells interact tightly through lateral and polar connections forming compact aggregates. These microcolonies are surrounded by a biofilm matrix, whose main component is the acidic exopolysaccharide (EPS). In addition, the PrsDE secretion system has been implicated in biofilm formation. This system is responsible for the secretion of Rap proteins that share one or two Ra/CHDL (cadherin-like-) domains.

Objectives

We aimed to explore the role of Rap(s) and other surface factors in biofilm development.

Methods

RapA was analyzed by biophysical and biochemical approaches. Functional studies were assessed by mutagenesis and gene overexpression.

Conclusions

We showed that RapA, which consists of two Ra/CHDL domains, is a unipolar calcium dependent lectin that specifically binds the EPS. RapA overexpression enhanced biofilm formation and expanded the distance between cells. In line with these observations, we showed that high levels of RapA secretion increases capsular polysaccharide (CPS) formation, which is structurally and genetically related with the EPS. We propose that RapA and the other Rap proteins, which are predicted to harbor one or two EPS/CPS-binding domains, play some role remodeling the biofilm matrix structure. Exploring the role of another important surface polysaccharide, the lipopolysaccharide (LPS), we found that the O-chain core region of the LPS is crucial for cell-cell cohesion. Mutants defective in the O-chain core moiety developed...
biofilms with an altered three-dimensional structure. Further studies are required to give insight into the interplay between the biofilm matrix components and Rap(s).
Background
Biofilms are microbial communities and highly relevant for water quality. In principle biofilms may affect human health in drinking water reservoirs and distribution systems hindering the efficient operation of these systems. Also they may also pose a health risk due the presence of pathogenic bacteria such as *Legionella pneumophila* and *Escherichia coli*.

Objectives
The aim of this study was to describe biofilms accumulating within controlled pilot plant after different treatment steps. The use of various methodological approaches should describe biofilms and the associated water phases in order to assess potential factors associated with human health risks.

Methods
In total 6 biofilm samples were collected from the PE-X pipes of the pilot drinking water treatment plant. The plant is designed for the drinking water treatment of a small rural community located in Upper-Austria (193 inhabitants in 2014; average daily water consumption: 20 m$^3$). The treatment has 4 successive steps: 1) oxygen enrichment (using pressurized air) 2) biological nitrification, 3) rapid sand filtration and 4) UV-disinfection.

Biofilms were characterized by chemical (TOC, DOC, heavy metals), microbiological (HPC, flow cytometry) and molecular biological (16S rRNA gene sequencing) and microscopic methodologies.

Conclusions
The multi-parametric approach applied in this study has shown valuable results with good correlations among chemical and microbiological characterization. The activity of biofilms (viability) could be described by flow cytometry and fluorescent microscopy. Phylogenetic analysis of sequenced culturable fractions revealed differing diversities in the microbial communities.
Biofilms are diverse microbial communities, bound by a matrix of extracellular polymeric substances, that allow cells to survive a variety of stressful, potentially lethal, conditions. To successfully avoid exposure to such toxic conditions, cells must transition rapidly from planktonic to sessile state, requiring quick acting regulatory mechanisms that can both inhibit motility and promote adhesion. We previously showed that the evolutionarily conserved type IV pili (TFP) are crucial for surface adhesion and microcolony formation in the model archaeon Haloferax volcanii. We also demonstrated that six conserved H. volcanii pilus subunits, the pilins PilA1-6, are involved in the regulation of flagella-dependent motility. Additionally, a subset of these pilins promotes microcolony formation, while at least two of the remaining pilins inhibit it. Recent studies suggest that pilin glycosylation catalyzed by the glycosyltransferase, AglB, plays an important role in the regulation of microcolony formation. Consistent with the pilins required for microcolony formation (PilA5 and PilA6) not being glycosylated, and the remaining pilins requiring glycosylation to function properly, we have shown that a ΔaglB strain forms microcolonies. Stress adversely affects AglB-dependent glycosylation, which is also required for flagella-dependent motility, hence inhibiting motility and promoting microcolony formation. Consistent with this we found that under low salt conditions H. volcanii is not motile and readily forms microcolonies. This differential post-translational modification that allows H. volcanii cells to respond quickly to changes in the environment that result in conditions favorable to sessile cells and biofilm formation rather than motile planktonic cells may be broadly conserved across prokaryotes.
The potential of halophilic microorganisms to convert waste glycerol to valuable compounds: glycerol carbonate (GlyC) and glycidol (GlyD)

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Background: Waste glycerol is the most important by-product in the production of biodiesel from biomass. As a direct consequence, waste glycerol (unpurified) was produced in quantities which soon will exceed current market demands leading to serious environmental problem by its storage.

Objectives: This work enlarges the application area for waste glycerol, proposing its usage as raw material in the production of glycerol derivatives (e.g. GlyC, GlyD and polyglycidol). Thus, alternative solutions for the storage problem of waste glycerol that can generate negative effects on the environment are developed. GlyC, GlyD and polyglycidol represent products of interest for fine chemical industry. Thus, GlyC is used as solvent for cosmetics and in medicine, while GlyD and polyglycerols appear as synthesis intermediates of plastics and resins in the polymer industry, but also in the pharmaceutical and cosmetic industries.

Methods: The products of interest were revealed in culture media using GC-MS and GC-FID techniques. Also, the polyglycidol products were evaluated (Mw, Mn and PI) based on GPC technique.

Conclusions: Preliminary results revealed ability of various moderately halophilic bacteria isolated from saline and hypersaline environments from Romania to convert waste glycerol to GlyC and GlyD. Lipase enzyme is responsible for GlyC production, while lipase and decarboxilase mixture convert the glycerol to GlyD. Polymerization of GlyD is performed under basic conditions.
Production of antimicrobial and anticancer substances by Bacillus pumilus SG-32 and Bacillus firmus P1-1 isolated from oil reservoirs in Brazil.

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BACKGROUND: Thirty strains isolated from Brazilian oil reservoirs were tested against each other for the production of antimicrobial substances. Two strains of Bacillus, B. pumilus SG-32 and B. firmus P1-1 were selected due to their ability to inhibit more than 60% of the strains tested. These strains were also investigated for cytotoxic activity against human cancer cell lineages.

METHODS: The screening of antimicrobial substances was performed by the agar diffusion method described for Rosado and Seldin (1993). Thirty strains previously isolated from oil reservoirs in Campos Basin (RJ, Brazil) were tested against each other for antimicrobial production. Two selected strains were growth in Marine Broth for 72 hours at 40°C to increase cell biomass. Anticancer screening was performed against nine different types of human cancer cell lineages.

RESULTS: Among the thirty strains tested, three of them (two strains of Bacillus pumilus, SG-30 and SG-32, and one strain of Bacillus firmus, P1-1) were able to inhibit more than 60% of the bacterial strains under study. Strains P1-1 and SG-32 were chosen for subsequent cytotoxicity assay against human cancer cells. Strain P1-1 presented better effect than the positive control doxorubicin against colon and breast cell cancer lineages. Strain SG-32 was not as efficient as the positive control; nevertheless five types of cancer cell lineage were inhibited.

CONCLUSIONS: Three different Bacillus strains from Brazilian oil reservoirs were able to produce antimicrobial substances, two of them with significant activity against cancer cell lineages, showing potential for further clinical trials.
SAFETY EVALUATION OF SOME COMMERCIAL SKIN COSMETICS USING A GENETICALLY MODIFIED ACINETOBACTER BIOLUMINESCENT BIOREPORTER DF4/PUTK2

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Background
Human skin is suffering from continued use of cosmetics, which may lead to ecosystem disruptions of useful skin microbiota. Therefore, a need to ensure its safety-in-use is important. Despite, microbiological assays employed to determine the lowest cosmetic concentration gave the lowest microbial contamination level during usage; toxicity assay must be performed first.

Objectives
In the present work, the Bioreporter DF4/PUTK2 was employed to assess the toxicity of eight local commercial cosmetic products. DF4/PUTK2 is a genetically modified strain belongs to the genus Acinetobacter that widely spread in nature and skin microbiota. Previously, DF4/PUTk2 was successfully employed to assess toxicity of phenolics and heavy metals.

Methods
Present results indicated that among the eight tested cosmetics, the Bioreporter was directly sensitive in order of decreasing sensitivity to aftershave balm, skin-refining toner, moisturizing cream and purifying cream with bioluminescence inhibition percent (BI%) equal to 64%, 58%, 50% and 30% respectively, Followed by eye makeup remover and sun cream with (BI%) equal to about 15% but after exposure time 300 and 700 min respectively. However, no decrease for bioluminescence with eye color powder and color trend – fresh foundation was observed. Also, the effect of exposure time on bioluminescence was tested. It was noticed that the cosmetics that expected to remain for a long time on skin seems most toxics.

Conclusions
In conclusion, the Bioreporter DF4/PUTK2 can be used to assay the toxicity of cosmetics and it is candidate to be a prescreening and quality control tool to pre-select the most toxic cosmetic for further chemical analysis.
Background

Considering the undeniable advantages of natural toxins from RIP family to produce anticancer immune-conjugates, well optimized recombinant expression of this category of proteins is of importance regarding industrial aspects. One of the proposed cellular mechanisms involved in EnBase fed-batch mode of cultivation is the change in protein expression via ribosomal manipulation. However, this system has not been applied to cytotoxic proteins.

Objectives

For the first time here, the expression profile of α-luffin, a ribosome inactivation protein (RIP) with an innate toxicity, was optimized in EnBase system. In fed-batch fermentation mode, the early incubation time was preferable at 30°C culture temperature whereas at 25°C the extended protein synthesis period (12 and 24h post induction) resulted in higher amount of soluble recombinant protein.
Methods

Mature α-luffin encoding cDNA was synthesized and subcloned into pET28a under the control of T7 promoter fused to the 6-HIS tag. The *E. coli* expression procedure was compared in traditional batch and newly developed fed batch; EnBase® Flo system at 25°C and 30°C incubation temperatures.

Conclusions
As the first study investigating the efficacy of EnBase fed-batch mode for the production of a toxic protein (α-luffin) it is concluded that in spite of the efficacy of this system in producing higher soluble protein ratios compared to batch cultivation growth rate, incubation temperature and time need to be optimized so as to cover innate cytotoxicity. The optimized condition proposed here is promising in terms of large scale soluble production of α-luffin without the need for refolding.
Background

Botulinum neurotoxin type A (BoNT/A) composed of three domain. One catalytic domain and one binding domain which are linked together by a translocation domain. In recent years, BoNT/A was used as a therapeutic agent for treatment some abnormal muscle contractions such as strabismus, blepharospasm, spasmodic or face wrinkles caused by ageing process. The injection of this drug causes some undesirable side effects such as irritation, pain, bleeding at the point of injection. Cell penetrating cationic peptides (CPPs) are three to 30 peptide residues which freely pass through cell membranes. Tat peptide is a CPP which is a favorite way to transduction of biological macromolecules (peptides, proteins or nucleotides) into cells.

Objectives

In this study, Tat\textsubscript{(47-57)} peptide directly fused to catalytic domain of BoNT/A. The penetration of this recombinant protein was analyzed \textit{in vitro}.

Methods

A genetic construct containing Tat gene sequence, residues 47-57, was fused with that of BoNT/A (residues 1-448) and cloned into a bacterial host for further expression. Obtained fusion protein, was purified and transferred into a 6 well plate seeded by HeLa cell line and was incubated at 37°C for 1 to 2 h. Transduction of protein was further analyzed by western blot.

Conclusions

Results showed that Tat\textsubscript{(47-57)} peptide can carry the BoNT/A\textsubscript{(1-448)} into HeLa cell line in comparison to BoNT/A\textsubscript{(1-448)} as control. Cell transduction was time dependent (protein band in western blot after 2 h was thicker than 1 h). CPPs are promising tools for
transferring different type of drugs directly into intact skin for therapeutic purposes through a non-invasive way.
CAN S. CEREVISIAE PRODUCE BIO-BASED POLYMERS FROM PENTOSE SUGARS?

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Background

A sustainable future necessitates an urgent transition from petroleum-based industries towards the production of bio-based bulk chemicals from renewable feedstock [1]. The bio-based polymers, such as polyhydroxyalkanoates (PHAs), can be produced from monomeric sugars. Poly-3-D-hydroxybutyrate (PHB) is one of the most studied PHAs and is a promising biopolymer produced naturally by several bacterial species [2]. In parallel, xylose is the main pentose sugar present in lignocellulosic biomass (LCB), which is considered the cheapest carbon source on earth [3]. Thus, LCB is a potential substrate for the bio-based production of bulk chemicals such as PHB.

Objectives

In this work, the capacity of recombinant Saccharomyces cerevisiae to produce PHB using pentose sugars (xylose) as the main carbon source was evaluated.

Methods

Engineered S. cerevisiae strains, capable of pentose utilization were transformed with the PHB pathway genes from the natural and well-characterized PHB-producer Cupriavidus necator. The two host strains carried genes for xylose utilization from the yeast Scheffersomyces stipitis, but two variants of the xylose reductase (XR) with different NADH/NADPH preference ratio were tested [4]. The resulting strains were evaluated for their PHB-production capacity.

Conclusions

The production of PHB from xylose-rich medium was achieved using S. cerevisiae. The highest PHB yield (g/g xylose) was achieved with the strains carrying the mutated S. stipitis XR with a higher NADH/NADPH preference, highlighting the importance of redox balancing for PHB production.

USE OF FUNGAL ROTATING DISK REACTOR (RDR) FOR BIODEGRADATION OF TEXTILE ANTHRAQUINONE DYES


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Background

Biodegradation potential of fungi for removal of dyes from textile wastewater has been established and applied in aerated reactors.

Objectives

The aim was to compare dye-degradation efficiency of a newly isolated Trametes suaveolens strain with different fungal species in RDR. Reactive RB19 (C.I.61200) and disperse DB3 (C.I.61505) dyes were used.

Methods

2-L RDR (6 disks, rotation speed 2 rpm, air flow 50L.h⁻¹) was used for dye decolorization (50mg.L⁻¹) in malt extract-glucose medium at 22°C. Decolorization was measured spectrophotometrically and the end-products by GC/MS. Bacterial luminiscence test, plant growth tests and Ames test were used to measure acute toxicity and genotoxicity.

Conclusions

Using batch mode, RB19 decolorization rates were: Irpex lacteus>T. suaveolens>Pleurotus ostreatus. When continuous mode was used, the dye degradation capacity of T. suaveolens was 2-fold compared to the batch mode. Majority of the degradation products were aromatic alcohols. No genotoxicity was detected after degradation. The acute toxicity measured with Vibrio fischeri, Sinapis alba and Lemna minor decreased 1.2-, 2.7- and 1.6-fold, respectively. The results demonstrated the efficiency of fungal RDR for removal of textile anthraquinone dyes.

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SULFATE-REDUCING BACTERIA AS WHOLE-CELL BIOCATALYSTS FOR HYDROGEN PRODUCTION

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Background
Sulfate-reducing bacteria (SRB) are a major group of environmental bacteria with important biotechnological applications in waste water treatment and bioremediation. These organisms have an extremely high hydrogenase activity and in natural habitats where sulfate is limited they produce hydrogen fermentatively and grow syntrophically with other organisms. However, their potential as H₂-producers has been poorly investigated.

Objectives
Given the high number of hydrogenases present in SRB genomes [1] we explored the potential of the model strain Desulfovibrio vulgaris Hildenborough for H₂ production from three substrates (lactate, ethanol, formate).

Methods
Among the substrates tested the highest H₂ production was observed from formate [2]. Formate has emerged recently as an environmental friendly storage of H₂ that can be easily transported and has relatively low toxicity. Therefore a lab-scale H₂ production process with gas sparging was designed to evaluate the potential of SRB as biocatalysts for formate-driven H₂ production [3].

Conclusions
By optimizing the operation conditions of bioreactor such as temperature, gas flow rate and cell load, a high H₂ production rate was obtained. In addition, a high specific hydrogen production rate and 100% efficiency of substrate conversion were achieved, demonstrating the high potential of SRB for H₂ production from formate [3]. Our studies reveal that SRB can be used as an efficient biocatalyst for the conversion of formate to hydrogen.

INVESTIGATING MICROALGAE AS A BIOTECH PLATFORM FOR HIGH-VALUE OILS

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Background

Natural oils are found in a diverse range of products such as soaps, paints, cosmetics, antibacterial treatments and nutritional supplements (fish oils).

Objectives

As part of a consortium with the University of Cambridge, the University of Aberdeen and Rothamsted Research we aim to establish the microalgae *Nannochloropsis gaditana* as a platform for the production of these commercially important oils. The advantages of microalgae as a production platform include low media costs, lack of toxins and fast growth rates; *N. gaditana* was chosen due to its naturally high lipid content.

Methods

The production of bespoke lipids will require a combination of metabolic modelling and nuclear genetic engineering to introduce or knock out specific elongase and desaturase genes. To test the genetic tractability of this species, two constructs were prepared that contained *ble-gfp* or *ble-HA* fusion genes; the *ble* marker encodes resistance to zeocin. *N. gaditana* was transformed by electroporation and colonies were selected on media containing zeocin. All of the colonies tested carried the expected transgene and accumulated Ble-GFP or Ble-HA fusion protein, as detected by western blotting.

Conclusions

The *N. gaditana* nuclear genome is amenable to genetic transformation by random integration, paving the way for our metabolic engineering studies over the next three years.
MALIC ACID PRODUCTION FROM RENEWABLE SOURCES BY ASPERGILLUS ORYZAE
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Background

L-malic acid is a C4 dicarboxylic organic acid and considered as a promising chemical building block. It can be applied as food preservative and acidulant, in rust removal because of its chelator properties and as polymerization starter unit due to its bifunctionality. Up to now it is produced chemically from crude oil via maleic anhydride. The mould Aspergillus oryzae produces malic acid in large quantities from glucose and other carbon sources. The microbial production of organic acids from renewable sources has the potential to be a sustainable alternative to petroleum and to reduce greenhouse gases as CO₂ fixation is involved in microbial biosynthesis.

Objectives

The potential of malic acid production from renewable resources is evaluated. Therefore, different carbon sources based on lignocellulosic biomass, e.g. fractions of pyrolysis oil and hemicellulosic sugars, are tested.

Methods

A. oryzae was cultivated in preculture medium in shaking flasks for 17 hours. Due to nitrogen limitation and an excess of glucose in the production medium the fungus started to produce malic acid. A bioreactor process using glucose as carbon source was already established¹ and used as basis for the evaluation of alternative carbon sources. Organic acid concentration was measured by HPLC.

Conclusions

A. oryzae proved to be a promising natural host for malic acid production with the potential to use several renewable carbon sources.

References:

EXPLORING NON-CONVENTIONAL YEAST SPECIES AS SOURCE FOR ENHANCING ANAEROBIC XYLOSE FERMENTATION IN METABOLIC ENGINEERED SACCHAROMYCES CEREVISIAE

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Background

Efficient conversion of xylose, a five-carbon sugar present in significant amounts in the hemicellulose fractions of a wide range of lignocellulosic biomass, is required for the development of second generation bioethanol. Xylose is not naturally consumed by the baker’s yeast, \textit{Saccharomyces cerevisiae}, therefore heterologous pathways has to be introduced by genetic engineering. This includes introducing one of the two known pathways for the conversion of xylose to xylulose: xylose isomerase (XI) or xylose reductase and xylitol dehydrogenase (XR-XDH). In most xylose-consuming fungal species, XR is an enzyme that uses NADPH as the dominant co-factor for xylose reduction to xylitol. It has been observed that \textit{S. cerevisiae} strains harbouring XR variants with an increased preference for NADH have more efficient xylose fermentation, therefore exploring the diversity of XR with different co-factor affinity is essential.

Objectives

In this work we have evaluated the xylose utilisation under anaerobic conditions from two constructed \textit{S. cerevisiae} strains carrying different varieties of xylose reductases.

Methods

The strain TMB3422, carrying a mutated version of the \textit{Scheffersomyces stipitis} XR gene and a constructed strain, carrying a novel XR gene from a fermenting yeast species that was isolated from rotting-wood samples, were compared for characteristics under fermentative conditions.

Conclusions
The best xylose fermenting yeast strain analysed revealed significantly higher ethanol yield and productivity, as well as lower xylitol yield under anaerobic conditions, which represents an advance towards the efficient fermentation of lignocellulosic hydrolysates to ethanol.
SOLID WASTE FROM COFFEE PROCESS FOR β-GLUCOSIDASE PRODUCTION BY BACILLUS SUBTILIS

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Background
The coffee production for the Brazil is a second important commodity however the solid and liquid waste during the process is a environmental problem. Some works focus on use these waste to produce organic acids, enzymes and other products

Objectives
The aims were optimize the β-glicosidase production for Bacillus subtilis in submerged fermentation using coffee pulp as substrate and evaluated the optimize condition culture in bioreactor.

Methods
The coffee pulp was dried at 60 °C for 72 h until reaching constant weight. After that, the coffee pulp was ground in Willey mill (1.0 mm). The microorganism used in study was Bacillus subtilis CCMA0087 belongs to Culture Collection of Agricultural Microbiology (CCMA) of Federal University of Lavras (Brazil), isolated from Brazilian Cerrado fruit namely Marolo. The enzyme production was done by Response Surface methodology. The media culture was based in Kasana et al. (2008) with modification (% 0.2 NaNO₃, 0.1 K₂HPO₄, 0.05 MgSO₄, 0.05 KCl, 0.02 peptone and coffee pulp (0.32 – 3.68) as a carbon source. The β-glucosidase activity as measured by p-nitrophenyl-β-D-glucopyranoside (PNPG) as substrate.

Conclusions
In bench scale, Bacillus subtilis UFLA BCEF1130 show high β-glucosidase production (22.59 UI/mL) at pH 3.64, 36.6 ºC and concentration coffee pulp of 36.8 g/L. However, in bioreactor the enzyme production was 2.5 less than bench scale in the same experimental conditions. The work continues to understand the scale up effect over the β-glucosidase production.
Background

Hyaluronidases (Hz) are enzymes that degrade predominantly hyaluronan (HA). Until recently, three groups of Hz were reported: hyaluronate 4-glycanohydrolases (found in Vertebrates and venom of some insects), endo-β-glucuronidases (leeches and crustaceans) and hyaluronan lyases (bacteria). Recently a new group of HA degrading enzymes from Micromycetes belonging to genus *Penicillium* was reported [Bakke M, Kamei J & Obata A., 2011, DOI: http://dx.doi.org/10.1016/j.febslet.2010.11.021]. These enzymes hydrolyze β-1,4 glycosidic bonds in HA, similar to Vertebrate Hz, however have different conserved domains. We report discovery of another producer of hyaluronate 4-glycanohydrolase from the Kingdom Fungi, the yeast *Pseudozyma aphidis*.

Objectives

To characterize *P. aphidis* extracellular Hz.

Methods

*P. aphidis* was cultivated in bioreactor Techfors-S with 20 L working volume (Infors AG, Switzerland). The enzyme was isolated from cultural broth filtrate and partially purified by diafiltration on Sartorius Hydra membrane cassette with 10 kDa cut-off and ion-exchange chromatography on DEAE-Sepharose Fast Flow (GE Healthcare). The products of HA enzymatic hydrolysis were analyzed by HPLC and LC-MS. Gel permeation chromatography, SDS-PAGE and Morgan-Elson colorimetric assay were used for enzyme characteristic.

Conclusions

*P. aphidis* produced extracellular enzyme with hyaluronate 4-glycanohydrolase activity. Temperature and pH optima of HA hydrolysis were 45 °C and pH 3.0 respectively. The enzyme didn’t hydrolyze neither chondroitin sulphate nor β-(1.3;1.6)-glucan schizophyllan. *P. aphidis* Hz showed high thermal stability that makes advantageous its application for enzymatic production of hyaluronan oligomers.
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PENICILLIUM CHRYSOGENUM B13, A FERULOYL ESTERASE FACTORY
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Background
Feruloyl esterases (FAEs) represent a diverse group of hydrolases catalyzing the
 cleavage of ester bonds between plant cell wall polysaccharide and phenolic acid.
These enzymes are key tools for degradation of plant cell wall and are widely
distributed in plants and microorganisms. FAEs release ferulic acid and other
aromatic acids from these polymeric structures and have received an increasing
interest in biotechnological processes for industrial and medicinal applications.

Objectives
In the worldwide market there are not fully purified FAEs or enough enzyme stocks.
Discovery of new FAEs with novel properties continues to be an important research
area that has increased drastically since 1990. Following this research line
(CTM2012-32026 project¹), genetic engineering techniques to improve the FAEs
production in the filamentous fungus Penicillium chrysogenum have been applied.

Methods
Several transformants, whose production of FAEs is significantly improved with
respect to the parental strain, have been obtained. Special interest deserves B13
transformant that multiplies the FAE activity more than 90 times when the substrate of
the enzyme is methyl ferulate and over of 14 when the substrate is sugar beet pulp.
Furthermore, this FAE activity from the B13 strain was compared with the activity that
is present in some commercial enzymes versus synthetic and natural substrates.

Conclusions
The analysis of the results revealed that the B13 enzymatic extracts triplicate the FAE
activity in comparison with the analyzed commercial enzymes.

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Competitiveness (MINECO) through the Subprogramme for Non-Guided
PARAMETERS AFFECTING THE PRODUCTION OF LIPASE ENZYME BY A NEW ISOLATE YEAST.

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Background

Lipases (E.C.3.1.1.3; tri glycerol acyl hydrolases) are hydrolizer enzymes which catalyze free fat into glycerol and triglycerid. Industrial lipase commonly is provided by external cellular activity from strains of microorganisms. Microbial lipases are produced in liquid phase and its activity is influenced by concentrations of carbon/nitrogen resources, pH, temperature and metal ions.

Objectives

In this study, fungi as a lipase source, isolated from the village which is located near Eskisehir city. In the production of lipase, environmental conditions and media components are investigated as important parameters.

Methods

Vegetable oils as carbon source, carbohydrate source and nitrogen sources are used for lipase activity investigation. To find the optimal condition, firstly between 3.0 – 9.0 pH and 10 – 40°C several experiments are carried out to detect the effects of these varieants on lipase production.

Conclusions

As a result of several experiments for production of lipase from Rhodotorula, the optimal condition is pH 5.0, temperature 30°C, 1% concentration of carbon from olive oil and the best source of nitrogen, the pepton has been considered. As a result of combination of carbon and nitrogen sources with olive oil, the activity of lipase was increased.
PRODUCTION OF LIPASE ENZYME BY A NOVEL FUNGAL SOURCE, TRICHODERMA SP.
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Background

The industrial use of enzymes obtained from animals, plants and microorganisms has developed recently and the most important source for enzyme production has been determined as microorganisms. Lipases has an important application areas in industry.

Objectives

In this research the lipase enzyme which has a industrial importance was obtained from new fungal source that was isolated from soil. Subsequently it was determined as *Trichoderma citrinoviride* by the analysis of 18S rRNA sequence.

Methods

Lipase activity was determined by titrimetric assay. Optimum conditions were detected for production of lipase.

Conclusions

The lipase enzyme by isolated fungi (*Trichoderma citrinoviride*) was extracellular and showed its highest activity on the 4. day of the incubation period. In the production of lipase, culture conditions and media components are investigated as important parameters. Various carbon and nitrogen sources were used for highest lipase activity. Also we detected that pH 5.5 and temperature 30 °C are optimum conditions for lipase production.
Background

The project **MySterI** (*Mycobacterial Steroids for Industry*), supported by the ERA-IB 3rd joint call, aims at producing high value steroid precursors using a novel bioconversion strategy that will yield a greening process with lower production costs (@MySterl ERA_IB; http://www.era-ib.net/mysteri-0). The bioconversion of phytosterols (low cost plant material similar to cholesterol) to desired steroid precursors is performed using engineered fast-growing mycobacteria in a single fermentation step.

Objectives

The targeted precursors are: androst-4-ene-3,17-dione (AD), 3β-hydroxyandrost-5-ene-17-one (DHEA) and then 11α-hydroxyandrost-4-ene-3,17-dione (11-α-OH-AD) and testosterone.

Methods

The key points tackled by the Consortium are: i) Genome sequencing and annotation of *Mycobacterium* sp. NRRL B-3805 (AD-producer) to identify key bioconversion genes and to enable 'omics tools; ii) Understanding of phytosterol bioconversion by means of ‘omics technologies; iii) Development of the genetic engineering tools for *Mycobacterium* sp. NRRL B-3805; iv) Construction of mycobacterial strains capable of producing 11-α-OH-AD, DHEA and testosterone; v) Designing more efficient and eco-friendly methods of production and downstream processing for the three selected compounds.

Conclusions

The MySterl pipeline is a top to bottom process that brings together six interdisciplinary research groups from Universities, Research Centres and Industrial companies in 3 different EU member states [Spain (INBIOTEC; Gadea Biopharma), Germany (Technische Universität Dortmund) and UK (University of York)], as
well as Norway (Stiftelsen SINTEF) and Russia (Pharmins Ltd.). Therefore, the MySterI results in progress are: i) novel strains capable of producing three valuable C19-steroid precursors from phytosterol ii) knowledge of the biochemistry of steroid biotransformations iii) optimized fermentation and eco-friendly downstream processes for the single-step production.
Background
Microbial biotransformation to generate new steroid drugs is a well-established application of industrial biotechnology. Thus, several worldwide enterprises are involved in steroid production (e.g.: Gadea Biopharma SL, Sanofi, Pfizer). Biotransformation can begin with different raw materials, but the mixture of plant sterols (phytosterols) have been the research focus in recent decades. However, the studies to date show that improvements in phytosterols broth culture solubility, product recovery and mainly in microbial strains are needed.

Objectives
Optimization of the whole molecular biology methodologies valid for a non-well characterized microbial strain has been carried out on the androstenedione (AD)-producer strain *Mycobacterium* sp. NRRL B-3805. Thus, i) growth conditions; ii) phytosterols solubility analysis; iii) growth titration in dirty media; iv) steroids quantitation by HPLC; v) genomic DNA extraction suitable for genome sequencing; vi) high quality RNA for transcriptomics (RNAseq, microarrays) analyses; as well as vii) secreted protein purification for 2D-DIGE secretome methods have been improved.

Methods
An example of successful optimized process is the genome sequencing pipeline defined for *Mycobacterium* sp. NRRL B-3805. This genome sequencing and annotation, as well as the understanding of phytosterols bioconversion by means of ‘omics technologies (RNAseq, microarrays, 2D-DIGE) are capital for the ERA-IB 3rd joint call supported project MySterI (@MySterI_ERA_IB; http://www.era-ib.net/mysteri-0).

Conclusions
Thus, key bioconversion genes and target genes suitable for the development and use of genetic engineering tools have been defined. This genome was fully sequenced using Ion Torrent and Sanger technologies. It comprises 5,421,338 bp in a circular chromosome with a GC content of 67 %.
Background
The use of fungal biomass for the preconcentration and separation of traces of heavy metals and some organic materials at trace levels are popular due to the good adsorption properties such as high surface area and high adsorption capacity of the fungus.

Objectives
This study describes the biosorption potential of *Helvella crispa* as a sorbent for solid phase extraction (SPE) and preconcentration of Pb\(^{2+}\) and Al\(^{3+}\).

Methods
The model solution was passed through the prepared SPE column at an adjusted flow rate with a peristaltic pump. Then, 10.0 mL distilled water was passed through the column. The retained Pb\(^{2+}\) and Al\(^{3+}\) on the fungal biomass was then eluted with 5.0 mL of 1.0 mol L\(^{-1}\) HCl. The concentration of Pb\(^{2+}\) and Al\(^{3+}\) in this solution was determined by ICP-OES.

Conclusions
The limit of detection (LOD) of Pb and Al was found to be 0.10 ng mL\(^{-1}\) and 0.03 ng mL\(^{-1}\), respectively. The sensitivity of ICP-OES improved 39.8-fold for Pb and 39.5-fold for Al. Linearity was obtained in the concentration range of 1.25–50.0 ng mL\(^{-1}\) for Pb and 0.5–50.0 ng mL\(^{-1}\) for Al. The relative standard deviation (RSD) of the method under optimum conditions was lower than 8.4% (n=5) for Pb and Al, which was validated through the analysis of certified reference tea and poplar leaves samples. The biosorption capacity of immobilized *Helvella crispa* for Pb and Al was found to be 31.2 mg g\(^{-1}\) and 45.7 mg g\(^{-1}\), respectively.
WHICH IS A BETTER APPROACH FOR ENZYME EXPLORATION?: CULTURE-DEPENDENT AND -INDEPENDENT (METAGENOMIC) APPROACHES TO THE SCREENING FOR AROMATIC-DEGRADING ENZYMES.
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Background
Although metagenomics is regarded as one of the best approaches for mining enzymes, its superiority over traditional culture-dependent approach is still controversial.

Objectives
Therefore, culture-dependent and -independent approaches were used for extensive retrieval of the extradiol dioxygenase (EDO) gene from the environment to investigate the relationship between the EDO genes from isolated bacteria and the metagenomic EDO genes from which they were isolated.

Methods
In our previous study, we identified 91 fosmid clones showing EDO enzyme activity using a metagenomic approach (Environ Microbiol 9, 2289-2297, 2007). In the present study, we newly isolated 88 phenol-utilizing bacteria from the same environmental sample and identified four EDO genes from them. Of these, two EDOs had amino acid sequences similar to those reported previously in aromatic-utilizing strains, and one EDO had a sequence almost identical to that of metagenomic EDOs identified in our previous study. Unexpectedly, one EDO showed no similarity to any class I EDOs and was categorized as class II, which has not been found in past any metagenomic approaches. Quantitative polymerase chain reaction (PCR) assay indicated that the low-abundance class II EDO gene can be enriched by culturing approaches.

Conclusions
We conclude that the combined use of the two approaches can explore the gene community more extensively than their individual use.

ENGINEERING STREPTOMYCES DIASTATOCHROMOGENES 1628 TO INCREASE THE PRODUCTION OF TOYOCAMYCIN

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Background
Toyocamycin (TM) is a member of the nucleoside antibiotics. It shows antifungal activity and might be used to treat plant diseases. TM is produced by Streptomyces diastatochromogenes 1628 but the production rate is always very low. Recently the gene toyG was cloned and its function in TM biosynthesis was reported. However, nothing was known about the influence of toyG on TM production.

Objectives
We studied the influence of three genes on TM production. Vgb, frr, and toyG were co-expressed, either in double- or triple-combinations.

Methods
Over-expression of these genes in double or triple leads to great significant increased production of TM in strain S. diastatochromogenes 1628. At the end, among all these different strains constructed in this work, the strain S. diastatochromogenes 1628-VGF, harboring vgb, frr and toyG that was placed under the control of PermE, respectively, exhibited the largest increase in TM production. Notably, the TM production of S. diastatochromogenes 1628-VGF reached the highest level at 831.2 mg/L at 72 h, while TM yield of original strain S. diastatochromogenes 1628 at 415.1 mg/L at 84 h.

Conclusions
Based on known knowledge, TM yield produced by engineered strain S. diastatochromogenes 1628-VGF was the highest as ever reported. The high-yield, energy-saving strain S. diastatochromogenes 1628-VGF constructed in this study shall be beneficial for the industrial production of TM.
Expression of the Cellulosomal Gene Cluster of Clostridium Cellulovorans in Butanol-Producing Clostridium Beijerinckii

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Background
Biofuel production from cellulosic biomass consists of pretreatment, saccharification, fermentation and distillation. However, production is hindered by the high cost compared with energy crops, such that innovative technologies are required. One approach is consolidated bioprocessing (CBP), in which pretreatment, saccharification, and fermentation are consolidated.

Objectives
Clostridium cellulovorans 743B, an anaerobic and mesophilic bacterium, produces a large extracellular enzyme complex (cellulosome) that efficiently hydrolyzes cellulosic biomass. The cellulosomal gene cluster of C. cellulovorans includes cbpA, which encodes a scaffold protein, and seven downstream glycoside hydrolase genes. Clostridium beijerinckii NCIMB 8052 cannot produce a cellulosome and does not degrade soft biomass, but instead produces butanol, acetone, and ethanol from mono- and disaccharides. The aim of this study was to express the cellulosome of C. cellulovorans in C. beijerinckii to obtain a bacterium able to carry out CBP.

Methods
The large cellulosomal gene cluster containing the cbpA gene was used to transform C. beijerinckii by electroporation. Protein-level expression of the gene cluster encoding the scaffold protein CbpA and mannanase A was demonstrated by western blotting. Cellulose and galactomannan activities on polysaccharide substrates were evaluated by Congo red staining.

Conclusions
The transformant was shown to be capable of hydrolyzing galactomannan and produced butanol directly. This butanol-producing bacterium provides the basis for the CBP of lignocellulose to biobutanol.
EFFECTS OF IRON AND MAGNESIUM IONS COMBINATION ON HYDROGEN PHOTOPRODUCTION BY RHODOBACTER SPHAEROIDES

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Background

Iron and magnesium are essential for growth of photosynthetic bacteria. Mg²⁺ participates in the structure of photosynthetic electron carriers and pigments. Fe²⁺ is known as the component of enzymes, involved in bio-hydrogen (H₂) production.

Objectives

In this work the effects of metal ions combinations on growth and H₂ photoproduction by Rhodobacter sphaeroides MDC6522, isolated from Jermuk mineral springs in Armenia, in compare to single ions effects, were investigated.

Methods

R. sphaeroides was cultivated under anaerobic conditions upon illumination in the presence of Fe²⁺ and Mg²⁺ various concentrations. The H₂ yield was calculated by the decrease of redox potential, measured by platinum electrode.

Conclusions

The results pointed out the concentration dependent effects of metal ions. R. sphaeroides was unable to grow well in the absence of Fe²⁺ and Mg²⁺, which indicates the importance of these ions for bacterial growth. The highest growth rates of R. sphaeroides cells were obtained in the presence of 0.08 mM Fe²⁺ and 5 mM Mg²⁺, in compare to control (0.04 mM Fe²⁺ and 1 mM Mg²⁺). The simultaneous addition of metal ions stimulated bacterial growth rate ~1.2-fold in compare to the single ions. The highest H₂ yields were obtained in the presence of 0.08 mM Fe²⁺ and 10 mM Mg²⁺, which were ~1.5-fold higher than control. However, the H₂ production was increased ~2.5-fold, when two metals were added simultaneously in concentration 0.08 mM Fe²⁺ and 5 mM Mg²⁺. Thus, combinations of metal ions in appropriate concentrations are preferable for H₂ production enhancement in R. sphaeroides.
HIGH-LEVEL EXPRESSION, RAPID ON-COLUMN REFOLDING AND PURIFICATION OF RECOMBINANT HUMAN VASCULAR ENDOTHELIAL GROWTH FACTOR 165 IN ESCHERICHIA COLI

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Background

Vascular endothelial growth factors (VEGFs) are a family of proteins that promote angiogenesis and participate in a variety of physiological and pathological processes. VEGF165 is the major isoform induced by hypoxia, oncogene mutations, and cytokines such as IL-1, IL-8, and TNF-α and secreted by many cell types. It is a potent angiogenic factor and mitogen that stimulates proliferation, migration, and formation of endothelial cells. Cancerous cells are able to grow and metastasize by VEGF secretion.

Objectives
A recombinant VEGF165 might be used in order to produce an antibody against it and also it could be used as a growth factor in some biological mediums.

Methods

A. PCR screening of the recombinant plasmid pET32a+ (VEGF165).

B. SDS-PAGE analysis of rhVEGF165 expression in BL21(DE3) cells
   U: uninduction (-IPTG)
   I: Induction (+IPTG) restriction sites.

C. The effects of induction time and temperature on soluble and insoluble rhVEGF165 after cell disruption
   I: Insoluble
   S: Soluble.

D. SDS-PAGE analysis and immunoblot with anti-His-tag antibody.

E. SDS-PAGE analysis of purified rhVEGF165 with On-Column refolding system.

F. SDS-PAGE and Western Blot analysis of purified rhVEGF165 using anti-His-tag antibody.

A recombinant VEGF165 might be used in order to produce an antibody against it and also it could be used as a growth factor in some biological mediums.
The RNA was extracted from Hela cell, and then used for cDNA synthesis. The gene encoding human VEGF isoform 165 (hVEGF165) was cloned into the expression vector pET32a (+) to construct a fusion expression plasmid that induced the thioredoxin (Trx) Gene. The expressed protein was purified by affinity chromatography using Ni-NTA resin. High concentration of the recombinant protein obtained from a single-step.

Conclusions
Fusion of VEGF to thioredoxin and careful codon optimization of the eukaryotic sequence could be improved to a high-level insoluble protein expression in comparison to an un-optimized, His-tagged construct. The thioredoxin-fused protein was successfully purified using an on-column Ni-NTA purification procedure. SDS-PAGE and Western Blotting analysis confirmed the desired expression. rhVEGF165 could readily and rapidly purify with on-column Ni-NTA system chromatography.
ADHESION OF ALKANOTROPHIC ACTINOBACTERIA TO SOLID SURFACES AND HYDROPHOBIC LIQUIDS

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Background
Mechanisms of adhesion of biotechnologically significant alkanotrophic actinobacteria are poorly studied.

Objectives
1. To study the adhesion of actinobacteria to a wide range of solid carriers and hydrophobic liquids under various growth conditions. 2. To determine physical–chemical and molecular factors of actinobacterial adhesion. 3. To define adhesion conditions providing the highest catalytic activity of actinobacteria.

Methods
100 strains from the IEGM Collection of Alkanotrophic Microorganisms (acronym IEGM, WDCM # 768, www.iegm.ru/iegmcol) were used in this study. The substrates for adhesion were polystyrene microplates, technical polymer tissue, industrial wastes (sawdust, poultry feathers, and leather), n–alkanes C10–C16, solvents, and different oils. Molecular genetic and biochemical mechanisms of adhesion were studied using targeted and in vivo Tn5 mutagenesis, chemical analysis of cell wall, and specific inhibition. Cell morphology, nanomechanical properties, adhesion forces, and distribution of actinobacteria on solid carriers were studied by interference microscopy and combined confocal and laser scanning atomic force microscopy. Thermodynamic data were obtained using infrared thermography.

Conclusions
Adhesion was shown to be a key mechanism in hydrophobic compound oxidation by...
actinobacteria. Involvement of glycolipid biosurfactants, proteins, and cell appendages in adhesion was revealed. Monolayer actinobacterial biofilms with high (115 mg·l⁻¹·h⁻¹) hydrocarbon–oxidizing activities were formed on solid surfaces hydrophobized with glycolipid biosurfactants (Figure). The results obtained could be used to develop biocatalysts for targeted biotransformations and pollutant degradation. The research was supported by the RFBR grant (14-04-96013), the RSF grant (14-14-00643), and the President of the Russian Federation for Leading Scientific Schools' grant (4607.2014.4).
BIOTRANSMFORMATION OF BETULIN USING RHODOCCUS ACTINOBACTERIA

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Background

Bacterial transformation of triterpenoids (in particular betulin) is an efficient method for single-stage production of biologically active compounds with high regio- and stereoselectivity.

Objectives

1. To demonstrate the possible use of Rhodococcus actinobacteria for selective biotransformation of betulin, a pentacyclic lupine-type triterpenoid. 2. To study the interaction mechanisms between rhodococci and betulin, and its biotransformation pathways. 3. To evaluate the possible application of products from bacterial betulin oxidation for subsequent synthesis of biologically active compounds using chemical methods.

Methods

The biological resources of the IEGM Collection of Alkanotrophic Microorganisms (acronym IEGM, WDCM # 768, www.iegm.ru/iegmcol) were used in this study. Conventional bacteriological methods, including betulin transformation by resting rhodococcal cells were employed. The mechanism of cell interaction with betulin was determined using methods of phase contrast, confocal lazer scanning, and atomic force microscopy. Rhodococcal viability was estimated using a LIVE/DEAD BacLight™ Bacterial Viability Kit (Invitrogen, USA).

Conclusions

Biotransformation of betulin (0.5 and 3.0 g/l) using Rhodococcus actinobacteria was demonstrated for the first time. Betulone was identified as a major metabolite. The optimal conditions for betulin bioconversion by resting cells of R. rhodochrous IEGM 66 were selected. Further chemical modifications of betulone resulting from bacterial betulin transformation lead to the formation of 3,4-secobetulone with a marked cytotoxic activity (IC50 3.05 мкМ) against melanoma.

The research was supported by the RFBR grant (14-04-96017), and the President of the Russian Federation for Leading Scientific Schools’ grant (4607.2014.4).
Background
Extracellular polysaccharides (EPS) of microbial origin are associated with the cell surface or excreted in the extracellular environment. Several food grade microorganisms, including lactic acid bacteria (LAB), synthesize EPS. The synthesis of EPS by LAB has gained remarkable interest because of LAB categorization as safe organisms and vast utilization of synthesized EPS in markets from food to health and industry. Dextran is a polysaccharide composed of D-glucose units which is synthesized by various LAB under appropriate conditions and widely used in several industries including food, pharmaceutical and chemical.

Objectives
Present study was designed to isolate LAB capable to synthesize good quality dextran and characterize the synthesized dextran.

Methods
In this study five bacterial strains were isolated from different vegetables and fruit samples on the basis of slime production in selected agar medium with 15% sucrose. When those five strains were cultivated in medium for the production and extraction of EPS, the highest crude yield of EPS was obtained from CMGDEX3 which was isolated from cabbage and identified as Weissella cibaria by 16S rRNA gene sequencing. EPS extracted from W. cibaria CMGDEX3 was purified and analyzed for structure by FTIR, $^1$H and $^{13}$C NMR spectroscopy. Molecular weight was determined by gel permeation chromatography.

Conclusions
Analysis of EPS demonstrated that W. cibaria CMGDEX3 synthesized commercially important linear dextran that predominately had α (1→6) glycosidic linkages with few (3.4%) α (1→3) linked branches. Molecular mass determination showed that it was a high molecular weight dextran of an average > 2,000,000 Daltons.
Background

Probiotic refers to viable, nonpathogenic microorganisms that, when ingested, are able to reach the intestines in sufficient numbers to confer health benefits to the host. Intake of probiotics reduces the amount of pathogens improving thus the intestinal microbial balance of the host and lowering the risk of gastro-intestinal diseases.

Objectives

To isolate potential probiotic bacterial strains inhibiting rota- and polio- viruses as well as pathogenic bacteria.

Methods

Three thousands bacterial strains isolated from various sources including human milk and fermented foods were screened for potential probiotics inhibiting rota- and polio-viruses as well as pathogenic bacteria such as enterotoxigenic *Escherichia coli*, *Clostridium perfringens*, *Listeria monocytogenes*, *Staphylococcus aureus*, *Salmonella Typhimurium*. The antibacterial activity of each culture filtrate was determined by agar-diffusion method and the antiviral activity of heat–treated culture broth was examined by reduction of plaque forming unit *in vitro* culture system. The properties of the bacterial strains with particular functions such as adherent ability to enterocyte, tolerances towards gastric juice, bile, and tolerances to heat and cold-dry conditions were further investigated to select strains for probiotic use.

Conclusions

One potential lactic acid bacterium with desirable probiotic characteristics as well as the highest anti-viral and anti-bacterial activities was selected and identified as *Lactobacillus plantarum* according to its 16rRNA gene sequence.
OPTIMIZATION OF THE SECRETION OF BACTERIOCINS AND THE BACTERIAL CELL GROWTH OF GEOBACILLUS SP. 15 STRAIN

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Background

Bacteriocins are natural peptides secreted by a variety of bacteria and provide the bacteria with a competitive advantage in their environment, eliminating competitors to gain resources. Bacteriocin production may be strongly influenced by growth environment and medium composition. Many different studies have indicated the potential usefulness of bacteriocins in food preservation, veterinary, medicine or as phytosanitary measures for plant protection. To date, only a few bacteriocin producing thermophilic bacterial strains of genus Geobacillus have been identified. Studies of factors affecting the production of bacteriocins by Geobacillus spp. bacteria are relatively scarce.

Objectives

This research was focused on the analysis effect of salts and to establish optimal conditions for bacteriocins production and bacterial cell growth for thermophilic bacteria Geobacillus sp. 15 strain.

Methods

Geobacillus sp. 15 strain was used as a bacteriocin producer. Geobacillus stearothermophilus NUB36187 (BGSC No. 9A11) was selected as the indicator strain for the bacteriocin assay. Nutrient broth (NB) liquid media were chosen for the strain growth and bacteriocin expression monitoring. It were supplemented with salts: KCl, ZnSO₄, MnSO₄, CuSO₄, MgCl₂, CaCl₂, NaNO₃, NaHCO₃. Bacteriocin activity was detected by agar well diffusion assay.

Conclusions

The greatest influence on bacterial growth was in NB medium with MnSO₄ or with KCl and MgSO₄. The best bacteriocin expression was achieved in media supplemented with two salts at a time: KCl and MgCl₂, MnSO₄ and NaHCO₃ or CaCl₂ and ZnSO₄.
METABOLIC ENGINEERING OF C. GLUTAMICUM THROUGH CONSOLIDATED BIOPROCESSING FOR SUCCINATE PRODUCTION FROM CO2-GROWN MICROALGAL BIOMASS AS CARBON SOURCE

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Background
This study presents the development of consolidated bioprocessing for succinate production from microalgal biomass using engineered Corynebacterium glutamicum.

Objectives
Our objective in this study is to develop the C. glutamicum strain capable of utilizing starch as carbon source using the synthetic biology-platform and to apply the strain for production of succinate from microalgal biomass as sole carbon source.

Methods
We developed the starch-degrading and succinate-producing C. glutamicum strains, which produced succinate (0.16 g succinate/g total carbon source) from a mixture of starch and glucose as a model microalgal biomass. Subsequently, the engineered C. glutamicum strains were able to produce succinate (0.28 g succinate/g of total sugars) from pretreated microalgal biomass of CO2-grown Chlamydomonas reinhardtii. For the first time, this work shows succinate production from CO2 via sequential fermentations of CO2-grown microalgae and engineered C. glutamicum.

Conclusions
In conclusion, consolidated bioprocessing based on microalgal biomass could be useful to promote variety of biorefineries.
Background
The impetus for research in the field of bioseparation has been sparked by the difficulty and complexity in the downstream processing of biological products. Indeed, 50% to 90% of the production cost for a typical biological products resides in the purification strategy. There is a need for efficient and economical large scale bioseparation techniques which will achieve high purity and high recovery, while maintaining the biological activity of the molecule. One such purification technique which meets these criteria involves the partitioning of biomolecules between two immiscible phases in an aqueous system (ATPS).

Objectives
The purification of xylanase from the strain *Jonesia denitrificans* BN13 using ATPS an efficient and economical bioseparation technique.

Methods
The Production of xylanases is carried out in 500ml of a liquid medium containing birchwood xylan. In each ATPS, PEG 1000 is added to a mixture consisting of dipotassium phosphate, sodium chloride and the culture medium inoculated with the strain *Jonesia denitrificans*. The concentration of PEG 1000 was varied : 8 to 16 % and the NaCl percentages are also varied from 2 to 4% while maintaining the other parameters constant, the xylanolytic activity was detected by zymogram coupled to SDS-PAGE.

Conclusions
The results showed that the best ATPS for purification of xylanases is composed of PEG 1000 at 8.33%, 13.14 % of K₂HPO₄, 1.62% NaCl at pH 7. We obtained a yield of 96.62 %, a partition coefficient of 86.66 and a purification factor of 2.9. The zymogram showed that the activity is mainly detected in the top phase.
Background

The filamentous actinobacterium *Microbispora* sp. ATCC-PTA-5024 produces the lantibiotic NAI-107 (Maffioli *et al.*, 2014), which is effective against multidrug-resistant Gram-positive pathogens (Jabés *et al.*, 2011).

In actinomycetes, the biosynthesis of antibiotics is generally elicited as a physiological response controlled by a complex regulatory network involving global regulators, playing pleiotropic roles, and pathway-specific regulators, which activate the biosynthesis of biologically active molecules (Bibb, 2005).

Objectives

The integration of bioinformatic tools and holistic technologies has allowed the development of consolidated strategies to manage huge amounts of complex molecular information on gene expression and biochemical capabilities deriving from "omic" investigations, ultimately leading to novel approaches to explore microbial physiology. In the perspective of using *Microbispora* strains for the industrial synthesis of a NAI-107, insights on the molecular physiology of this actinobacterium would be beneficial to develop robust and economically-feasible production.
processes.

Figure 1. Synoptic scheme summarizing the signalling systems and the regulatory cascades associated with the physiological differentiation processes in *Microbispora* sp.

Methods

An extensive investigation on proteomic changes associated with lantibiotic production was performed on the *Microbispora* sp. ATCC-PTA-5024 wild type strain at different growth stages by using combined two-dimensional difference in gel electrophoresis and mass spectrometry approaches. To evaluate the effect of NAI-107 on bacterial vitality, comparative proteomic experiments were also performed on a not producing strain following NAI-107 addition. The obtained results were then integrated with bioinformatics, fluorescence microscopy and molecular genetic experiments.

Conclusions

The results of this study elucidate the regulatory networks, the biochemical pathways and the molecular processes occurring during growth and lantibiotic production (Figure 1), thus providing the first functional picture of a member of the *Microbispora* genus.
OPTIMISATION OF THE BIOFLOCCULANT PRODUCED BY PANTOEA SP., A NOVEL BACTERIUM ISOLATED FROM SEDIMENT FROM THE BEACH AT MTUNZINI, KWAZULU-NATAL, SOUTH AFRICA

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Background

We screened microorganisms with potential for bioflocculation. An organism that secretes large amounts of bioflocculant was isolated from a sediment sample collected from the beach at Mtunzini on the coast of northern Kwazulu-Natal, South Africa.

Objectives

The primary objective of this study was to optimised the bioflocculant production by the Pantoea sp. Further studies on the characterisation and applications of the bioflocculant produced by this organism will be done to underscore the biotechnological importance of this species as a producer of secondary metabolite useful for water and wastewater treatment.

Methods

From detailed morphological and biochemical analysis, the isolate was identified as Pantoea sp. with 91% probability. Cultivation studies revealed that the isolate produced bioflocculant optimally with sucrose as a source of carbon (92.4% flocculating activity), inoculum size of 3% (v/v), at an initial pH of 6.0, with Ca\textsuperscript{2+} as an aid to coagulation, and a production time of 96 h.

Conclusions

We proposed that this organism has great potential as a producer of bioflocculant due to its ability of producing high flocculating activity when cultivation conditions are varied.
Background
The bacterial cellulose (BC), produced by Gluconacetobacter xylinus, has been presented as a useful alternative to replace the vegetable cellulose as well as their use in many industrial applications. A carbon source and a system optimized for efficient aeration is important for a greater production of BC.

Objectives
This study has as its objective determine the effect of molasses in the production of BC by Gluconacetobacter xylinus IFO 13693 under static conditions and static with aeration.

Methods
The synthesis of bacterial cellulose (BC) by Gluconacetobacter xylinus was carried out in static culture discontinuous with and without aeration intermittent at room temperature, in the presence of molasses as the main carbon source at initial concentrations of 13.3 % (w/v). The concentration of BC concentrations, sucrose, glucose and fructose were determined each week. To determine the formation of cellulose and the coefficient of performance of the product was used the Microcal Origin software 6.0®.

Conclusions
By the fourth week, BC values found in the static and static and aeration experiments were around 103 g/L and for static 78.7 g/L, respectively. The kinetics for the hydrolysis of sucrose in the medium fixed the model of Michaelis-Menten, with a Vmax of 0.0041 moles/L/h and 0.0036 moles/L/h, and a Km of 0.021 M and 0.019 M for each culture condition, respectively. The yield coefficients have values of 0.013 to 8.7 g dry cellulose/g of glucose consumed. This shows the importance of the substrate and aeration on the synthesis of cellulose.
Background
Laccases are polyphenol oxidases with numerous industrial and bioremediation application, most laccase activity is demonstrated in fungal species.

Objectives
The study evaluated the potential of fungi isolated from selected agro waste for laccase production

Methods
Fungi cultures were screened for laccase production by plate test using 2, 2, azinobis (3 ethyl benthiazoline 6 sulphonate) (ABTS) and submerged culture using synthetic medium.

Conclusions
Utilisation of selected agro wastes (sawdust, planatian and banana peel) residue for laccase production was evaluated. Five out of twelve isolates were postive and identified as GEOTRICUM Spp,CEPHALOSPORIUM Spp, TRICHODERMA Spp, TRAMETES Spp and FUSARIUM Spp. Optimum enzyme activity was observed using TRICHODERMA Spp at 57.1U/ml followed by TRAMETES Spp with 51.99U/ml, FUSARIUM Spp had 29.2U/ml while GEOTRICUM Spp and CEPHALOSPORIUM Spp had 28.04U/ml and 9.72U/ml respectively. TRICHODERMA Spp was selected as the most potent in producing enzyme and therefore used for further studies. Effect of carbon source, inoculum sizes, pH and shaker speed was evaluated. Sawdust gave the highest yield with 151.17U/ml followed by plantain peel with 62.49U/ml,banana peel was lest with 54.94U/ml. The optimum pH for sawdust, plantain peel and banana peel was 5.95, 5.94 and 5.83 respectively. using shaker incubator @ 150rpm with sawdust as carbon source,Laccase yield of 310U/ml was obtained at pH of 6 and temperature of 25 degree celcius inoculum size of 1000000 spores per ml. Thus sawdust can serve as cheap substrate for laccase production
IDENTIFICATION OF CELLULOSOMAL ENZYMES FOR THE CONSTRUCTION OF CELLULOSOMES FROM CLOSTRIDIUM CELLULOVARANS

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Background

*Clostridium cellulovorans* 743B, an anaerobic mesophile, produces a multienzyme complex, called the cellulosome, for the efficient degradation of cellulosic biomass. The cellulosome consists of carbohydrate enzymes and scaffolding proteins. The scaffolding protein CbpA consists of nine hydrophobic repeat domains (cohesin domains) for the binding of enzymes containing dockerin domains (cellulosomal enzymes).

Objectives

The expression pattern of the cellulosomal enzymes in the presence of various carbon sources was previously shown by proteome analysis, but the constituent enzymes of the cellulosome and their arrangement as a complex have yet to be determined. In this study, we identified the cellulosomal enzymes involved in the cohesin-dockerin interaction.

Methods

*C. cellulovorans* was cultivated on cellobiose and its cellulosome was purified from cell extracts using Avicel affinity chromatography. Fourteen enzymes were found to make up the cellulosome. Nine glutathione-S-transferase (GST) fusion mini-scaffolding proteins, each containing a cohesin domain of CbpA, were expressed in *Escherichia coli*. Cellulosomal enzymes that specifically or non-specifically bound cohesin domains were detected by GST pull-down assay, which was also used to qualitatively analyze the interaction between five types of dockerin domains and all cohesin domains.

Conclusions

The interaction was found to be significantly dependent on the type of dockerin domain and the particular cohesin domain. These results imply a specific arrangement of enzymes in the cellulosome.
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TOWARDS BIOTECHNOLOGICAL APPLICATION OF PSEUDOMONAS SPP. LIPASES
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Background

Microbial lipases catalyze hydrolysis of lipids, enabling bacteria to use vegetable and
other oils as a carbon and energy source. Lipid hydrolysis share common
intermediates with synthesis of valuable biopolymer polyhydroxyalkanoate (PHA).
Bringing together these two processes in a single microorganism could further
increase its potential for application in industrial biotechnology.

Objectives

The objective was to screen Pseudomonas strains for lipase activity and capability to
accumulate PHA in order to identify or to genetically modify these microorganisms to
use waste cooking oils for production of valuable biopolymers.

Methods

Seven Pseudomonas strains were tested for lipase activity on agar plates containing
either Tween 80 or olive oil as carbon source. Bacterial capability to utilize reused
fryer oil as a sole carbon source was tested in liquid medium. For selected
Pseudomonas strains lipase activity was quantified using para-nitrophenyl palmitate
enzyme assay and genetic determinants of lipase activity were amplified. In order to
select appropriate host for the heterologous expression of lipase all Pseudomonas
strains were tested for their ability to accumulate PHA.

Conclusions

Among seven tested Pseudomonas strains Pseudomonas aeruginosa PAO1 showed
highest lipase activity, while both P. aeruginosa PAO1 and Pseudomonas putida
KT2440 could accumulate PHA. Since P. aeruginosa PAO1 is known opportunistic
pathogen, P. putida KT2440 was more appropriate host for the biopolymer
production. *P. aeruginosa* PAO1 lipase-encoding genes were amplified and cloned into *P. putida* KT2440, and heterologous expression was optimized. Further analyses should reveal if these constructs have potential for the industrial application.
Background

Steroid based pharmaceuticals are extremely important for health, and conversion of cheap agricultural waste into highly valuable steroids is therefore an attractive prospect. Previous studies have indicated that in order to achieve this several areas need improvement, including the microbial strains used for bioconversion.

Objectives

By utilizing the bacterium Mycobacterium sp. NRRL B-3805 we aim to replace the current microbial and chemical multistep processes with a single-step production of several steroids including androstenedione (AD), 11α-hydroxy-androstenedione (11α-OH-AD), dehydroepiandrosterone (DHEA) and testosterone from a cheap phytosterol feedstock.

Methods

We have developed genetic engineering tools and procedures to manipulate Mycobacterium sp. NRRL B-3805 to improve the bioconversion of phytosterols. Mycobacterium sp. NRRL B-3805 metabolises steroids through the action of cholesterol oxidases, which modify the A-ring of the steroid core before cleaving the side chain through multiple enzymatic steps to produce AD. The genome of Mycobacterium sp. NRRL B-3805 was sequenced and was mined for potential cholesterol oxidases, hydroxysteroid dehydrogenases and other known enzymes involved in cholesterol catabolism. The genome mining enabled strain manipulation, as a means to optimise steroid production. Quantitation of steroid production was determined using GCMS.

Conclusions

Production of 11α-OH-AD is being investigated by amplifying a putative 11α-hydroxylase and the associated oxidoreductase from Aspergillus ochraceus cDNA and placing both coding sequences under bidirectional inducible promoters for expression in Saccharomyces cerevisiae and 11α-OH-AD production in media.
containing AD. If active these enzymes will be expressed in Mycobacterium sp. NRRL B-3805.
Background

In the future, petrochemical industry may be replaced by biotechnological processes producing building block chemicals from renewable carbon and employing genetically engineered bacteria and other microorganisms. However, besides searching for suitable microbial production platforms, a major hurdle for a large-scale implementation of bioprocess-based technologies is the availability of economic and sustainable carbon sources.

Objectives

An alternative substrate not competing with food or fodder production might be methanol due to its high synthesis capacities and a potentially sustainable production from natural gas, agricultural waste materials and biogas. However, main weaknesses of this C1 compound are its high volatility and toxicity. Its use in fermentation processes therefore calls for an innovative real-time control strategy.

Methods

In this study, we decided on biocalorimetry as a basis for optimizing C1 substrate feed regimes in fermentations with the methylotrophic bacterium Methylobacterium extorquens. According to Hess' law every metabolic flux is quantitatively related to the heat production rate. Thus, calorimetry provides real-time stoichiometric and kinetic information of the fermentation process and allows reacting fast and efficiently to any metabolic changes.

Conclusions

By comparing different calorimetry-based control strategies we established a procedure leading to the highest growth and product formation rates of M. extorquens on methanol. Additionally, we could show that this approach can be extended to other, even more toxic substrates such as formic acid. In conclusion, this study demonstrates that biocalorimetry is a very efficient tool for process control and can be applied to optimize any microbial product formation process.
Physicochemical and Biological Properties of the Antifungal Polyene Antibiotic Roseofungin

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Background

Due to the upward trend of fungal infections and development of resistance to existing drugs, the requirement for efficient antifungal agents has increased.

Objectives

Strain Streptomyces roseoflavus var. roseofungini 1-68, a producer of the antibiotic roseofungin.

Methods

Methods for spectrometry, NMR- and mass spectroscopy, elemental analysis, ozonolysis, and biological testing were used.

Conclusions

Roseofungin is an original natural highly efficient antifungal antibiotic. Roseofungin is amorphous powder, it decomposes at t>130°C; fluoresces in UV, is quite soluble in pyridine, dimethylformamide, acetic acid, lower alcohols, soluble in water-saturated butanol and wateracetone. UV-spectrum has 2 absorption maxima: at 263 nm and 363 nm. Roseofungin in concentrations of 0.5-12.5μg/ml inhibits the pathogenic agents of superficial and deep mycoses-trichophytosis, microsporia, candidiasis, cryptococcosis, sporotrichosis, chromomycosis, aspergillosis, and others. High virus-inhibiting activity of roseofungin has been established against various strains of influenza virus, Sendai parainfluenzavirus and Newcastle disease virus.
DEVELOPMENT OF NEW BIOLOGICAL PREPARATIONS OF THE “RIZOVIT-AKS” SERIES BASED ON NODULE BACTERIA OF LEGUMINOUS PLANTS CULTIVATED IN KAZAKHSTAN

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Background
Development of biological preparations based on active strains of indigenous nodule bacteria of leguminous plants improves their productivity and soil capabilities.

Objectives
Strains of nodule bacteria isolated from the rhizosphere of leguminous plants: Bradyrhizobium japonicum M-1 from soybean, Sinorhizobium meliloti L5-1 from alfalfa, Rhizobium leguminosarum G2 from peas, Mesorhizobium cicer U-2O from chickpeas, Rhizobium leguminosarum B-1 from lentils.

Methods
Biotechnological and microbiological methods were used in the study.

Conclusions
Modification of the culture media containing bean broth or yeast extract has been carried out, and effect of carbon and inorganic salt sources on the growth and biomass accumulation of indigenous strains of nodule bacteria from soybean, alfalfa, peas, chickpeas, lentils studied. The best carbon source for most strains of root nodule bacteria is sucrose at a concentration of 4-10.0 g/L. A number of salts (CaCO₃, MnSO₄, ZnSO₄, CoCl₂) stimulated the biomass accumulation of nodule bacteria strains up to 10¹¹ CFU/ml.

Application of biological preparations of the “Rizovit-AKS” series to inoculate the seeds of leguminous crops reduces the time required for passing phenophases by legumes: on average 2 to 3 days for soybean; 2 to 5 days for peas; up to 2 days for lentils, 2 to 4 days for alfalfa. Biopreparations increase the plant growth, green mass and number of nodules under all tested soil and climatic conditions of Kazakhstan. With the use of biopreparations, an increase in the yields of leguminous crops: for soybeans by 3.2 t/ha (34.4%), lentils – by 3.3 t/ha (41.3%); peas – by 8.3 t/ha (140.7%).
FROM ORGANIC WASTE TO RENEWABLE ENERGY: A NOVEL HYBRID DARK FERMENTATION-PHOTOHETEROTROPHIC BIOHYDROGEN PRODUCTION APPROACH

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Background
Our current society is confronted with a series of global geopolitical, economic, energetic and climate crises. While there is no common solution for all these issues, there is an obvious main connection, the need for a novel renewable and environmentally friendly energy carrier. Hydrogen gas is considered an attractive alternative to fossil fuels with certain methods of biohydrogen production being able to utilize various low-priced industrial and agricultural wastes, thus coupling organic waste treatment with renewable energy generation.

Objectives
By designing a novel hybrid dark fermentation-photoheterotrophic degradation biohydrogen production process using industrial wastewater as a substrate we aim to resolve the major scientific and technical bottle-necks preventing the biohydrogen production systems to become economically feasible, bringing thus the technology closer to the industry.

Methods
A laboratory-scale bioreactor experimental setup was developed in order to identify the most suited microbial and micro-algal consortia, as well as the key metabolic pathways involved. In addition, an advance statistical design of experiments approach was used to identify the influence and interactions of key micro-environmental factors.

Conclusions
The obtained preliminary results prove that this novel system has the potential to produce considerably more H₂ yields compared to traditional systems as well as generate valuable oils through the micro-algal metabolic activity. In addition, such a novel technology could capture the generated CO₂ produced during the dark fermentation step, and used it by the micro-algal species together with the dark fermentation effluent.
EXTRACELLULAR N-ACETYLGLUCOSAMINE INCREASES HYALURONAN YIELD AND ITS MOLECULAR WEIGHT IN CULTURE OF STREPTOCOCCUS ZOOEPIDEMICUS

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Background

Hyaluronan (hyaluronic acid, HA) is a linear polysaccharide composed of alternating D-glucuronic acid (GlcUA) and N-acetylglucosamine (GlcNAc) linked by β(1,4) and β(1,3) glycosidic bonds. This macromolecule is found in various tissues of vertebrates, where it has different biological functions. Industrially, HA has been manufactured by fermentation of group C streptococci.

This polysaccharide is produced by processive synthase from the activated UDP-sugars (UDP-GlcA and UDP-GlcNAc). A positive correlation between molecular weight (MW) and concentration of UDP-GlcNAc was previously reported [1].

Objectives

To increase hyaluronan yield or its molecular weight by strenghtening UDP-precurors biosynthesis.

Methods

Fermentations were carried out in fermentor Sixfors (Infors HT, Switzerland) with working volume of 300 ml. HA yield was determined by isopropanol precipitation. HA Mw was analysed by SEC-MALLS.

Conclusions

In order to increase yield or molecular weight of hyaluronic acid in Streptococcus zooepidemicus fermentation, cultivation medium was supplemented with GlcUA and GlcNAc. These molecules are involved in hyaluronan biosynthetic pathway.

Extracellular GlcNAc significantly increased both HA yield and molecular weight (35% and 21% respectively). HA yield positively correlated with GlcNAc concentration up to 1g/l. Higher concentrations had detrimental effect on HA production. No significant
effect on HA yield was observed for GlcUA addition.
Background

Because of the ease of their synthesis and the greatest variety of colors, azo dyes are the largest class of synthetic dyes. Generally they are very recalcitrant to biodegradation. Thus, in the current study the biodegradation of an azo dye namely Remazol Blue (RB) by wastewater- isolated bacteria (Bacillus megaterium, Micrococcus luteus and Bacillus pumilus) has been investigated in media containing molasses as a carbon and energy source.

Objectives

The goal of this work was to identify decolorization and degradation properties of Remazol Blue dye by bacterial strains at batch scale.

Methods

Enzyme activity: The reaction mixture contained 400 μl of 50 mM sodium phosphate buffer (pH 7.0), 200 μl of the sample and 200 μl of Remazol Blue. The reaction was initiated by the addition of 200 μl NADH (final concentration 2 mM) and followed photometrically.

Bioremoval assays: Experiments were carried out at different (from 28.7 to 97.9 mg/L) dye concentrations at pH 7 throughout 3 days.

Conclusions

Bacillus-derived azoreductases were responsible for biodegradation of RB. The maximum azoreductase activity was obtained as 39.9 U/ml for B. pumilus at 2nd day. The removal yields of B. pumilus were 71.0%, 74.3%, 67.2% and 69.9% for 28.7, 57.9, 78.3 and 97.9 mg/L dye, respectively. There were also high azoreductase activities for B. megaterium at the first and second days of incubation time such as 37.4 and 33.6 U/ml while M. luteus did not show any azoreductase activity.
Background

Among many heavy metals, Cr(VI), Ni(II) and Cu(II) are the ones having many usage fields, and therefore wastewaters including them have to be treated.

Objectives

This study aimed to isolate Ni(II), Cu(II), Cr(VI) resistant-bacteria from Ankara Stream (Turkey) and determining EPS production and bioremoval capacities of them under different conditions.

Methods

Experiments were done with bacterial cultures in different media (active sludge: AS; active sludge with molasses: MAS) including Ni(II), Cu(II), Cr(VI) at varied pH values (6-9). Heavy metals were determined spectrophotometrically at 340 nm for Ni(II); 460 nm for Cu(II); 540 nm for Cr(VI). EPS amount was also investigated.

Conclusions

Mixed bacterial cultures had higher bioremoval than the pure cultures. Six heavy metal-resistant strains were purified. Strain 5 had the highest Cu(II) (69.1%) and Cr(VI) (43.1%) removal under 25 mg/l heavy metal at pH 7. The same strain was the efficient bacterium in bioremoval and EPS production. It had EPS amount as 1.05 g/l at 25 mg/l Cr(VI), and 0.74 g/l at similar Cu(II) concentration. Especially mixed and also pure bacteria could be used as a biosorbent in treatment of heavy metals.
BIOETHANOL PRODUCTION FROM AGRICULTURAL WASTES BY SACCHAROMYCES CEREVISIAE

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Background

The depletion of fossil fuels and the concerns about environment have led scientists to find alternative renewable energy sources. Bioethanol is the most widely used liquid biofuel at present among these alternative fuels. Feedstock costs are the major part of the process. Thus, considerable work has been performed toward production of bioethanol using various kinds of feedstocks such as starch rich agricultural wastes and cellulosic biomass.

Objectives

To demonstrate the utilization capacity of agricultural wastes (apple and carrot pomaces) as feedstocks for bioethanol production.

Methods

Feedstock preparation: Appropriate amounts of apple and carrot pomaces were mixed with distilled water and homogenized to obtain desired concentration of feedstock solutions. Different pretreatment methods were performed to find the most effective way to obtain fermentable sugars in pomaces.

Fermentation conditions: The yeast cells were precultured in YPG medium at pH 6. 10% yeast suspension was aseptically transferred to anaerobic fermentation medium which is prepeared by pomace sugar containing distilled water.

Biomass, sugar and ethanol determination: Yeast biomass was determined by measuring optical density. The sugars were determined by phenol-sulphuric acid method. The bioethanol concentration was analyzed using gas chromatography.

Conclusions

Higher ethanol production values were obtained from apple pomace rather than carrot. The pretreatments performing with acid were more effective than the ones
performed with base. The highest ethanol production was obtained when the apple pomace was pretreated with 0.25% (v/v) H$_2$SO$_4$. 
Background

Boron (B) is a naturally occurring element found in earth’s crust, rocks, soil, and water. Although B is an essential element and used at many industries, it could be toxic for organisms in excess concentrations.

Objectives

In the present study we aimed to remove B from aqueous media via biosorption by *Rhodotorula mucilaginosa*, which was isolated from B-contaminated wastewater.

Methods

Batch culture experiments including pH (4-7), increasing B (15-20 mg/L), biomass concentrations (1-4 g/L) and different biosorption methods (wet, dried and formaldehyde-treated cells) were carried out at 25±2 °C, at 100 rpm for 120 min. Boron concentrations were determined spectrophotometrically by measuring the absorbance at 585 nm with using carmine.

Conclusions

According to the data obtained, the optimum pH was found as 6 for the maximum B removal. Under 15.35 mg/L B, the highest biosorption was determined as 23.78%. Biosorption was increased with increasing biomass and the maximum yield (31.92%) was found at 4 g/L biomass. Wet cells removed B more effectively than formaldehyde-treated or dried cells and the yields were 23.78%, 19.31% and 12.07%, respectively. It can be concluded that *R. mucilaginosa* might be an effective biosorbent in B removal processes.
A NOVEL PATHWAY FOR ITACONIC ACID PRODUCTION IN USTILAGO MAYDIS

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Background

Itaconic acid is a promising bio-based platform chemical for the production of pharmaceuticals, adhesives and polymers. \textit{Ustilago} is a promising fungal host for the production of itaconic acid. Contrary to established filamentous itaconate producers, \textit{Ustilago} grows unicellularly. This has distinct process advantages relating to morphology control, viscosity and aeration. However, the itaconate production yield, titer and rate of \textit{Ustilago} are below that of the commercial production hosts. In order to optimize itaconate production in \textit{Ustilago}, detailed biochemical knowledge is needed.

Objectives

To characterize the itaconate production pathway in \textit{Ustilago} in order to enable metabolic engineering.

Methods

An itaconate gene cluster was identified in \textit{Ustilago}. The function of the cluster genes and their encoded proteins was characterized by knockout and overexpression. The activity of two essential catalytic proteins was determined \textit{in vivo} by permeabilized cell assays and \textit{in vitro} by \textit{E. coli} expression and purification.

Conclusions

The genes \textit{tad1} (\textit{trans}-aconitate decarboxylase), \textit{itp1} (Major Facilitator Superfamily extracellular itaconate transporter), \textit{adi1} (aconitate-\textit{Δ}-isomerase), \textit{mtt1} (mitochondrial tricarboxylate transporter), and \textit{ria1} (transcriptional itaconate regulator) are involved in the itaconate biosynthesis and possibly its further conversion.

In contrast to the known itaconate biosynthesis pathway of \textit{Aspergillus terreus}, \textit{Ustilago}'s itaconate production proceeds first via an isomerization from \textit{cis}- to \textit{trans}-aconitate, followed by decarboxylation of the \textit{trans}-aconitate. First metabolic engineering attempts were successful, enhancing \textit{U. maydis}' itaconate production twofold by overexpression of \textit{ria1} or \textit{mtt1}. This work lays the foundation for further
optimization of *U. maydis*’ itaconate biosynthesis and is therefore a further step towards industrial application of this promising biocatalyst.
PURIFICATION AND CHARACTERIZATION OF A LIPASE WITH HIGH SYNTHETIC ACTIVITY FROM RHIZOPUS STOLONIFER

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Background
Filamentous fungi are extensively studied due to their exoenzyme producing ability. Since lipases are effectively utilizable in various industrial processes, special attention is paid for these enzymes. Development of environmentally friendly technologies requires lipases able to catalyze the rapid synthesis and translocation of esters. Isolation and analysis of such enzymes contributes to the knowledge of these catalytic processes, and provides data on new lipases with potential biotechnological interest.

Objectives
A Rhizopus stolonifer isolate has been identified as high-yield lipase producer in our previous tests. Further studies revealed that the crude enzyme has significant transesterification activity. Therefore, our present work has focused on the purification and characterization of this R. stolonifer lipase.

Methods
Isolate was grown on liquid medium containing wheat bran. To purify the enzyme, after filtration and centrifugation, ammonium sulphate precipitation, size-exclusion and ion-exchange separations were performed. Biochemical characterization assays including substrate specificity, temperature and pH tolerance studies, and examination of the effect of some ions and organic solvents on the activity were carried out.

Conclusions
The optimal temperature and pH for the activity were about 50 °C and pH 5.0, respectively. The lipase has broad substrate specificity because it has effectively hydrolyzed the substrates containing fatty acids from C6 to C16. Enzyme activity was stimulated by 5% ethanol, butanol and propanol. Results showed that certain alkanes could stabilize the active conformation of the enzyme. The isolated R. stolonifer lipase exhibited high esterification and transesterification activity at 40-50 °C. This research was supported by the Hungarian Research Fund (OTKA PD 112234).
Investigation of the transesterification and esterification properties of extracellular lipase enzymes from Mucoromycotina fungi

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Background

Recent years, there is a fast-growing interest on the catalytical activities display by lipase enzymes, especially focusing on their synthetic activity in organic solvents. Translocation and synthesis of ester linkages result various aroma-, phenyl- and alkyl esters and different polymers. Many of these compounds have important role in the food-, pharmaceutical-, and oleochemical industries as well as in the biodiesel production. In these industries, development of modern environmentally friendly biotechnological technologies requires the exploration and utilisation of new microbial enzymes.

Objectives

Lipases can be produced by certain Mucoromycotina fungi as well, but only some of these enzymes have been tested for their synthetic activity in organic media. In this work, we evaluated the esterification and transesterification capacities of Rhizomucor, Rhizopus, Mucor and Mortierella lipase enzymes through alkyl ester synthesis, and we characterise of their fatty acid preference during these reactions.

Methods

Reactions were carried out in non-aqueous condition using lyophilised crude enzyme preparations. Transesterification was studied between p-nitrophenyl-palmitate and ethanol, while esterification reactions were examined with different chain length fatty acids and methanol. The effect of the reaction time on the ester production was also evaluated. Synthetic esters produced were detected by a GC-FID technique.

Conclusions

Each lipase catalysed the transesterification significantly faster than esterification. High ethyl-palmitate production was reached by lipases from Rhizopus stolonifer and Mucor corticola strains. During esterification reactions, the tested enzymes showed increased alkyl ester synthesis in the presence of medium-chain fatty acids. This
research was supported by the TÁMOP-4.1.1.C-12/1/KONV-2012-0012 and the Hungarian Research Fund (OTKA PD 112234).
NOVEL SMALL-SCALE AND ENZYMATIC TEST SYSTEMS TO ANALYSE FULL OPERATING BIOGAS PLANTS

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Background

One important renewable energy source is biomass from energy crops and organic waste that is used for the production of biogas. Biogas mainly consists of CH₄ and CO₂ and is generated by anaerobic fermentation of organic matter.

Objectives

For a more precise and targeted optimization it is crucial to understand the biological processes during biogas production.

Methods

Using authentic samples from full operating biogas plants it was possible to develop batch and continuous reactors mirroring the conditions and the performance of the full-scale biogas plant. With these test systems it was possible to observe effects of different substrates for syntrophic bacteria and for methanogenic archaea on biogas formation.

Furthermore, the development of novel sensors in biogas plants to quantify the activities of key enzymes is desirable. A potential candidate is the heterodisulfide reductase (Hdr). Methanogenesis finally results in the formation of the heterodisulfide, which is regenerated by the Hdr. In aceticlastic methanogens the Hdr is tightly bound to the cytoplasmic membrane but in hydrogenotrophic methanogens it is located in cytoplasm.

Conclusions

Most biogas sludge samples were stimulated by the addition of ethanol or acetate, which leads to increasing methane production by 35-126%. These results indicate that syntrophic ethanol oxidation and aceticlastic methanogenesis were not rate limiting in routinely operating biogas plants.
Our test system allows a quantification of hydrogenotrophic/aceticlastic methanogens in biogas sludge based on Hdr activity. In summary 26% of total Hdr activity was found in the membranes while the cytoplasmic fraction contained 74% of total activity.
EXPRESS OF AGARICUS BISPORUS MANGANESE PEROXIDASES IN SCHIZOPHYLLUM COMMUNE

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Background
White rot fungi are thought to degrade lignin using AA2 enzymes like lignin peroxidase, manganese peroxidase (MnP) and versatile peroxidase. It is unclear if S. commune is a white rot or brown rot fungus. This basidiomycete is a poor lignin degrader. No genes encoding AA2 enzymes are found in its genome, while it is enriched in AA9 genes. This would place S. commune in between brown and white rot fungi. We here introduced two A. bisporus mnp genes in S. commune.

Objectives
Introduction of A. bisporus MnPs in S. commune and its effect on the lignin degrading capacity of S. commune.

Methods
qPCR verified expression of these genes in S. commune. Decoloration of RBBR by mnp expressing S. commune strains indicated production of active MnP. This was confirmed using the MBTH/DMAB assay. Addition of hemin to standing cultures increased MnP activity 5-fold. Pyrolysis analysis indicated that a S. commune strain expressing both MnPs of A. bisporus degraded lignin in birchwood.

Conclusions
A. bisporus MnPs were successfully introduced and expressed in S. commune. The G subunit of lignin seems to be preferentially degraded by S. commune MnP expressors. The ligninolytic pathway of litter degrading fungi can be reconstituted in S. commune.
Background

Enzyme 4-oxalocrotonate tautomerase (4-OT) encoded by xylH gene is a part of aromatic compounds degradation pathway in Pseudomonas putida mt-2. Due to its promiscuous activity, 4-OT was described to catalyse Michael-type addition of acetaldehyde to β-nitrostyrene, the isomerisation of cis-nitrostyrene to trans-nitrostyrene as well as aldol condensation and dehydratation as free enzyme. Whole cell system based on recombinantly expressed 4-OT has been developed and shown to be effective biocatalyst of asymmetric Michael addition of acetaldehyde to β-nitrostyrene.

Objectives

The objective of this study was to improve biocatalytic production of 4-nitro-3-phenyl-butanal employing two strategies: biocatalyst immobilization and product recovery using Amberlite XAD-2 polymeric adsorbent.

Methods

Whole cell biocatalyst was immobilized in 4% alginate gel. Capsules of 1 and 2 mm in diameter were made. Liquefaction of the capsules core was performed by addition of sodium citrate. Depletion of β-nitrostyrene was monitored by reduction of absorbance at 320 nm. Product was recovered from aqueous reaction buffer by addition of XAD-2 beads, followed by elution in minimal volume of ethylacetate.

Conclusions

Biocatalytic production of 4-nitro-3-phenyl-butanal was improved by whole-cell immobilization in alginate capsules. Capsules were used in 12 reaction cycles, and retained 20% of the activity after two months of storage. Bioprocess was further improved by using XAD-2 beads for improved product recovery. With this
modification, the amount of organic solvent was reduced 20-fold in comparison to previously reported method making this process more environmentally friendly.
COMPARATIVE GENOMIC ANALYSIS OF SOLVENT PRODUCING CLOSTRIDIUM ACETOButYLYCUM AND CLOSTRIDIUM BEIJERINCKII STRAINS

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Background
The clostridial acetone-butanol-ethanol (ABE) fermentation pathway is complex and represents one of the oldest industrial fermentations. Interest in the production of solvents from lignocellulosic sources has increased in recent years. However, low production yields, end-product inhibition and phage infections remain as disadvantages in the ABE fermentation.

Objectives
Genetic and metabolic engineering strategies leading to a stable, high-yielding process require prior genomic exploration of diverse solventogenic Clostridial strains.

Methods
Ten strains of C. acetobutylicum and C. beijerinckii were selected from batch fermentation experiments according to their solvent yields. Genomes and plasmids (when present) were sequenced using the Illumina HiSeq platform. Complete genomes were obtained through de novo and reference-guided assembly comparison with coverages of ~ 100x. Annotation was performed using RAST (http://rast.nmpdr.org). Key enzymes for solvent production where mapped in the genomes and/or plasmids to allow variant detection and comparison in future RNA-seq experiments. Raw Illumina reads were used as input for CRISPR structure detection using the Crass software (http://ctskennerton.github.io/crass/). Putative CRISPR fragments were assembled and queried against the CRISPR database (http://crispr.u-psud.fr/crispr/). Complete CRISPR structures were detected in 7 out of 10 genomes (average 2.5 CRISPR structures per strain), and repeat/spacer units were detected in all 10 sequenced strains. An alignment-based approach revealed the existence of uneven distribution of types (11 detected) across their genomes.

Conclusions
In combination, our results provide a sound basis for directed generation of engineered Clostridial solventogenic strains, with the potential to re-launch the ABE fermentation as a competitive industrial fermentation.
REROUTING OF CARBON FLUX TOWARDS GLYCEROL PRODUCTION IN YEAST SACCHAROMYCES CEREVISIAE

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Background
Currently there is an interest in development of microbial strains effectively converting cheap feedstocks to glycerol. Facultative anaerobic yeast *Saccharomyces cerevisiae* can be a good platform for development of recombinant strains overproducing glycerol under low-aeration conditions. In *S. cerevisiae* glycerol is synthesized from dihydroxyacetone phosphate by the action of glycerol-3-phosphate dehydrogenase (Gpd1) and glycerol-3-phosphate phosphatase (Gpp2). Other road of dihydroxyacetone phosphate catabolism occurs by its isomerization to glyceraldehyde-3-phosphate with triose phosphate isomerase (Tpi1), resulting to ethanol formation.

Objectives
We aimed to construct recombinant strains with reduced Tpi1 activity and increased Gpd1 and Gpp2 activities.

Methods
Homologous recombination was used for partial substitution of *TPI1* gene promoter region with selective marker. Obtained strains contain 100, 50 or 25 base pairs of native *TPI1* gene promoter before *TPI1* ORF, and revealed corresponding sequential decreases in Tpi1 activity. Multicopy integration module was used for expression of hybrid *GPD1-GPP2* ORF (encoding artificial fusion of both enzymes) under the control of strong constitutive promoter of the alcohol dehydrogenase gene.

Conclusions
Recombinant *S. cerevisiae* strains with 50 or 25 bp version of *TPI1* promoter revealed up to 2 times increase in glycerol production as compared to the WT strain. Recombinant strains expressing *GPD1-GPP2* fusion produced approximately 4 times more glycerol than that of WT strain. Combination of both approaches resulted to 5-fold increase of glycerol production as compared to the WT strain.
Background
The long-term goal of this research is to develop a robust microbial platform for conversion of greenhouse gases (CH$_4$ and CO$_2$) into valuable chemicals as an alternate approach to widespread (bio)gas-flaring.

Objectives
The main objective of this project is to generate a comprehensive vision of methane oxidation in a model methanotroph (*Methylomicrobium alcaliphilum* 20Z) using modern systems biology approaches.

Methods
A multi-tiered systems-level approaches (genomics/mutagenesis, transcriptomics, metabolomics and fluxomics) were used to validate the metabolic network in *M. alcaliphilum* 20Z grown on methane and methanol during both, steady state and transitions from restricted to active utilization of C1-substrate.

Conclusions
Comparisons of different growth conditions highlighted functions important for methane oxidation and for the re-activation of methane oxidation machinery. Complete genome sequences of the methanotrophic strain enabled genome-wide metabolic reconstruction. The first stoichiometric flux balance model of methane utilization was constructed and validated. A new vision of the core methanotrophic functions will be presented. The majority of the data (methane oxidation parameters, growth yield, mutant phenotypes) suggest that direct coupling between methanol and methane oxidation is the most compelling mode of methane activation. The reconstruction of spatial organization of the metabolic network coupled with the computational modeling enables a mechanistic understanding and systematic analysis of the interplay between core methane oxidation processes and overall performance of the biological system. New data were used to improve conversion of the C1-substate into values-added chemicals and fuels.
OPTIMIZATION OF LEVAN PRODUCTION BY BACILLUS LICHENIFORMIS ANALYSED BY THE RESPONSE SURFACE METHOD

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Background

Levan is a fructose based exopolysaccharide produced by microorganisms as an energy reserve and defense. It has present low viscosity, high solubility in water, biocompatibility and other properties that have industrial and medicine applications. Levan-type polymers have been reported as hypocholesterolemic, cell-proliferating, anti-inflammatory and antitumour agent, an immune modulator, thickener, stabilizer, flavors, and a aroma carrier [1].

Response surface methodology (RSM) is the most popular optimization method used in recent years because the application of statistical designs for experiments and its modeling defines the effect of various factors and its interaction [2].

Objectives

The aim of this study was to optimize fermentation medium composition for levan production by \textit{Bacillus licheniformis} strain.

Methods

Basic medium for levan production contains sucrose as carbon source, yeast and beef extract as nitrogen source, and its pH level is important for excretion of polysaccharide. In this work it has been shown that high levan production by the studied strain could be achieved with ammonium chloride as a sole nitrogen source. Influence of three independent variables (pH, sucrose and ammonium-chloride concentration) was investigated using Box-Behnken statistic design.

Conclusions

Based on response surface model that we obtained, in medium that contain 200 g/L sucrose, 1.44 g/L ammonium-chloride and pH 7.5 maximum concentration of levan (41.29 g/L) is reached, which was in agreement with experimental data.

References:

MOLECULAR BREEDING OF YEAST CO-DISPLAYING ENDO- AND EXO-TYPE ALGINATE LYASES TO COMPLETELY UTILIZE MACROALGAE
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Background
Brown macroalgae is characterized by a large size and high productivity without requiring arable land, irrigation water, or fertilizer. It is a raw material suitable for productivity of bioenergy and biochemical productions. However, alginate, which is the main component of brown macroalgae, is an undegradable polysaccharide with poly (M)-, poly (G)-, and poly (MG)-blocks composed of α-l-guluronic acid (G) and its C5 epimer, β-d-mannuronic acid (M), as monomeric units.

Objectives
In order to degrade alginate polymers effectively, both endo- and exo-type alginate lyases are necessary. Therefore, we co-displayed endo- and exo-type alginate lyases from the alginate-degrading marine bacterium *Saccharophagus degradans* [1] on the cell surface of *Saccharomyces cerevisiae* with our original method, cell surface engineering technology [2].

Methods
The alginate lyases from *S. degradans* were produced as fusion proteins with cell wall anchoring domain of α-agglutinin in *S. cerevisiae*. The production and localization of alginate lyases on the yeast cell surface were confirmed by immunofluorescence microscopy. The enzyme activities of the displayed alginate lyases were measured by using the dinitrosalicylic acid method.

Conclusions
The results suggested that the engineered yeasts co-displaying endo- and exo-type alginate lyases could lead to efficient production of monomeric units from alginate polymers [3]. This system is a unique easily feasible to utilize any biomass.

References
Background
Contrary to temperate regions, knowledge on laccase producing fungal species from ecosystems allocated at neotropical regions is still scarce. Search for ligninolytic fungi in these ecosystems can provide laccases with novel biotechnological potential.

Objectives
The goal of this study was to explore novel fungal species with laccase activity from different forest ecosystems.

Methods
Fungal species were isolated from soil and basidiocarp samples collected from five different geographic regions with dominant vegetation of Pinus spp., Abies spp., and Quercus spp. Extracellular laccase activity by fungal isolates were analyzed by guaiacol assay. Identification of laccase positive fungi was carried out by sequence comparison and phylogenetic analysis of internal transcribed region (ITS1F-5.8S-ITS4B) of rDNA gene with reference taxa.

Conclusions
A total of thirty (30) laccase producing fungi, representing twenty one (21) species of fifteen (15) genera, were purified. Fifteen strains (15) with high laccase activity of basidiomycetes were identified in thirteen genera i.e., Bjerkandera, Corticiaceae, Coriolopsis, Echinodontium, Ganoderma., Hexagonia, Irpex, Limonomyces, Pseathyrella, Peniophora, Phlebiopsis, Trametes and Trichaptum. Seven (7) Trichoderma and only one Penicillium species were found as low laccase producer, isolated from different soil and basidocarp samples. Trichoderma tomentosum was recorded as most isolated laccase producing isolate from different soil samples
followed by *T. atroviride* from different regions. However, *Penicillium pinophilum* was only laccase producing species of genus *Penicillium*, isolated from soil sample of *Pinus* spp., and *Quercus* spp., dominant area.
COMPLEX PROBIOTIC FEED SUPPLEMENT BASED ON CONSORTIUM OF MICROBIAL STRAINS FOR INDUSTRIAL POULTRY BREEDING

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Background
Large-scale poultry breeding often provokes outbreaks of various bacterial, viral and mixed infections leading to fowl loss, while shortage of vitamins and hardly digestible feed ingredients reduces nutritive value and downgrades commodity outlook for meat and egg products.

Objectives
Aim of this study – development of technology for production and application of complex probiotic feed additive possessing antimicrobial, antiviral and antioxidant action allowing to prevent and cure fowl diseases, to raise nutritive value and digestibility of fodder, to promote poultry resistance to stress factors.

Methods
Microbiological, biochemical, genetic engineering, physical-chemical methods were used in this research: engineering a strain-producer of chicken leukocyte alpha-interferon, submerged fermentation of microorganisms, lspectrometry, freeze-drying and etc.

Conclusions
To broaden the activity spectrum of probiotic feed supplement several bacterial species capable to complement each other were applied. Active principle of the feed additive — strain *Bacillus subtilis* produces surfactins, polylene and peptide compounds displaying antagonistic activity against strictly and facultatively pathogenic microbial species colonizing avian gastrointestinal tract. It was found that *Bacillus subtilis* bacteria generate cellulases, proteases and other enzymes facilitating assimilation of fodder. Enterobacteria *Pantoea agglomerans* synthesize carotenoid pigments – sources of antioxidant activity and precursors of vitamin A in the body. The protein of chicken leukocytic alpha-interferon synthesized by *Escherichia coli* strain possesses pronounced non-specific antiviral and immune-stimulating activities.

Technology of producing complex probiotic feed supplement in dry form for poultry was developed.

Biological trials *in vitro* and *in vivo* demonstrated its safety and high efficiency in feeding experiments with broilers and hens.
Background

Background: In the frame of the BIOSUR project (LIFE11 ENV/IT/075), a one-year monitoring of an industrial tannery wastewater treatment plant was performed, both from chemical-physical and biological points of view.

Objectives

Objectives: This study is conducted to investigate the evolution of the bacterial community of activated sludge in the same treatment plant during 2013.

Methods

Methods: Bacterial diversity is analyzed by the use of the Illumina MiSeq platform for sequencing hypervariable regions V3-V4 in the 16S rRNA genes. Bioinformatic analysis of the obtained 12,429,502 paired-ends reads is performed using the QIIME pipeline. The assembled 4,039,284 contigs are binned into 5,156 Operational Taxonomic Units (OTUs) that are assigned to taxonomic groups by the use of the Silva 111 database.

Conclusions

Conclusions: Preliminary results indicate that the bacterial diversity of active sludge is maintained at very high levels throughout the whole year. The dominant bacterial phylum is Proteobacteria, which accounts for 40%. Multivariate analysis shows that there is a discontinuity between two groups of samples. The first group, corresponding to samples collected from January to July 2013, is detached from the second group, corresponding to samples collected from September, after a period of drastic decrease of work in tanneries. The Shannon diversity index ($H$), used to calculate the diversity of bacterial communities, indicates a slight increase in the diversity level in the second period. Further analysis will better clarify the complexity
of the microbial community and the influence of specific environmental parameters on its structure and evolution.
Background

Glycerol is widely used in cosmetics, food, tobacco, pharmaceutical, leather and textile industries. In addition, it is considered as a cheap raw material for microbial fermentation. That is why the construction of yeast strains-producers of glycerol became an actual objective for modern metabolic engineering. Our strategy comprised the deletion of \textit{ADH1} gene, encoding alcohol dehydrogenase in \textit{S. cerevisiae} and overexpression of both glycerol-3-phosphate dehydrogenase and glycerol-3-phosphate phosphatase genes (\textit{GPD1}, \textit{GPP2}) in order to redirect the carbon flux to glycerol instead of ethanol formation.

Objectives

Our goal was to design recombinant strains with deletion of \textit{ADH1} gene combined with increased Gpd1 and Gpp2 activities.

Methods

Homologous recombination was used for deletion of \textit{ADH1} gene with selective marker. The integrative module was used for expression of hybrid \textit{GPD1-GPP2} ORF (encoding artificial fusion of both enzymes) under the control of strong constitutive promoter of the alcohol dehydrogenase gene.

Conclusions

Glycerol concentration during the course of fermentation was approximately 4.7-fold enhanced in a \textit{adh1}_GPD1-GPP2 recombinant strain if to compare with BY4742 (2.57 g/L), reaching 12.14 g/L (0.168 g/L/h) on the third day of fermentation. Furthermore, the glycerol production in a recombinant \textit{adh1}_GPD1-GPP2 strains with deletion of \textit{ADH1} gene and overexpression of \textit{GPD1} and \textit{GPD2} genes was nearly 1.2-fold increase in comparison with \textit{adh1} strain, which contained only the disruption of \textit{ADH1} gene.
MODIFICATION OF METHABOLIC PATHWAY FOR GLYCEROL PRODUCTION IN YEAST SACCHAROMYCES CEREVISIAE

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Background

Several processes are known for the microbial synthesis of glycerol from carbohydrates that use osmotolerant yeasts, algae and a number of bacteria. However, all these utilize aerobic conditions, so the glycerol production demands air or oxygen purging throughout the fermentation, which considerably increases production costs. Therefore the development of the Saccharomyces cerevisiae strains capable of efficient glycerol production from glucose under anaerobic conditions is of great interest.

Objectives

We aimed to increase glycerol production by derepression of ILV2 in part with GPD1-GPP2 fused genes.

Methods

Homologous recombination was used for construction of the truncated version (deficient 5’-165 bp and lacking a mitochondrial targeting signal) of the yeast ILV2 gene, encoding for acetolactate synthase with a strong constitutive promoter. Obtained strains shown increased acetolactate synthase activity. Multicopy integration module was used for expression of hybrid GPD1-GPP2 ORF (encoding artificial fusion of glycerol-3-phosphate dehydrogenase and glycerol-3-phosphate phosphatase) under the control of strong constitutive promoter of the alcohol dehydrogenase gene.

Conclusions

Overexpression of the truncated version of ILV2 with presumably cytosolic localization, strongly activates glycerol production under anaerobic conditions. Preliminary results possessed that the glycerol production by recombinant strains co-expressing ILV2 and GPD1-GPP2 was increased as compared to the wild type strain and strain expressing solely ILV2.
AN ANTIFUNGAL PEPTIDE FROM ACTINOBACTERIA (STREPTOMYCES SP. TKJ2): ISOLATION AND PARTIAL CHARACTERIZATION

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Background
Actinobacteria are of special biotechnological interest since they are known to produce chemically diverse compounds with a wide range of biological activity. This distinct clade of Gram-positive bacteria include some of the key antibiotic producers and are also sources of several bioactive compounds.

Objectives
The aim of our study is the purification and the partial characterization of an antifungal protein produced by Streptomyces sp. TKJ2 isolated from Algerian soil.

Methods
A newly filamentous bacteria was recovered from Tikjda forest soil (Algeria) for its high antifungal activity against various pathogenic and phytopathogenic fungi. The nucleotide sequence of the 16S rRNA gene (1454 pb) of Streptomyces sp. TKJ2 exhibited close similarity (99%) with other Streptomyces 16S rRNA genes. Antifungal metabolite production of Streptomyces sp. TKJ2 was evaluated using six different fermentation media. The antifungal protein produced by Streptomyces sp. TKJ2 on PCA medium has been purified by ammonium sulfate precipitation, SPE column chromatography and high-performance liquid chromatography in a reverse-phase column.

Conclusions
The UV chromatograms of the active fractions obtained at 214 nm by NanoLC-ESI-MS/MS have different molecular weights. The F20 Peptidique fraction obtained from culture filtrat of Streptomyces sp. TKJ2 precipitated at 30% of ammonium sulfate was selected for analysis by infusion ESI-MS which yielded a singly charged ion mass of 437.17 Da.
Background

In recent years public emphasis has been focused on the problems of rapid arable land degradation: structural deterioration of ploughed layer, reduced humus content, rising acidity – all leading to loss of soil suppressive potential. Tillage quality downward trend requires urgent measures for preservation and remediation of agricultural resources. A vital method to promote soil fertility and harvests of cultivars envisages ploughing-in of straw from crop rotation precursor treated by phytoprotective microbial preparation accelerating decomposition of plant residues and regeneration of soil biocenoses.

Objectives

Aim of this study was to assemble a consortium of microbial species distinguished by phytoprotective and hydrolytic potential.

Methods

Various investigation methods were used: microbiological, biochemical, molecular-genetical.

Conclusions

500 microorganisms isolated from diverse natural sources were screened to yield 33 antagonists of crop soil pathogens. 3 antagonistic bacterial strains were found to show the highest levels of antimicrobial and cellulolytic activities. The selected variants were characterized by the following properties:

- growth suppression of phytopathogens belonging to genera *Fusarium*, *Botrytis*, *Colletotrichum*, *Chaetomium*, *Pseudomonas*, *Xanthomonas*;
- increased seedling/root size and crude weight;
- production of hydrolytic enzymes – endo-1,4-β-glucanase and xylanase;
- 10-12% reduction of straw fiber content by 1 month;
- upgrading soil cellulytic activity;
- stimulating effect on major ecologo-trophical microbial groups in soil (ammonifying, oligonitrophilic, cellulytic, micromycetes).

The obtained data evidence attractive application prospects of microbial consortium as active ingredient of biopreparation to trying down infectious background, to restitute soil microbial cenoses and to promote plant growth.
SULFUR OXIDIZING BACTERIA COMMUNITY: CHARACTERIZATION AND MONITORING IN A REACTOR FOR SULFIDE REMOval FROM INDUSTRIAL WASTE.

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Background

Tannery wastewater contains high concentrations of pollutants, mostly sulfides and sulfates, which are usually chemically removed by expensive and inefficient methods. An innovative oxidizing bioreactor for biological removal of sulfide from gaseous industrial waste streams is developed and set up in the frame of the BIOSUR project (LIFE11 ENV/IT/075).

Objectives

This is achieved thanks to the development of a specialized chemolithoautotrophic sulfur-oxidizer biomass, which has been studied in order to elucidate its evolution.

Methods

The involved microbial community is selected in a side reactor by incremental addition of sulfides in selective conditions: low pH (2-4) and a concentration of sulfides of 2-4 mg/l. Such a specialized biomass was characterized and monitored by means of T-RFLP fingerprinting, clone-library construction of 16S rRNA coding genes and isolation in pure culture.

Conclusions

Preliminary results show the evolution of a selected, specialized sulfur-oxidizer biomass from the onset of the reactor to the stationary phase. In this scenario, about 65% of screened clones are represented by bacteria whose 16S rRNA gene sequence shows a similarity higher than 97% with that of members of the genus Halothiobacillus, well-known as Sulfur Oxidizing Bacteria (SOB). The same results has been also confirmed by isolation in SOB selective medium. Finally, obtained data have been comparatively analyzed in order to monitor microbial community evolution from the start-up throughout the end of the experiment.
IMPROVEMENT OF GLUTATHIONE PRODUCTION IN METHYLOTROPHIC YEAST HANSENULA POLYMORPHA

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Background

Glutathione (γ-L-glutamyl-L-cysteinyl-glycine; GSH) is the most abundant non-protein thiol compound of the most living organisms that protects cells from environmental and oxidative stresses. This tripeptide is an important natural compound for medicine, biotechnology and food industry. Microbial GSH overproduction is limited by mechanisms of feedback inhibition of γ-glutamylcysteine synthetase (GCS), the first and rate-limiting enzyme of GSH biosynthesis, by the end product. In addition the expression of gene coding for GCS is repressed by GSH. Thermotolerant methylotrophic yeast H. polymorpha is considered as promising organism for genetic modification and design of competitive GSH producer.

Objectives

To obtain a competitive producer of GSH in methylotrophic yeast H. polymorpha by overexpression of modified GCS with eliminated repression-inhibition mechanisms normally exerted by GSH.

Methods

Standard methods of yeast molecular genetics, yeast biochemistry and physiology have been used. The modified versions of GSH2 gene were obtained by error prone PCR.

Conclusions

The modified versions of GSH2 gene obtained by error prone PCR were cloned under the control of strong constitutive promoter of glyceraldehyde-3-phosphate dehydrogenase in replicative plasmid pYT3. Selected transformants were analyzed for their resistance to different prooxidant agents (1,2,3-triazole, diethylmaleate, ethionine) as compared to strains carrying unmodified GSH2 gene. Selected strains possessed growth on medium supplemented with triazole. Strains providing more intensive growth on the selective medium revealed higher GSH accumulation as compared to strains carrying unmodified GSH2 gene, indicating the reduction of Gsh2 feedback inhibition. In this work the selection scheme providing generation of
Gsh2 insensitive to feedback inhibition was developed.
HOW TO INTERPRET GENOMIC UNITS INSTEAD OF COLONY FORMING UNITS: A CASE STUDY WITH LEGIONELLA PNEUMOPHILA

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Background
Legionella pneumophila has been proven to be the causative agent of the potential deadly Legionnaires’ disease since 1977. In most of the European countries, the reference method for enumeration of Legionella in water is culture (ISO 11731). Due to the long incubation time of this method, more and more laboratories use quantitative PCR (qPCR). Since 2012 the normalization for the Legionella qPCR increases considerably: the ISO 12869 was developed, there exist different interlaboratory tests and reference standards. Different reports showed that this technique is accurate enough to be implemented as a reference method. One remaining obstacle is the data interpretation: the culture is expressed in colony forming units (CFU) and the qPCR in genomic units (GU). Although there is a correlation between both units, there is no consensus on how GU can be translated to CFU.

Objectives
Based on a database of hot and cold sanitary water samples and a literature screening an action level was defined for the monitoring of Legionella in sanitary water.

Methods
200 water samples were analyzed by culture and qPCR following the ISO 11731 and ISO 12869 respectively. A correlation analysis was performed and the variance was calculated by the logarithmic differences between the culture and the qPCR results. Together with the results from literature, action levels for the Legionella monitoring are proposed.

Conclusions
Although the culture has been widely used for many years, one should consider the advantages of qPCR over culture and acknowledge its suitability as a reference method.
A NOVEL BETA-GALACTOSIDASE FROM ARTHROBACTER SULFONIVORANS: PURIFICATION, CHARACTERIZATION AND GENE CLONING

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Background

Beta-galactosidase (EC 3.2.1.23) also known as lactase is the enzyme that typically catalyzes hydrolysis of beta-1,4-D-galactosidic linkages in beta-D-galactosides, including disaccharide lactose, with glucose and galactose as end reaction products. This enzyme is able to catalyze synthesis of oligosaccharides, in particular galactooligosaccharides via galactosyl transfer reaction.

Arthrobacter sulfonivorans beta-galactosidase of unique for prokaryotes extracellular localization may find application in food industry for manufacturing lactose-free dairy products and in pharmacology as bioactive principle of medicines prescribed for patients suffering from lactase deficiency.

Objectives

The study was aimed at cloning of the gene encoding \textit{A. sulfonivorans} beta-galactosidase, purification and characterization of the enzyme.

Methods

Fast protein liquid chromatography, electrophoretic analysis, enzyme assay, PCR, DNA sequencing.

Conclusions

A novel extracellular beta-galactosidase from \textit{A. sulfonivorans} was recovered with an overall 207-fold purification, a 7.7\% yield and specific activity 16 300 U·mg\textsuperscript{-1} protein. The subunit molecular mass of the enzyme determined by SDS-PAGE analysis equalled 125 kDa. It was found that the enzyme displays pI 5.35, prefers ortho-nitrophenyl-beta-galactoside as substrate (Km 27 mM) and shows maximum activity at 40ºC and at pH 7.5–9.5.

The beta-galactosidase gene was isolated from the genomic DNA library of \textit{A. sulfonivorans}, sequenced, cloned and deposited in the GenBank database under
accession number KM277894.1. It was established that the gene carries an open reading frame consisting of 3132 bp (1043 amino acids) and encodes beta-galactosidase referred to Glycosyl Hydrolase Family 2 (CAZy database).
**FUNCTIONALITY ANALYSIS OF STRUCTURAL DOMAINS FROM GD-95 LIPASE BY SITE SPECIFIC AND RANDOM MUTAGENESIS**

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**Background**

The rapid evolution of bioconversion and ecotechnology strongly increases the focus on the enzymes which possess novel properties. One of the most interesting enzymes are lipases from *Geobacillus* bacteria, because they can be active at extreme conditions. The ability to manipulate these lipases depends on the knowledge about the importance on the functionality of their individual domains and amino acids.

**Objectives**

Determination of influence conservative amino acids located at C-terminal end on activity GD-95 lipase without 10 C-terminal amino acids (GD-95-10) and construction new GD-95 lipase variants using error-prone PCR.

**Methods**

Lipase from *Geobacillus* sp. 95 without 10 C-terminal acid was analyzed by Ala scanning mutagenesis. New variants of recombinant GD-95 lipase were created using several error-prone PCR (epPCR). Ala mutants and GD-95 lipase variants after epPCR were cloned into pTZ57R/T and pET-21c(+) vectors. The recombinant proteins were expressed in *Escherichia coli* BL21(DE3) and purified using affinity chromatography. The activity and thermostability analysis of new lipases was performed spectrophotometrically.

**Conclusions**

GD-95-10 lipase was analyzed in this work using Ala mutagenesis. New results confirmed, that higher influence on activity and functionality of GD-95-10 lipase make Asp371 and Tyr376. Also the new GD-95 lipase variants were created using epPCR. The analysis of lipases obtained after random mutagenesis was showed that only a few changes in amino acids sequence can result new physicochemical properties of *Geobacillus* lipases. Therefore our results are basis for further enzyme engineering experiments and for creation of new lipases for bioconversion and ecotechnology.
USE OF SIGMA FACTORS FROM BACILLUS SUBTILIS IN THE DEVELOPMENT OF AN ORTHOGONAL EXPRESSION SYSTEM IN ESCHERICHIA COLI.
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Background

Technological advances in synthetic biology, systems biology, and metabolic engineering have boosted applications of industrial biotechnology for an increasing number of complex and high added-value molecules. In general, the transfer of multi-gene or poorly understood heterologous pathways into the production host leads to imbalances due to lack of adequate regulatory mechanisms. Hence, fine-tuning expression of synthesis pathways in specific conditions is mandatory.

Objectives

Here we develop a new genetic circuit for regulated expression specifically in stationary phase due to clear advantages during this period (reduction of toxicity, competition).

Methods

This circuit consists of a heterologous sigma factor (σ) recognizing specific promoter sequences, which are not recognised by the native σ factors of E. coli and is expressed upon entering the stationary phase. First, several σ factors of B. subtilis were tested for their orthogonality in E. coli on the level of promoter recognition, by using a red-fluorescent reporter system. Secondly, the potential of σ factors of B. subtilis to work together with the E. coli core RNA polymerase was tested, by expressing these proteins together with their promoters. Based on the results a specific factor will be chosen for further optimisation and the corresponding gene can be cloned in the σS factor operon of E. coli, which is most abundantly expressed in stationary conditions.

Conclusions
Combining all these elements should allow us to create an orthogonal genetic circuit that is able to transcribe specific genes under stationary phase with a limited influence on the host cell's metabolism.
COMPARISON OF POLYETHYLENE TEREPHTHALATE DEGRADING POLYESTER HYDROLASES FROM ACTINOMYCETES

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Background

Polyethylene terephthalate (PET) is a synthetic aromatic polyester that is degraded by several hydrolases from actinomycetes. The heterogeneous enzymatic PET hydrolysis occurs at mild temperature and pH conditions and offers an environmentally friendly alternative to chemical plastic recycling processes. LC cutinase, TfCut2 and Cut190 are homologous polyester hydrolases produced by actinomycetes obtained from a compost metagenome, from \textit{Thermobifida fusca} KW3 and \textit{Saccharomonospora viridis} AHK 190, respectively.

Objectives

The enzymatic degradation of PET films by the three polyester hydrolases was compared at various reaction conditions to evaluate the effects of buffer composition, pH, ionic strength and metal ions on their enzymatic activity.

Methods

Genes coding for LC cutinase, TfCut2 and Cut190 were cloned into pET-20b(+) vector for heterologous protein expression in \textit{Escherichia coli} BL21(DE3). Purified enzymes were used to degrade PET films at 60 to 70 °C for 1 to 50 h in different buffer systems as well as in the presence of Ca\textsuperscript{2+} and Mg\textsuperscript{2+}. The released products were analyzed by reversed phase high performance liquid chromatography. The weight losses of PET films following an enzymatic hydrolysis by the different enzymes were determined gravimetrically.

Conclusions

The LC cutinase from the compost metagenome and TfCut2 from \textit{T. fusca} showed significantly higher PET hydrolytic activity compared to Cut190 from \textit{S. viridis}. The addition of both Ca\textsuperscript{2+} and Mg\textsuperscript{2+} stabilized the enzymes and thus facilitated an effective PET degradation. The enzymatic activity of the polyester hydrolases against PET films was strongly depending on the type and ionic strength of the selected buffer.
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A DISRUPTIVE INNOVATION FOR AMBIENT TEMPERATURE PRESERVATION OF MICROORGANISMS
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Background
Because of the increase in the number of samples of microorganisms to be stored and shipped, classical preservation in freezers, subjected to risks of technical failure, is becoming more and more costly in space, energy and maintenance. Freeze-drying offers an alternative to cold preservation. However, it is time-consuming and requires precise cycle adjustments. Moreover, glass vials are subjected to breakage and can become leaky resulting in decrease in bacterial viability over time. Imagene innovation aims at preserving dehydrated bacteria at ambient temperature (AT) in hermetic minicapsules that maintain an anoxic and a low-controlled humidity environment. The minicapsules are resistant to impacts and have a unique, engraved datamatrix code allowing a tamper-proof traceability of the samples.

Objectives
This study aims at demonstrating the effectiveness of a novel and rapid process for a reliable long term AT storage of microbial strains.

Methods
Two bacteria, Pantoea dispersa and Aeromonas Salmonicida, a freeze-drying sensitive strain, were desiccated by evapo-concentration in presence of different stabilization solutions and encapsulated in minicapsules, which were stored either at AT or 37 °C (long-term storage simulation). Bacteria were recovered by simple rehydration.

Conclusions
The residual viability rates post-dessication were ~80 % for P. dispersa and ~60 % for A. salmonicida. Both strains kept a residual viability rate >50 % after 4 weeks at 37 °C and at AT in Imagene minicapsules. Viability rates following Imagene process were comparable to that of freeze-drying. In conclusion, our work demonstrated that this new process is suited to AT preservation of bacteria.
Background
Antrodia cinnamomea grows only on Cinnamomum kanehirai Hay (host specificity) and it is a unique fungus in Taiwan. In recent studies, the major components of Antrodia cinnamomea are triterpenoids whose functions were anti-inflammation and anti-cancer. So it is a valuable fungus in pharmaceutical potential. However, Antrodia cinnamomea grows slowly and does not obtain the fruiting body form easily under artificial cultivation.

Objectives
Based on this consideration, this study modified the plant factory as the new fungus fermenter to increase the triterpenoids products.

Methods
We isolated the new Antrodia cinnamomea strain (CUST-AC901) from the trees in the mountainous areas of Hualien in Taiwan. It was fermented on this new established fermenter without light, which is one type of closed plant production systems (CPPS) consisting of a thermally insulated, air conditioners, a CO2 and nutrient solution supply unit. The triterpenoids were analyzed by HPLC.

Conclusions
We established a new high-efficient solid-state cultivation with CUST-AC901 strain and obtained the optimal conditions (temperature at 20°C, CO2 at 10%, humidity at 85%, and with 1% peptone in whole grains medium). We also obtained compositions on fruiting bodies similar to the wild ones. It only took two months and reduced the producing cost. With HPLC analysis, we confirmed that total triterpenoids products were increased by four-folds than they were under traditional culture method. To our knowledge, this is the first report to omni-functional solid-state fermenter with high triterpenoids product efficiency and low cost as well as reaching to automatic mass production without expensive automatic production equipment.
THE IMPACT OF CYTOSOLIC AND PEROXISOME LOCALIZED TRANSALDOLASE AND TRANSKETOLASE ON XYLOSE METABOLISM AND ALCOHOLIC FERMENTATION IN METHYLOTROPHIC YEAST HANSENULA POLYMORPHA

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Background

Lignocellulose is currently considered as the most promising renewable feedstock for production of liquid biofuels. Xylose is the second most abundant sugar in nature, being a component of hemicellulose, which is in turn a part of lignocellulose. Yeasts and most of mycelial fungi can metabolize xylose by the so called xylose reductase – xylitol dehydrogenase pathway including xylose reduction to xylitol and subsequent xylitol oxidation to xylulose which is then phosphorylated by a specific kinase and enters pentose phosphate pathway (PPP). A simultaneous improvement of xylose conversion to xylulose and in the activities of PPP enzymes is required for efficient xylose utilization in different microorganisms. Xylose fermenting yeast Hansenula polymorpha contains in addition to cytosolic transaldolase (gene TAL1) and transketolase (gene TKL1) also peroxisomal transketolase (also known as dihydroxyacetone synthase, gene DAS1) and putative peroxisomal transaldolase (gene designated by us as TAL2).

Objectives

To investigate the role of cytoplasmic and peroxisomal transaldolase and transketolase in xylose metabolism and alcoholic fermentation in H. polymorpha.

Methods

Molecular-biology techniques

Conclusions

In the wild type strain of H. polymorpha overexpression of DAS1 and TAL2 turned out to be beneficial for xylose alcoholic fermentation. Moreover, mutants with knock out of at least one of these genes were impaired in xylose fermentation as compared to the
wild-type strain. To investigate the role of cytosolic transaldolase and transketolase in xylose metabolism of *H. polymorpha* recombinant strains overexpressing *TAL1* and *TKL1* genes were constructed. However, such strains revealed no substantial difference in ethanol production as compared to the wild-type strain.
Background

Wood is a direct food contact material used in the food industry since ancestral times. In Europe, wooden food contact surfaces are subject to the European regulation n°1935/2004, which specifies that food contact material must not interfere with foodstuff characteristics (Anonymous, 2004).

Today, no standard recovery method was defined for wooden food contact surface. Therefore, it might be important to provide an efficient method to quantify the microbial load on the wooden surfaces in direct contact with food.

Objectives

The aim of this study was to compare three methods of recovering microorganisms from wooden surfaces: grinding, brushing and planning (Ismaïl et al., 2014).

Methods

Three microorganisms, well-known as risk along the food chain, were tested: *Listeria monocytogenes*, *Escherichia coli* and *Penicillium expansum*. We chose three wooden species - pine, poplar and spruce - which are mainly used to manufacture wooden packaging. We analyzed the influence of wooden moisture content, contact time and wood timbers on microbial recovery rates.

Conclusions

We identified that factors cited above influenced the microbial recovery rates from wooden surfaces. Grinding was the most reliable method with the best recovery yield: 30.1% for *Listeria monocytogenes* on spruce and *Escherichia coli* on poplar, and 30.4% for *Penicillium expansum* on poplar. Planing was chosen to be applied to thicker wooden as cheese ripening shelves and tested, then, for analyzing the microbial load of 54 various wooden ripening shelves. We did not found common
pathogens known as risks for dairy products. Our results suggest that wood is suitable for food contact.
Background
GRAS status, the availability of different types of mutants and the ability to perform eukaryotic post-translational modifications, including complex glycosylation and protease processing, make *Saccharomyces cerevisiae* an ideal host for the expression of recombinant proteins. Among recombinant enzymes, lipases are used in many different industrial applications including pharmaceutical synthesis, biodiesel production, detergent formulation and food industry.

Objectives
In the present work, lipase A from *Bacillus subtilis* was expressed in the *S. cerevisiae* CEN.PK113-5D strain as a fusion protein with the yeast cell-wall mannoprotein Pir4. In order to compare the effect of different promoters on the levels of lipase activity secreted into the growth medium, three different YEplac195 based constructions were created, differing only in the promoter controlling the expression of the gene fusion; the original weak and constitutive PIR4 promoter, the TEF1 promoter, strong and constitutive, and the inducible GAL1 promoter.

Methods
The resulting three strains were grown in rich non-selective medium and the levels of secreted lipase activity and plasmid loss were determined after 48 hours of growth.

Conclusions
Our results show that the use of a very strong constitutive promoter is a poor strategy, since plasmid loss occurs in over 80% of cells and consequently total activity levels remain low in comparison with the strain transformed with the construction including a weaker promoter, where plasmid loss occurs in around 20% of cells. Finally, maximum levels of activity were achieved using the GAL1 promoter in conditions of induction for 24 hours after 24 hours growth in non-inducing conditions.
ENGINEERED SALMONELLA ENTERICA TYPHIMURIUM CONTROLS TUMOR GROWTH IN MURINE MELANOMA MODEL

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Background
The cancer is the second most frequent cause of death. Melanoma, a malignancy that arises from melanocytes, accounts for approximately 10% of skin tumors and in recent years its incidence has increased. The mortality rate currently stands at 80%, while 5-year survival rate is less than 5%. The factors that affect cancer outcomes, such as disease recurrence, risk of second malignant neoplasms, and the late effects of cancer treatments, becomes more important. For almost 200 years has been known that bacteria have the ability to colonize solid tumors and induce tumor shrinkage. The bacteria Salmonella enterica Typhimurium are facultative anaerobes and has a particular promise as a cancer therapeutic because it can be manipulated and has been shown to preferentially accumulate in tumors compared with other organs after systemic injection. S. enterica strains can have the anti-tumor activity enhanced by genetic manipulation.

Objectives
This study aims to evaluate the anti-tumor potential of attenuated new mutants of S. Typhimurium in murine models.

Methods
Six –week-old female C57Bl/6 mice were subcutaneously injected with 5x10^5 B16F10 (murine melanoma). After 12 days mice were injected intratumoral with 10^7 CFU/mL of S. enterica LGBM127.

Conclusions
Our results showed a reduction in tumor growth and an increased survival rate in mice treated with S. enterica when compared to the control group (PBS). There were no signs of prostration after LGBM127 inoculation. More tests are needed, but our preliminary data demonstrate a high potential for the use of the attenuated strain of S. Typhimurium as antitumor agents.
PROTEIN ENGINEERING TO INCREASE THE THERMOSTABILITY AND ACTIVITY OF THE GH-10 XYLANASE FROM THERMOASCUS AURANTIACUS, BY SITE-DIRECTED MUTAGENESIS

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Background

The enzymatic saccharification of lignocellulosic biomass and biobleaching paper pulp are attractive strategies for the bioprocess industry where xylanases have been heavily applied. These processes require enzymes that tolerate high stress environmental conditions such as extreme temperatures. The wild xylanase from *T. aurantiacus* (*xynA*) has been described as a potential enzyme for rational protein engineering studies. In the first step of this study, two no silent mutations were detected in the *xynA* sequence, cloned in *S. cerevisiae* strain, and it may explain the reduced expression and activity.

Objectives

New strategy was proposed to overcome the expression issues and enzyme improvement. First, *xynA* gene was rebuilt and it was used as template for constructions of mutants by site-directed mutagenesis (SDM) to increase thermal tolerance and activity.

Methods

For this, the original gene was amplified and cloning into pET28a. For the SDM reactions were used the Q5® SDM Kit. Primers were constructed according to the desired mutations and each one used for its changing. In the total, 13 reactions were prepared, in which only the first two were done to the reconstruction of *xynA*. All the mutagenic xylanases were characterized in pH (3.5-10) and Temperature (40-80°C) by enzymatic assay.
Conclusions

Regarding the optimum temperature, the enzymes are active at 65°C, and pH 5. About the stability temperature, the most of them are stable from 40°C to 65°C, and four mutants until 75°C, both in wide range of pH. These results helped the selection of the best mutagenic xylanases for future assays.
NOVEL BIOINSECTICIDES BASED IN BACILLUS THURINGIENSIS SPORES TO CONTROL PLAGUE DISEASES IN PLANTS

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Background

The massive use of synthetic chemical agents of biocontrol (i.e. insecticides) with potential toxicity to the users and the environment pushes for the development of alternative ( ecological and natural) compounds. To this respect, the use of natural and human-friendly bacteria with insecticide (i.e. larvicide) activities (biocontrol agents) represents an interesting alternative.

Objectives

*Bacillus thuringiensis*, is a ubiquitous Gram-positive, spore-forming bacterium that forms soluble Vip toxins a parasporal crystal (Cry toxin) during its growth cycle. These *B. thuringiensis* toxins are efficient for the control of certain plant-detrimental insect species among the orders *Lepidoptera*, *Diptera*, and *Coleoptera*.

Methods

One hundred nineteen *B. thuringiensis* strains isolated from different agricultural regions of Argentina were characterized for Vip and crystal protein production and evaluation of toxicity against Lepidopteran insect's larvae. Finally the genomic sequencing of the 16S ribosomal subunit genes plus the PCR identification of cry and vip subclasses of selected isolates were performed.

Conclusions

Several of the novel isolates were able to simultaneously control *Spodoptera frugiperda* and *Rachiplusia nu* larvae under laboratory and open field conditions. Interestingly, many of the isolates harbored both insecticidal genes (*vip, cyt*). The role of the master transcription regulator Spo0A on insecticidal protein production is presented. The understanding of the genetic network under Spo0A command open new avenues for the development of intelligent biocontrol agents in the scope of integrated pest management for sustainable agriculture.
NOVEL BACTERIAL INOCULANTS, BASED IN BACILLUS SPORES, TO INCREASE AND PRESERVE THE NUTRITIONAL QUALITY OF ALFALFA IN LIVESTOCK FODDER

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Background

Many problems associated with haymaking would be solved by reducing hay-drying time or if hay could be stored at high moisture without fungal spoilage. Bacterial inoculants are an ecological alternative for improving hay (and silage) preservation.

Objectives

The aim of this study was to identify, isolate and select the best spore-forming Bacillus strains capable of inhibiting the growth of undesirable fungus and accelerate the dehydration of forage in order to conserve its original nutritional value.

Methods

Seventy Bacillus strains were isolated from alfalfa fields of Argentina. Small bales and open field assays were prepared and tressed with each Bacillus strain in absence and presence of Fusarium verticilliodes. These bales were daily monitored throughout the trial for evidence of mold growth, dehydration of alfalfa and bale organoleptic quality. Germination rates / outgrowth and biofilm / spreading were evaluated in AFGK-buffer and LBY medium, respectively. Genomic identification of selected isolates was performed by sequencing of the gene coding for the 16S ribosomal RNA. Five isolates (initially identified as B. subtilis, B. pumilus and B. amylobacter) showed a broad spectrum of fungal control and an accelerated dehydrating capacity of alfalfa that improved the retention of its nutritional values in the forage. Viable spores were showed to be needed for these effects and the capacity of alfalfa colonization by mechanisms of biofilm formation and spreading were identified.

Conclusions

The novel Bacilli presented in this work, and the understanding of the displayed molecular mechanisms, are suitable for the production of novel inoculants to conserve forages of high nutritional value.
GENE DISRUPTION ANALYSIS FOR ETHANOL PRODUCTION IN GLYCEROL FERMENTATION IN GLYCEROL-ASSIMILATING KLEBSIELLA VARIICOLA AND ITS METABOLIC CHANGES

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Background

The gram-negative enteric bacterial genus Klebsiella can utilize glycerol as a sole carbon source and is known to produce some valuable metabolites, such as ethanol and 1,3-propanediol (1,3-PD). Previous studies, we have obtained streptomycin-resistance K. variicola mutant, strain TB-83D, as an ethanol producer from glycerol, and have demonstrated that it showed higher ethanol production than parent strain (Suzuki et al. Bioresour. Technol. 2015.). To further increase ethanol productivity, metabolic changes and inhibition of byproduct production by gene disruption were required.

Objectives

To further improve ethanol production, the aim of this study is to increase gene induction efficiency for gene disruption and to suppress byproduct production by gene disruption.

Methods

Gene disruption was performed by using modified Red recombination method. Metabolite concentrations were measured by HPLC (Waters alliance 2695) and F-kit D-/L-lactic acid (Boehringer Mannheim).

Conclusions

Since strain TB-83D produced mainly D-lactate under optimum conditions, we considered that suppression of D-lactate production is effective in improving ethanol production. To suppress D-lactate production, we disrupted lactate dehydrogenase gene (ldhA) by Red recombination. Gene disruption efficiency of Klebsiella species is low than that of other Enterobacteriaceae family. Therefore, we modified the conventional Red recombination, and succeeded in improvement of gene disruption efficiency. Although ldhA disruptant did not produce lactate in glycerol fermentation, ethanol production increased than TB-83D. Metabolic flux analysis and metabolic changes are in progress to optimize the ethanol production process, the results of which will be described in detail in due course.
DESIGN OF SACCHAROMYCES CEREVISIAE PRODUCING CELLULOSE-BASED CELLULO lytic Enzyme Complexes FOR LIGNOCELLULOSIC BIOMASS UTILIZATION

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Background
Heterogeneous structure of lignin imparts plants with structural rigidity and also serves to protect cellulose and hemicellulose from degradation. Thus, prior to fermentative production of ethanol from the cellulose by yeast strains, the materials are degraded and hydrolyzed to release monomeric sugars.

Objectives
In this study, designer cellulosome was assembled in yeast Saccharomyces cerevisiae for utilizing of cellulose as the substrate. For utilizing of cellulose part in lignocellulosic biomass by simultaneous saccharification and fermentation, a recombinant scaffolding protein from Clostridium cellulovorans and a chimeric endoglucanase E from Clostridium thermocellum were assembled as complex system. Compared to the results for single subunit, assembly of cellulosome-based enzyme complexes caused a noticeable increase such as 2.1-fold-higher in the level of enzyme activity. The resulting strain was able to ferment cellulose part in pretreated barley straw into ethanol with the aid of beta-glucosidase A from C. thermocellum. In the fermentation assay at 10 g/L initial substrate, approximately 2.1-folds higher ethanol than that of wild type was produced.

Methods
The use of complexed enzyme systems is one of the strategies for effective lignocellulosic biomass hydrolysis. Enzyme complexes were formed via the interaction of a dockerin domain with cohesin modules in the scaffolding protein.

Conclusions
Accelerating the biological degradation of lignocellulosic materials will benefit from the development of useful recombinant enzymes with hydrolysis ability. In future research, construction of designer enzyme complexes containing other lignin degrading enzymes could be used to develop biocatalysts that can completely degrade lignocellulose into single sugars.
CHARACTERIZATION OF PHYSIOLOGICALLY ACTIVE COMPOUNDS AND CO2 REDUCTION IN RHODOBACTER SPHAEROIDES MUTANT STRAINS

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Background:

The purple nonsulfur photosynthetic bacteria, Rhodobacter sphaeroides, are capable of growing under a variety of environmental conditions. Particularly this microorganism can use carbon dioxide as a carbon source to grow. In addition, this bacterium also has the capability to produce all kinds of the polyphenol, nucleic acid material, vitamin, bio-active substances and polyhydroxybutyrate (PHB). Therefore, this study focused on analyzing the properties of organic compounds and reduction of CO₂ in R. sphaeroides mutant strains.

Objectives:

In this study, relying on the ability to produce physiologically active compounds (PACs) and reduce CO₂, four different chemically mutated strains: R. sphaeroides KCTC 1434, MBTLJ-8, MBTLJ-13 and MBTLJ-20 were screened in order to select the most optimal strain.

Methods:

R. sphaeroides KCTC 1434, MBTLJ-8, MBTLJ-13 and MBTLJ-20 that are all chemically mutated strains, were used in this study. To be more precise, the comparison among these strains in terms of their ability to produce physiologically active compounds (PACs) and reduce CO₂ was carried out.

Conclusions:

The result showed that the mutant strain MBTLJ-8 performed the best ability of CO₂ fixation. In addition, it also showed that the CO₂ reduction and cell growth of mutant strains enhanced under the blue colored light emitting diode (LED) conditions. Therefore, our data indicated that the wave length of special light probably affected the cell growth, CO₂ reduction and organic compounds production of mutant strains.
Acknowledgments: This work was carried out with the support of “Cooperative Research Program for Agriculture Science & Technology Development (Project No: PJ01051502)” Rural Development Administration, Republic of Korea.
EFFECT AND ANALYSIS OF RHODOBACTER SPHAEROIDES EXTRACT UNDER VARIOUS CULTURE CONDITIONS

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Background:

*Rhodobacter sphaeroides* is a gram negative purple nonsulfur photosynthetic bacterium, facultative photoheterotrophic bacterium capable of growing phototrophically or chemotrophically as either a heterotroph or lithotroph in the presence or absence of O₂, depending on the energy source. In addition, it owns capability to produce all kinds of amino acids, nucleic acid materials, vitamins, bio-active substances, polyphenol and polyhydroxybutyrate (PHB).

Objectives:

In this study we focus on analyzing the properties of organic compounds and reduction of CO₂ in *R. sphaeroides* under various culturing conditions. Moreover, it is another goal of this study to confirm the effect on promoting growth of other microorganisms caused by the complement of *R. sphaeroides* extract in various culture conditions.

Methods:

*R. sphaeroides* KCTC1434 were used in this study. More precisely, the analysis of growth promoting effect of the extract of *R. sphaeroides* was conducted under various culture conditions.

Conclusions:

Our data indicated that addition of the *R. sphaerodes* extract into culture media accelerated the growth of microorganisms. Therefore, there was a strong evidence showing that the *R. sphaerodes* extract has multifunctional potential to increase the physiological activity of other organisms. It is, thus, determined as an enhanced material which is able to force to activate the growth promotion of other organisms.

Acknowledgments: This work was carried out with the support of "Cooperative
Research Program for Agriculture Science & Technology Development (Project No: PJ01051502) Rural Development Administration, Republic of Korea.
Background
Thermostable cellulases offer several advantages like higher rates of substrate hydrolysis, lowered risk of contamination and increased flexibility with respect to process design. The production of cellulases from a thermophilic fungus identified as *Thermoascus aurantiacus* RCKK (Acc. No. JN676149) has been attempted.

Objectives
In order to increase production level of cellulases and eventually economize the process, heterologous expression of these genes in *Pichia pastoris* was carried out.

Methods
β-glucosidase gene from *T. aurantiacus* RCKK was cloned and overexpressed in *P. pastoris* X-33.

Conclusions
A 2.5 fold higher production of recombinant β-glucosidase was achieved at shake flask level in comparison to natively expressed enzyme. Expression of β-glucosidase of about 96kDa was confirmed by SDS PAGE, western blot and zymogram analysis. The purified recombinant β-glucosidase was found to be stable in wide range of pH (3.0-8.0), temperature (up to 50% activity after 3 h at 80°C) and tolerant to the presence of ionic liquid (1-ethyl-3-methylimidazolium acetate [C2mim][OAc]. These properties make the recombinant enzyme an important catalyst for carrying out an efficient hydrolysis of cellulose to sugars and enzymatic deinking of recycled paper pulp. This will eventually open up potential of this enzyme.
RAPID DEGRADATION OF LIGNOCELLULOSIC BIOMASS

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Background

The main obstacle to lignocellulose bioconversion is the presence of lignin and crystalline cellulose in plant cell wall. However, major factors have not been definitely determined between lignin and crystalline cellulose in enzymatic lignocellulose recalcitrance. The correlative information promotes a deeper understanding of lignocellulose bioconversion characteristics.

Objectives

Our aim is to determine the major rate-limiting factor in the hydrolysis of popping pre-treated rice straw (PPRS) by examining cellulase adsorption to lignin and cellulose, re-hydrolysis, and amorphogenesis.

Methods

Lignin was prepared from PPRS by enzyme treatment until complete hydrolysis of carbohydrates and used to measure enzyme adsorption. Re-hydrolysis experiments were repeatedly performed with 15.75 or 31.50 FPU g\textsuperscript{-1}-biomass cellulase for 1 or 3 hour until complete hydrolysis of 1 or 2\% PPRS. Amorphogenesis of PPRS was carried out with 80\% phosphoric acid on ice for 1 hour.

Conclusions

Lignin isolated from PPRS adsorbed 20\% of enzyme loading from 3.15-31.50 FPU g\textsuperscript{-1}-biomass, adsorbing low levels of exoglucanases, endoglucanase and xylanase. Marked inhibition by cellulose structural effect was occurred during re-hydrolysis steps. Amorphogenesis of PPRS resulted in twofold higher cellulase adsorption and increased the yield of the first re-hydrolysis step from 13\% to 46\%. The total yield was increased to 84\% in 3 h. These results provide strong evidence that cellulose structure, not lignin, has a major effect on the enzymatic hydrolysis of lignocellulose.
Background
Poly(L-lactide) (PLA) is a biodegradable polymer. It is synthesized from lactic acid that is produced by microbes from agricultural products. It has much attention since it plays an important role to resolve the global warming problem. In general, PLA is polymerized commercially by chemical processes. However, these processes need severe conditions, i.e. high temperature, high solvent and catalyst loading. Recently, biological polymerization methods have been presented using lipases for the biocatalytic reaction.

Objectives
This work we aimed to apply the biological process for recycle of PLA.

Methods
Using a protease produced by Actinomadura keratinilytica strain T16-1. 1 g/L PLA powder was incubated at 77 °C for 24 h using an enzyme concentration of 100 mg/L. As a result, 750 mg/L lactic acid was obtained and used as substrate for re-polymerization of PLA. This was done by using commercial lipase under 0.1 vvm nitrogen atmosphere at 60 °C for 6 h.

Conclusions
Oligomers with molecular weight of 450 (n=4) were obtained. This is the first report to demonstrate the recycling of PLA wastes biologically.
OPTIMIZATION OF EXTRACELLULAR LIPASES PRODUCED BY NEWLY ISOLATED BACILLUS SAFENSIS STRAIN PSR

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Background

Lipases are an important enzyme for many biotechnological applications due to they are biocatalysts which have an activity at mild condition. Recently, bacterial lipase was very interesting because it can be used for re-polymerization of biodegradable plastics such as poly(L-lactide).

Objectives

The present work was aimed to screen lipase-producing bacteria from oil-contaminated soil in South of Thailand and study the optimization of medium composition for lipase-production.

Methods

Lipase-producing bacteria with 662 strains were isolated from 100 soil samples according to opaque zone formation on Tween 80 agar. Strain PSR exhibited the highest lipase activities in both agar plate and production medium. It was identified as Bacillus safensis strain PSR based on 16S rRNA gene sequencing. Moreover, the factors influencing lipase production were investigated in shake flask such as carbon and nitrogen sources, pH, temperature and incubation time. The maximum enzyme activity 63.86 U/ml was obtained by using 0.5% (w/v) rice bran oil and 0.12% (w/v) yeast extract as carbon and nitrogen sources, respectively, at pH 6.85, 37°C and 60 hours cultivation.

Conclusions

The study provides a high bacterial lipase production which is a good candidate to apply for recycle of biodegradable plastic waste in the near future.
Background
Medium-chain-length polyhydroxyalkanoate (PHA\textsubscript{MCL}) are biodegradable elastomeric polymers produced from renewable resources. \textit{Pseudomonas} sp. LFM046 is a PHA\textsubscript{MCL} producer from carbohydrates sources, presenting a better performance than the most studied \textit{Pseudomonas putida} KT2440.

Objectives
In this work, based on 16S rDNA sequences, \textit{Pseudomonas} species phylogenetically close to \textit{Pseudomonas} sp. LFM046 were identified and their sequenced genomes were searched for genes involved in carbohydrates catabolism and PHA biosynthesis, allowing reconstructed a core metabolic network. To confirm this network, the whole genomic DNA of \textit{Pseudomonas} sp. LFM046 was sequenced.

Methods
For this, genomic DNA was extracted and fragmented using Covaris. Paired-end sequencing library was constructed using TruSeq DNA PCR-Free LT Sample Preparation Kit. The library quantification was performed using QUBIT, Agilent Bioanalyzer and real time PCR. The library was sequenced on an Illumina MiSeq sequencer.

Conclusions
\textit{De novo} assembly generated 34 contigs using 3,032,982 reads, and the average size of 50\% of the contigs (N50) was 640,128 bp, with the maximum size of 1,038,386 bp. The genome size was estimated at 5,970,318 bp. All genes involved in carbohydrate metabolism and PHA biosynthesis previously detected in other \textit{Pseudomonas} species were also identified and annotated in the genome of \textit{Pseudomonas} sp. LFM046 using RAST software. It includes genes from Entner-Doudoroff (ED) and pentose phosphate (PPP) pathways, tricarboxylic acid (TCA) and glyoxyl acid cycles (GAC), \textit{de novo} fatty acids (FAB) and PHA biosynthesis. Thus, a core metabolic network of \textit{Pseudomonas} sp. LFM046 was reconstructed and is going to be used to determine flux distribution based on exchange flux measurements.

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Background

Modern waste water treatment plants utilize activated sludge systems to reduce soluble compounds in waste water. The diversity of the biocenosis is primarily affected by the composition of the treated waste water. Especially the distribution of carbon and nitrogen leads to a characteristic diversity. Information about the specific abundance of essential microorganisms can be used to improve the plant operation and stability of the biological degradation.

Objectives

While available quantitative methods like MPN (most probable number) and qPCR (quantitative polymerase chain reaction) are time consuming, this work led to the development of a rapid and quantitative variation of the fluorescence in situ hybridisation (qrFISH). The main objective was to design a method for a daily industrial application.

Methods
Fluorescence in situ hybridisation was carried out according to Nielsen (2009). Fluorescence particles were added to the sludge samples and used as a reference for quantification. Thereby the unknown loss of biomass throughout the FISH process could be compensated.

Conclusions

The current results showed a strong correlation between cell counts before and after the hybridisation process. Figures 1 shows the comparison of quantification results by fluorescence microscopy and conventional counting in a Thoma chamber. The experiments were conducted with enriched cultures of bacterial cells. The primary limitation of quantitative FISH was overcome, and the results were achieved in the targeted time frame of 8 hours.
CHARACTERIZATION OF RECOMBINANT LACCASE (BPCOTA) FROM BACILLUS PUMILUS MK001 AND ITS POTENTIAL FOR PHENOLICS DEGRADATION

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Background

Bacterial laccases are very less explored despite their superior properties such as high thermostability and wide pH stability. In the present study, Putative laccase (CotA) from Bacillus pumilus MK001 has been cloned and expressed in E. coli BL21(DE3).

Objectives

Hyper-production of recombinant bacterial laccase was carried out to utilize its potential in bioconversion of phenolics.

Methods

BPCotA was expressed in E. coli BL21(DE3) and purified to homogeneity. Purified enzyme was characterized for its thermal and pH stability. The 3D model of BPCotA was constructed based on the crystal structure of B. subtilis CotA (PDB Id: 1GSK) by homology modelling (Modeller 9.11). Modeled structure was refined and validated for stereochemical quality using PROCHECK, ERRAT, Verify 3D and PROSA servers. In silico interaction between enzyme and phenolics was studied out to predict in vitro phenolics degradation ability of the recombinant enzyme.

Conclusions

The recombinant BPCotA was purified with ~82% recovery exhibiting ~230 IU/mg specific activity. The recombinant BPCotA retained more than 50 % of the original activity after incubating at 80°C and 90°C for 90 min and 30 min, respectively, and more than 80% of its activity in the wide pH range 5.0-9.0 for 240 h. Increase in temperature from 30-90°C resulted in significant changes in secondary structure as predicted by far UV thermal CD spectra. Docking analysis showed that both ferulic acid and vanillin bind in the vicinity of predictive active site of recombinant protein. Further, in vitro action of the enzyme on phenolics resulted in bioconversion of both ferulic acid and vanillin.
THE EFFECT OF METHANE DIGESTED SLURRY FOR ETHANOL PRODUCTION FROM GLYCEROL BY KLEBSIELLA VARIICOLA STRAIN TB-83D.

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Background
Biodiesel fuel (BDF) waste contains large amounts of crude glycerol as a by-product, and its treatment is an environmental problem. In this study, it was attempted to produce bio-ethanol from crude glycerol. In previous study, we isolated *Klebsiella variicola* strain TB-83D that could produce ethanol from glycerol. This strain has high potential of ethanol production, and its production was 32 g/L by using yeast extract (YE) as a nutrient. However, YE is very expensive, so it is not suitable for an industrial use.

Objectives
The objective of this study is to decrease its culture cost. And we attempted to use methane fermentation digested slurry (MFDS). MFDS is residue of methane fermentation and its cost is very low.

Methods
The experiment was conducted by 1 L fermenter at 25°C and the culture pH was maintained at around 8.0 with 6 N NaOH. Basal medium consist of 50 g/L of glycerol and 600 g/L of MFDS is used for ethanol production by strain TB-83D.

Conclusions
The ethanol production was decreased to 17 g/L by replacing YE with sterilized MFDS. However, we think that this result seems to be a promising start to establish a low cost ethanol production from BDF waste. Next, we used non-sterilized MFDS to reduce sterilizing cost. Unfortunately, ethanol production was decreased but 1,3-propanediol and acetate production were observed. It may be caused by other bacteria survived in the MFDS. Now we are trying to optimize the culture condition that strain TB-83D is dominant when using non-sterilized MFDS.
CAT8 GENE IS INVOLVED IN REGULATION OF XYLOSE ALCOHOLIC FERMENTATION IN THE THERMOTOLERANT YEAST HANSENULA POLYMORPHA
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Background
Efficient alcoholic fermentation of hexose and pentose sugars by yeast is essential to achieve maximal ethanol yield from lignocellulosic hydrolyzates. It is known that CAT8 encodes carbon source-responsive transcriptional activator that controls expression of genes involved in gluconeogenesis. Deletion of CAT8 in the non-conventional yeast Pichia guilliermondii activated alcoholic fermentation.

Objectives
The aim of this work was to isolate mutants of H. polymorpha with knock out of the gene CAT8 and study their alcoholic fermentation. The strains were planned to be constructed in the backgrounds of the wild-type strain and of the mutant with elevated ethanol production from xylose due to overexpression of the genes encoded the key enzymes involved in xylose metabolism (xylose reductase, xylitol dehydrogenase, xylulokinase) and resistance to 3-bromopyruvic acid (glycolysis inhibitor).

Methods
The cassettes for CAT8 gene deletion was constructed by used of homologous recombination method with gene HphNT1 conferring resistance to hygromycin B as the selective marker. Fermentation was carried out at a temperature of 45°C with limited aeration. Concentrations of ethanol in the medium were determined using alcohol oxidase/peroxidase-based enzymatic kit 'Alcotest".

Conclusions
Deletion of CAT8 gene did not have effect on glucose alcoholic fermentation. At the same time, constructed knock out strains showed an improved xylose alcoholic fermentation relative to the parental strains. The Δcat8 strains isolated from the best available ethanol producer accumulated up to 12.5 g of ethanol/L at 45°C after 3 day fermentation without correction for evaporation. This is the 30 fold increase as compared to the wild-type strain.
CHIMERIC ROTAVIRUS AND HEPATITIS A VIRUS-LIKE PARTICLES INDUCE VIRUS-SPECIFIC HUMORAL AND CELLULAR IMMUNE RESPONSES IN MICE
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Background
Rotavirus and hepatitis A virus (HAV) are transmitted by the fecal-oral route and continues to be endemic throughout the world. Virus-like particles (VLPs) is safe and represents an economical approach to combined multivalent vaccine development.

Objectives
D2/VP7 VLPs demonstrated excellent combined vaccine candidate and may lead to the development of a commercialized vaccine worldwide for preventing both rotavirus and HAV.

Methods
In the current study, we evaluated the humoral and cellular immune responses in mice vaccinated with hepatitis A virus/rotavirus VLPs vaccine manufactured in methylotrophic yeast Pichia pastoris strain GS115. The VLPs were comprised of the hepatitis A virus D2 epitope and rotavirus ΔVP7 proteins. BALB/c mice vaccinated with chimeric VLPs.

Conclusions
VLPs produced high levels of neutralizing antibodies in sera against rotavirus and HAV. Induction of the secretion cytokines were detected from anti sera. In addition, splenic CD4+ and CD8+ T cell accessory molecules increased and produced cytokines after vaccination.
Background

Biopolymers are becoming more and more attractive for the industry due to their biological/natural origin, sustainability, biodegradability and low toxicity. This results in an increased demand for novel biopolymers, including exopolysaccharides (EPS) for different biotechnological purposes. It is worth to mention a broad spectrum for applications ranging from human health, over food and fodder production to chemical industry and environmental applications.

The ability to synthetize EPS is quite common among bacteria. Thus, as described exopolysaccharides differ in their physical and chemical properties, it may be surprising that only few of those products were successfully commercialized.

Objectives

In our studies we focused on Acidobacteria, highly divers and environmentally widespread but not well studied bacteria phylum, as a source of valuable compounds. The ability to synthetize EPS has been previously reported for some members of this phylum. However, its chemical composition and properties were not investigated up to date.

Taking into consideration a total lack of information on acidobacterial EPS, the aim of the present work was first of all to gain insight into the nature of those polymers and second to explore some of the characteristics potentially novel and superior for industrial applications.

Methods

We thus, focused on isolation, purification and physico-chemical characterization of EPS. Additionally, the stability of emulsions formed with selected oils and hydrocarbons.

Conclusions
The study proved different composition of EPS produced by closely related bacteria translating into differences in its properties but also a high stability of emulsions suggesting potential of EPS as stabilizing agents and/or bio-emulsifiers.
DETERMINATION AND CHARACTERIZATION OF PHYTASE FROM A NEWLY ISOLATED BACILLUS SP.

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Background
Phytate is the primary storage of phosphate in plants, and abundant in legumes, cereals and nuts. However, phytate make a polyanionic chelating agent which reacts with proteins, amino acids and divalent cations in humans and animals. Resulting in malnutrition and several health problems. Phytase is used as additive for degradation of antinutritional phytate and the enzyme is desired to be highly thermostable for it to withstand feed formulation conditions.

Objectives
The production of phytase is aimed from a new microbial source that was isolated from natural sources.

Methods
12 different soil samples taken from barn and coop surroundings were used for isolation of microorganism. To identify microorganism, we used 16srRNA analysis. For phytase production, strains were inoculated to Phytase-Screening Medium for 3d at 30°C. After phytase activity detection, enzyme was purified by ammonium-sulphate precipitation and DEAE-Sepharose. Samples were analyzed by SDS–PAGE.

Conclusions
Isolate (K12) was found Bacillus subtilis according to 16S rRNA sequencing. Several production parameters were optimized in order to ensure high enzyme production. In production medium, glucose was found as the most suitable C-source and ammonium nitrate (NH₄NO₃) was found to be most suitable N-source. In the presence of glucose, NH₄NO₃, sodium phytate and stirred speed (150 rpm), 72 hours incubation at 30°C were the most suitable conditions for phytase production. Phytase obtained from optimized production conditions, at pH 6.5 at 60°C and enzyme substrate ratio of 1:9 has the highest activity. MgCl₂, CuCl₂, CoCl₂, ZnCl₂ solutions and EDTA causing a loss of phytase activity whereas CaCl₂ solution has been found to be effective as an activity enhancer.
APPLICATION OF TYROSINASE PPO2 AS A BIOMARKER FOR THE FITNESS EVALUATION OF GENOME-REDUCED SACCHAROMYCES CEREVISIAE CELLS

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Background

In recent years, the availability of explosive whole-genome information has thoroughly reshaped the strain reconstruction studies for potential industrial applications. Consequently, the concept of minimum genome factories (MGFs) was proposed, which can be defined as recombinant strains whose metabolism have been streamlined to the optimal minimal subset applicable to specific utilities. The construction of MGF generally involves multiple large-scale genomic manipulations leading to a highly reduced genome. To this end, a wide range of genome engineering systems have been developed and applied in various microorganisms. One new issue raised thereafter is the fitness assessment of numerous resulting genome-reduced cells.

Objectives

Establish a simple and efficient system to evaluate the genome-reduced Saccharomyces cerevisiae chassis as platform for the production of value-added secondary metabolites.

Methods

A set of S. cerevisiae cells harboring large-scale deletions of different chromosomal regions was obtained using the recently developed mazF-based scarless genome engineering system. On the other hand, the coding sequence of tyrosinase PPO2 from Agaricus bisporus was amplified and cloned into yeast expression vector pLC12. The recombinant plasmid was transformed into all deletion cells by lithium acetate method. The product of PPO2 can convert intracellular tyrosine into colored substance melanin, which can be measured through spectral.
Conclusions
The tyrosine formation of yeast deletant cells can be readily deduced from the formation of melanin and this will be helpful in strain optimization for biosynthesis of valuable chemical based on tyrosine.
Background

Four varieties of *Aureobasidium pullulans* (Dothideales, Ascomycota), a cosmopolitan melanised yeast-like fungus with good stress tolerance, were recently redefined as four separate species: *A. pullulans*, *A. subglaciale*, *A. melanogenum*, and *A. namibiae*. These species inhabit various environments, including those with temperature and pH extremes, high UV radiation and high salt concentrations – and are thus considered to be polyextremotolerant. *Aureobasidium* spp. are biotechnologically important organisms, mainly due to their production of the extracellular polysaccharide pullulan, antimycotic aureobasidin A, siderophores, and various extracellular enzymes. *Aureobasidium pullulans* is also used in agriculture as a biocontrol agent while *A. melanogenum* is implicated in human disease. The recent sequencing of the genomes of the four above listed *Aureobasidium* species opened new possibilities for numerous novel applications in biotechnology, medicine, pharmacy, food industry, biofuel production, and in other fields.

Objectives

The aim of the study was to develop a yeast strain with good amylolytic activity under normal and stressful (industrially relevant) conditions.

Methods

In this study, we have cloned all alpha-amylase and glucoamylase encoding genes from the *Aureobasidium* species, used in the genome sequencing, into *S. cerevisiae*. The amylolytic performance of the transformants at different temperatures, low water activity and other relevant conditions was then evaluated. The best amylases were expressed in an industrial *S. cerevisiae* strain.

Conclusions

Yeast strains with good amylolytic activity under the conditions of increased salt concentration, low water activity, low temperatures and other relevant conditions were developed.
Background
Hydrophobins are amphipatic proteins with high surface activity produced by ascomycetes and basidiomycetes. Their interfacial properties make them very interesting to use them as emulsifiers and stabilizers for the food industry. However, a major drawback is the low production of protein without the use of genetic techniques. Here we report the use of an innovative biofilm reactor which uses a metallic packing system as a substrate to grow on.

Objectives
- Increase the production of hydrophobin HFBI from *Trichoderma reesei* using a biofilm reactor system

- Design of a reproducible, easy to scale up and relatively cheap method to increase the production of a fungal hydrophobin from *Trichoderma reesei*.

Methods
- Fungal strain and culture medium: *Trichoderma reesei* MUCL 44908 were...
inoculated in 20L of culture medium supplemented with glucose.  

-Bioreactor set up: Along with the fermentation under biofilm system a submerged fermentation was also carried out in order to compare the increase in the production. In the case of the biofilm, the reactor was filled with a metal structured packing (Sulzer, Chemtech) where a peristaltic pump recirculated the medium evenly over the top of the packing system, this cycle was continued for the total time of the fermentation.

Conclusions
- The protein production was 2.16 times higher that when submerged culture was used.
- The total weight of the fungal biomass was 33% higher that in submerged culture.
- The separation process between medium culture and fungal biomass was greatly enhanced, more than 99% of the biomass was attached to the biofilm.
MICROBES AS TOOLS FOR MYCOTOXIN DEGRADATION
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Background
Mycotoxins are secondary metabolites produced by several fungi. These toxic chemicals play a major role as feed and food contaminants. They can severely impact animal health, which is associated with high costs in livestock industry. Approximately 25% of all commodities worldwide are polluted by mycotoxins. The most prominent mycotoxins in contaminated food are Aflatoxins (hepatotoxic and cancerogenic), Deoxynivalenol (immunosuppression, protein synthesis inhibition), Zearalenone (infertility, abortion), Fumonisins (hepatotoxicity, nephrotoxicity) and Ochratoxin A (Neurotoxicity and cancerogenicity).

Objectives
To counteract this problem, several strategies are applied, which involve prevention of fungal contamination during production and storage, followed by physical, chemical, and biological methods to reduce mycotoxin levels.

Methods
Biological methods such as the use of microbial detoxifiers have the highest potential for feed decontamination. Bacteria capable of metabolizing mycotoxins allow decontamination and preserve nutritional value at the same time. To identify microbial detoxifiers, contaminated soil samples are cultured with a mycotoxin of interest to enrich potential degrading strains. This eases the screening of single colonies for detoxification, which is a time consuming procedure and associated with extensive labor. Once the organism responsible for detoxification has been isolated and identified, further experiments are necessary to ensure the organism is fermentable, non-pathogenic and can be applied as direct feed additive.

Conclusions
To date several detoxifying microbes have been found and characterized. An example for such a detoxifier is the strain BBSH 797 which degrades trichothecenes and is the only detoxifying feed additive on the market.
PENIOPHORA SP. CBMAI 1063 AS LACCASES PRODUCER: A MARINE ADAPTED STRAIN

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Background
Laccases are oxidoreductases enzymes that use molecular oxygen to oxidize phenolic and non-phenolic compounds. Laccases’ applications are related to bioremediation of hazards compounds, biobleaching and mill water treatment on pulp/paper industry, as component for phenolic detection, and for the synthesis of new “eco-friendly” polymers, antibiotics and cosmetics. Although its importance, it is still lacking ways to produce high amounts of laccases. Therefore, the search of new laccases and new producers are highly important for its commercial application. The marine-derived basidiomycete Peniophora sp. CBMAI 1063, isolated from the sponge Anphimedon viridis (Brazilian coast) is able to produce significant amounts of laccase. Previous studies showed that the laccase produced by this fungus has a good pH/thermal activity range and metallic ions resistance.

Objectives
To evaluate the laccase production by Peniophora sp. CBMAI 1063 under the presence of CuSO₄ and saline conditions.

Methods
The fungus was cultivated in 50 mL of optimized medium under different concentrations of CuSO₄ and artificial seawater (ASW) during 7 days at 140 rpm and 28 °C. Laccase was quantified spectrophotometrically using ABTS as substrate.

Conclusions
The highest laccase activity (around 2000 U L⁻¹), was obtained in the presence of 2 mM of CuSO₄ and 65% (v/v) of ASW. There was no laccase activity in the absence of ASW, suggesting its importance in fungal metabolic pathway. Peniophora sp. CBMAI 1063 has shown a great potential to be a laccase producer under saline conditions. The produced laccase are being characterized and its genes are being studied for heterologous expression.
EFFECTS OF ENVIRONMENTAL CONDITIONS ON THE EXOPLOYSACCHARIDE PRODUCTION OF A NOVEL BACILLUS STRAIN ISOLATED FROM SOIL

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Background

Microbial exopolysaccharides (EPS) have wide range of applications in food, pharmaceutical and other industries due to their stabilizing, gelling and texturizing properties. Various EPSs from different microorganisms including yeast and bacteria have been determined. They can be either homopolysaccharides (e.g. cellulose, dextran, pullulan, levan, curdlan) or heteropolysaccharides (e.g. xantan, gellan).

Objectives

In this study, exopolysaccharide production of a novel Bacillus strain ZBP4 on molasses was investigated. Effects of some environmental conditions on the production were also determined.

Methods

The bacterial growth was carried out in Nutrient Broth at 35°C for 24 h. The medium for EPS production contained 60 g molasses, 5 g yeast extract, 5 g peptone, 1 g K₂HPO₄, and 0.1 g MgSO₄.7H₂O in one liter of distilled water. Effects of temperature (30 to 45°C), pH (4.0- to 9.0), substrate concentration (10, 20, 30, 40, and 60 g/L molasses), nitrogen sources (ammonium sulfate, peptone, tryptone, and yeast extract), and aeration (80, 100, 150, and 200 rpm) on the EPS production were determined. For the isolation of EPS, cells were removed by centrifugation and supernatant was boiled and proteins were precipitated with trichloroacetic acid. Protein free supernatant was mixed with cold ethanol and centrifuged to precipitate EPS. Amount of EPS was determined with phenol-sulfuric acid method using glucose as standard.

Conclusions
The strain was produced highest amount of EPS at 60 g/L molasses concentration, 33 °C, pH 5.0. Tryptone was the best nitrogen source for EPS production. At 100 rpm agitation speed EPS production was the highest.
ASSEMBLY AND GRAFTING OF PATHWAYS FOR SYNTHESIS OF BERRY HIGH-VALUE PHENOLICS IN LACTOCOCCUS LACTIS.

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Background

Plant phenolics demonstrate promising effects in the combat of cardiovascular disease, certain types of cancer, neurodegenerative diseases, allergies, diabetes and inflammation. Accordingly, the food industry actively searches for natural alternatives, such as anthocyanins, to replace synthetic dyes. The increasing consumers demand for plant-derived natural products with health-promoting (eg. nutraceuticals) and/or biotechnological applications (eg. food colorants) requires the implementation of innovative solutions to the large-scale production of such compounds. One such opportunity is offered by the exploitation of microbial hosts as cell factories.

Objectives

Our aim was to reconstruct the pathway for plant anthocyanin pelargonidin-O-glucoside production from flavanone naringenin in Lactococcus lactis.

Methods

In order to assemble a functional pathway, native genes from various plants and synthetic codon-optimized genes were cloned in L. lactis, and their expression was tested in this bacterium. Then, highly expressed genes were assembled in one anthocyanin production cluster in L. lactis.

Conclusions

In this study, we present the construction of an artificial gene cluster consisting of four native and codon-optimized plant genes for synthesis of anthocyanin pelargonidin-O-glucoside from naringenin in L. lactis.
Background
In our previous work three methanogenic consortia were selected from environments with high concentration of methane with biological origin: (i) anaerobic digester biogas plant (AD), raw sewage sludge waste (RSS), cattle slurry (CS). Those consortia are highly specialized in stable and effective biogas production during mesophilic anaerobic digestion from maize silage.

Objectives
The aim of this study was verification of: (i) the ability to effective production of biogas from maize silage by the selected microbial consortia under various temperature and pH conditions, and (ii) the stability of microbial consortia community under various temperature and pH conditions.

Methods
Lab-scale anaerobic digestion process. Biogas yield: daily biogas production, methane content were analyzed by gas chromatography GC-MS. Physical and chemical analysis: pH, volatile fatty acids (VFA), chemical oxygen demand (COD), ammonium, total solids (TS), volatile solids (VS). Determination of total cell counts by fluorescence microscope at a magnification of 600, using the filter set WU for DAPI detection. DNA isolation and purification. PCR amplification. Denaturing gradient gel electrophoresis (DGGE) of archaeal and bacterial 16S rRNA.

Conclusions
The obtained results showed that the selected consortia are able to produce biogas in broad range of environmental conditions. However, the process is running with a lower methane yield in psychrophilic and acidogenic condition. These results also demonstrated that selected consortia have broad spectrum of activity and could be used as a “biostarter” in start-up trial operation in different biogas plant technology process.
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COMPARATIVE EFFICIENCY OF ADDING ETHANOL OR POTASSIUM METABISULPHITE IN FUEL ALCOHOL FERMENTATIONS CONTAMINATED WITH DEKKERA BRUXELLENSIS: EFFECT ON THE CELL NUMBER AND ETHANOL YIELD
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Background
Fuel alcoholic fermentations contaminated with Dekkera bruxellensis result in decreased ethanol yield.

Objectives
The aim of this work is to compare the efficiency of two cell treatments carried out prior to the fermentation to control the growth of D.bruxellensis without affecting the starter yeast Saccharomyces cerevisiae and the ethanol yield, in pure and mixed cultures.

Methods
The treatments consisted in the addition of 13% (v/v) ethanol or 250mg/l potassium metabisulphite (PMB) to the acidic solution (pH2.0), for 2 hours at 30°C, under shaking, in which the cells were introduced. After treatment, the cells were washed twice with sterile water, centrifuged and inoculated in sugar cane juice for 12 hours. The cell number was estimated by plating the samples in WLN medium with and without actidione to count the CFU number of D.bruxellensis and S.cerevisiae, respectively. Alcohol production was also determined.

Conclusions
In the mixed culture, the addition of ethanol to the acidic solution had similar effect over S.cerevisiae and D.bruxellensis, however the cell number increased after the fermentation time only for S.cerevisiae. The ethanol production was reduced by 6%. When PMB was used, the ethanol production was decreased considerably (74%) although the number of S.cerevisiae was not affected. D.bruxellensis was not affected at all. The interaction between PMB and S.cerevisiae to inactive the toxic effect of this substance may be the reason why the fermentation yield was affected considerably. The addition of 13% ethanol in the cell treatment was the most efficient and the least harmful. Support: FAPESP
LIGNINOXYTIC ENZYME ACTIVITIES AND CORN STRAW DELIGNIFICATION BY THE FUNGAL STRAIN CMU-196 OF PARACONIOPHYRIUM SP.
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Background
Filamentous fungi degrade lignin by producing extracellular oxidative enzymes such as laccase (LAC), lignin peroxidase (LiP) and manganese peroxidase (MnP). These enzymes have diverse biotechnological applications, including straw delignification intended for biofuels production or ruminant feed. Paraconiothyrium is an ascomycete genus recently described whose species can produce ligninolytic enzymes; however, until now only few strains within the genus have been analyzed for this purpose.

Objectives
The aim of this work is to analyze the production of the extracellular LAC, LiP and MnP in the strain CMU-196 of Paraconiothyrium sp., and to evaluate the corn straw delignification by this strain.

Methods
Basal and induced enzyme activities were determined by incubating the strain CMU-196 in potato dextrose broth (PDB) at 24 °C and 128 rpm. Induced media were supplemented with ground corn straw (2% w/v), an aqueous extract of it (10% v/v) and CuSO₄ (150 µM). Corn straw delignification in a solid state fermentation was followed by scanning electron microscopy (SOM).

Conclusions
Maximum activity for MnP (83.72 U/ml) and LAC (103.64 U/ml) was shown at 5th day of incubation in PDB supplemented with ground corn straw. Maximum activity of LiP (25.66 U/ml) was observed at 6th day of incubation in basal medium. SOM images reveal an increase in delignification of vascular tissue of the corn straw inoculated with the fungus since week 4th until the end of treatment at week12th. The strain CMU-196 produce high levels of the three extracellular ligninolytic enzymes and has potential for straw delignification.
Background

The contamination of fuel alcohol fermentations with Dekkera bruxellensis brings about decrease in ethanol productivity and yield.

Objectives

Growth and fermentation profiles of two strains of D. bruxellensis and one strain of S. cerevisiae were evaluated under varying conditions of shaking in synthetic medium with glucose or sucrose as carbon source in different concentrations. We aimed to verify if the conditions to obtain higher alcohol production are similar for both yeast species.

Methods

Erlenmeyer flasks containing yeast cells in 200 ml of synthetic medium containing glucose or sucrose (50, 100 and 150g/l) were maintained at 30°C for 96 h under 0, 150 or 250 rpm. Growth was monitored by optical density at 600 nm and alcohol production was determined by density.

Conclusions

The strains of D. bruxellensis displayed similar results concerning growth: it was higher under shaking conditions regardless the carbon source type and concentration and was higher in sucrose than in glucose. The best conditions to achieve higher alcohol production were not similar and it was dependent on the shaking and the carbon source concentration. For S. cerevisiae, the growth was higher with shaking and no effect of the carbon source type and concentration was detected. Alcohol
production was substantially higher in static cultures regardless the carbon source. Sucrose at 150g/L, which is the basic fermentation medium in static cultures, favors alcohol production by \textit{S.cerevisiae} but it does not for \textit{D.bruxellensis}, whose growth is stimulated by sucrose. These results confirm the role of this yeast as contaminant and shows the variability exhibited among strains. Support: FAPESP
Background
Membrane proteins (MPs) play a crucial role in cell biology of any living organism. Nearly 30% of all open reading frames encode MPs and more than 50% of currently available pharmaceuticals target MPs. Despite their importance, the function of most MPs has not been assigned, yet.

Objectives
Since production of MPs is commonly limited by their hydrophobic nature, it is inevitable to generate novel expression systems that allow the high-level production of this class of enzymes. Here, we present an alternative expression system based on the bacterium *Rhodobacter capsulatus*, which is – due to its phototrophic nature – especially suited for the synthesis of MPs.

Methods
The facultative anaerobic purple bacterium *R. capsulatus* is able to grow either chemotrophically or phototrophically. Under latter conditions, it forms an intracytoplasmic membrane system (ICM) and vesicles, harboring the proteins of the host’s photosystem and heterologously produced MPs. To characterize the expression properties of this system, several genes encoding monooxygenases, diagnostic-relevant MPs as well as GPCRs were cloned into a comprehensive set of novel broad host range expression plasmids of the pRho series. Subsequent accumulation, localization and activity studies were conducted to evaluate the alternative expression system.

Conclusions
With *R. capsulatus* and the pRho plasmids we have developed an alternative, powerful expression system for the synthesis of heterologous MPs. Our system is particularly suited for the production of monooxygenases, diagnostic-relevant proteins and GPCR-(like) proteins with yields of more than 10 mg per liter culture.
INNOVATION IN THE CONJUGATION PROCESS OF THE CAPSULAR POLYSACCHARIDE PRODUCED BY HAEMOPHILUS INFLUENZAE TYPE B LINKED TO TETANUS TOXOID

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Background

*Haemophilus influenzae* type b is a Gram-negative bacterium causing pneumonia and meningitis. Its capsular polysaccharide - PRP is the main virulence factor. Vaccines based on PRP conjugated to protein are very efficient. The low yield of the conjugate has motivated the development of conjugation process to attend the demand of low-income countries. DMT-MM (4-[4,6-dimethoxy-1,3,5-triazin-2-yl]-4-methylmorpholinium chloride) used as activation molecule is an alternative to improve the conjugation reaction and the anti-PRP IgG level was statistically similar to commercial vaccine.

Objectives

To improve the conjugation process to make feasible the production of conjugate vaccine in large scale (g).

Methods

PRP (2.3g at 10g/L) was oxidized by NaIO₄, quenched with glycerol and purified by tangential ultrafiltration resulting PRP-Oxi. PRP-Oxi (1.9g at 8g/L) was mixed with adipic acid dihydrazide (ADH) followed by addition of NaBH₄CN. After 4 hours, NaBH₄ was added and submitted to ultrafiltration resulting PRP-AH. PRP-AH (15g/L) was reacted with TT (15g/L) in the presence of DMT-MM (0.1M) and the conjugate product was purified by size exclusion chromatography.

Conclusions

In large scale some adjustment were required in order to achieve the desired yield. For instance, glycerol amount was increased to stop the oxidation (83%). In the next step the amount of ADH was raised to avoid intermolecular reaction with 94% of PRP-AH. These yields are in agreement with those obtained in small scale (mg). The last step (mg scale) yielded 46% and now we are working on gram scale. Further studies considering immunological aspects will be performed.
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GENOME SHUFFLING OF LACTOBACILLUS RHAMNOSUS FOR IMPROVED PRODUCTION OF LACTIC ACID

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Background
The development of bio-based production leads to an increasing demand for rapid engineering of multiple complex phenotypes into a single production host. The approach of genome shuffling is providing a powerful platform for improving multiple complex phenotypes in ill-characterized hosts.

Objectives
The main objectives of this study were to improve acid tolerance and lactic acid production from Lactobacillus rhamnosus through genome shuffling.

Methods
In the present study, the genome shuffling was used to improve the acid tolerance of while simultaneously enhancing lactic acid production. A total of 10 yoghurt samples were randomly collected in sterilized glass bottles from farmers directly and were processed immediately for isolation of the lactic acid bacteria. The isolated 5 strains of Lactobacillus rhamnosus treated for adaptation of Low pH. Adapted wild-type strain in pH 4.0 medium was then used as the parental, or starter, for genome shuffling. All the isolated Lactobacillus rhamnosus strains were mutagenized using nitrosoguanidine (NTG) while genome shuffling was carried out using standard method. From the results, it was observed that, the mutants showed a small increase, from 6.1 to 11.2 g/l, in production of l-lactic acid in comparison with the Wild type strain.

Conclusions
In conclusion, genome shuffling successfully improved the tolerance of L. rhamnosus towards acid. The research here demonstrated that genome shuffling could greatly accelerate the improvement of important phenotypes of microorganisms by developing their ability to circumvent the extreme process condition.
MORPHOLOGY OF ASPERGILLUS TERREUS NRRL 1960 IN RELATION TO EXTERNAL MANGANESE (II) ION CONCENTRATION

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Background

Filamentous fungi in general and Aspergilli in particular are amongst the most important cell factories in biotechnology, as they have an intrinsic potential to produce a variety of organic acids such as citrate, gluconate and itaconate in high quantities. In order to obtain high yields of citric acid by A. niger, the elimination of Mn (II) ions from the medium is a prerequisite. Mycelia grown under manganese deficiency form dense and very small pellets. In this study, A. terreus NRRL 1960, an itaconic acid producer strain was used to study the effects of external manganese (II) ion concentration on fungal morphology.

Objectives

Our primary objective was to monitor time-course changes in the morphology of A. terreus, grown under different external Mn²⁺ ion concentrations.

Methods

Well-controlled, strongly aerated lab-scale batch fermentations were performed. Changes in fungal morphology was monitored by image analysis. External Mn (II) ion concentrations were determined by ICP-MS.

Conclusions

The overwhelmingly mycelial morphology of A. terreus NRRL 1960 progressively turned to pellet-like below 50 µgL⁻¹ Mn²⁺ ion concentration. At 5 µgL⁻¹ Mn²⁺ ion concentration and below, morphology was completely dominated by yeast-like, swollen cells.
Background

*Bacillus pumilus* present a biotechnological and pharmaceutical relevance highlighted by their ability to produce compounds with different biological activities. Moreover, they can be also found as troublesome contaminants in industrial settings and also be associated with food poisoning. Recently, phylogenetic analysis supported on different biomarkers determined the reassignment of most *B. pumilus* as *Bacillus safensis* or *Bacillus invictae*. The absence of a resistance profile to antibiotics of human and veterinary importance and also of toxin encoding genes are pre-requisites specified by EFSA when their use is intended in the food sector.

Objectives

To evaluate the antibiotic susceptibility, and the presence of antimicrobial resistance or entero- and emetic-toxins encoding genes in a clonally diverse *B. pumilus* group collection.

Methods

Minimum inhibitory concentrations to different antibiotics were determined and interpreted according to clinical breakpoints and microbiological cut-offs, in a collection comprising *B. safensis* (n=27), *B. invictae* (n=9) and *B. pumilus* (n=4). Additionally, acquired genes encoding resistance to relevant human and veterinary antibiotics (tetracylines, macrolides, aminoglycosides, glycopeptides, phenicols and oxazolidinones) and also entero- and emetic-toxins (cytotoxin K, haemolysin, non-hemolytic and emetic toxins) were screened by PCR.

Conclusions

Susceptibility to all antibiotics included in interpretative criteria for their testing on *Bacillus* spp. was observed. Nevertheless, high MIC values (8-64 mg/mL) were observed for cefotaxime, a therapeutically relevant antibiotic. All *B. pumilus* group species revealed the absence of acquired antibiotic resistance and virulence genes, suggesting that there is no recognized risk of their application in the food sector or in other relevant biotechnological uses.
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BIOTECHNOLOGICAL POTENTIAL OF BURKHOLDERIA GLUMAE PG1

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Background

Microorganisms represent prolific sources for biotechnological relevant compounds which often need to be produced in heterologous hosts like Escherichia coli. Here, we present the Gram-negative bacterium Burkholderia glumae PG1 as a promising alternative production strain.

Objectives

B. glumae PG1 is known to secrete the lipase LipA which is used by the BASF SE for the production of enantiopure building blocks. A detailed genome comparison of B. glumae PG1 [1] with other representatives of this genus revealed that this phytopathogenic strain lacks the gene cluster encoding the biosynthesis of the important virulence factor toxoflavin.

Methods

Furthermore, we identified 25 clusters including PKS and NRPS for the production of secondary metabolites as well as genes encoding rhamnolipid biosynthesis enzymes resulting in the production of 20 mg/L rhamnolipids under non-optimized conditions.

We analyzed the genome of the industrial lipase production strain B. glumae LU8093 which was generated by random mutagenesis and identified 72 single nucleotide polymorphisms compared to the wild-type. Two of them were associated with the lipAB operon and affect its transcription as well as the efficiency of lipase secretion.

Conclusions
The identification of the putative SGNH hydrolase LipG and the construction and functional analysis of a T7 polymerase based expression strain further underline the biotechnological potential of \textit{B. glumae} PG1.

OPTIMIZATION AND KINETICS OF THE PHYTOHORMONE PRODUCTION BY INONOTUS HISPIDUS IN STIRRED TANK REACTOR SCALE


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Background
In this study, firstly, the ability of plant growth hormones (PGH) production capacity of more than a hundred Basidiomycetes isolates was screened.

Objectives
Plant growth regulators are commonly used by agricultural manufacturers in world wide. In our study, we have tried producing some of these plant growth regulators from a macro fungi, Inonotus hispidus.

Methods
Inonotus hispidus was firstly recorded as a PGH producer species and was selected for following experiments based on its 1033.65, 125.11, and 10.91 mg/L gibberellic acid (GA3), absorbic acid (ABA) and indole acetic acid (IAA) production values, respectively. Secondly, PGH producing capacity of Inonotus hispidus isolate was determined while the growth of the mushroom on different culture types (static, submerged and solid state). On 15th day of submerged fermentation, maximum plant growth hormones production was determined as 2478.85, 273.26, and 30.67 mg/L for GA3, ABA and IAA, respectively. Then, Plackett-Burman and response surface experimental designs were used for optimization of PGH production by Inonotus hispidus. Finally, the determined optimum conditions was investigated in stirred tank reactor scale to produce PGH in higher volume and reached results were, GA; 5441.54, ABA; 390.39 and IAA; 44.79 mg/L. PGH production by free and immobilized cells was also compared.

Conclusions
Consequently, production amounts of GA3, ABA and IAA by Inonotus hispidus in reactor scale were increased respectively 5.26, 3.12 and 4.1 fold according to screening step. The kinetic parameters of PGH production and growth of Inonotus hispidus (m, r_s, Q_s, r_p, Q_p, Y_p/s, Y_p/x) were also determined in optimize and non-optimize conditions in Erlenmeyer flask and stirred tank reactor scales.
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DNA APTAMERS BLOCKING ACTIVITY OF ANTHRAX LETHAL TOXIN

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Background

Anthrax is major biosecurity threat. Apart from natural outbreaks, lethality of anthrax and recalcitrance of B. anthracis spores to envioronmental factors and disinfectants made it the most feared bioterrorist weapon.

Anthrax lethal toxin (LeTx) consisting of receptor-binding (protective antigen), and protease (lethal factor) subunits is main target for development of anthrax diagnostic and therapeutic tools. Aptamers represent important platform for discovery of target-specific affinity reagents. Particularly, aptamers known to be the source of efficient allosteric protease inhibitors [1]. Immuno-aptameric PCR possesses the highest known detection sensitivity [2].

Objectives

The purpose of the present study was to develop new technique aimed at rapid isolation of high-affinity aptamers suitable as diagnostic and therapeutic candidates against LeTx.

Methods

In the aptamer library screening, key elements to isolate high-affinity binders are: prevention of carryover of target-specific aptamers by nonspecific oligonucleotides, and early elimination of unbound and weakly bound species. Using LeTx as the target, we developed efficient, cost and time saving protocol of enabling isolation of high-affinity aptamers by using on-rate selection, tandem affinity purification of target-specific aptamers by double proteolytic elution that leaves tightest bound aptamers associated with the eluted target, whereas the majority of binders with intermediate affinity already dissociated and removed during the competitor-based off-rate selection.

Conclusions

New aptamer selection technique is useful in isolation of ultra-high affinity aptamers targeting bacterial toxins.

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References

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SELECTION OF NANNOCHLOROPSIS OCEANICA CELLS WITH HIGHER INTRACELLULAR LIPIDS CONTENT BY FLUORESCENCE ACTIVATED CELL SORTING.
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Background
Microalgae are now the focus of interest due to their ability to produce an intracellular lipid feedstock that is suited to conversion in biodiesel. Nannochloropsis oceanica (N.O.) is a unicellular green alga intensely investigated because of the natural ability to form intracellular lipid bodies that contain triacylglycerol. As a way to select the cells with spontaneous higher amount of intracellular lipids were separated those cells from a heterologous population using the technique of fluorescence activated cell sorting (FACS).

Objectives
Visualization of intracellular lipid droplets of N.O. by epifluorescence microscopy.
Visualization and selection of cells with high intracellular lipids content from heterologous populations of N.O. cultures by FACS.
Quantification of total lipids in selected cellular populations of N.O. under different culture conditions

Methods
The microalgal cultures where suspended in DMSO and stained with Bodipy 505/515. Flow Cytometer (BD Influx™ cell sorter) was used to detect the fluorescence signal of Bodipy. Neutral lipids stained showed a particular emission peak at 530 nm. Total lipid fatty acid profiles were determined by GC of methyl esters.

Conclusions
Intracellular lipid droplets stained with Bodipy 505/515 was easily visualized by epifluorescence microscopy in N.O. cells.
The FACS technique allowed the visualization and separation of cellular populations enriched in intracellular lipids from liquid cultures of N.O.
Analysis of lipids quantification confirmed the obtaining of cellular populations with higher amounts of total lipids and fatty acids reaching 46% of total lipids by mg of dry weight.

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Fungicidal activity of exo- and endo-polysaccharides from Amazonian fungi

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Background:
Endophyte fungi are usually found in Amazon Rainforest with high biodiversity, and biological and chemical potential. They can be found as endophytes, phytopathogens or saprophytes. Candida is a fungal pathogen responsible for opportunistic infections in humans.

Objectives:
To produce exo- and endo-polysaccharide extracts from Pestalotiopsis sp. and Talaromyces sp., and assay them against Candida albicans.

Material and Methods:
Two fungi from the GEMMA group fungal collection were cultivated for 18 days on PDY medium. Then the mycelium was separated from the medium. After add to culture medium from each fungus 1L of methanol, the mixture was left for 48 hours at 4 °C. The precipitate formed was separated by centrifugation and the solvent in the supernatant was evaporated in rotary evaporator. The aqueous phase was subjected to liquid-liquid extraction with ethyl acetate and the acetate extract was concentrated by rotary concentration.

Conclusions:
Three samples were selected for candidical assays: (1) Talaromyces inorganic partitioned, (2) Pestalotiopsis mycelium, and (3) Talaromyces culture aqueous. All extracts had revealed candidical activity and were subsequently tested at the concentrations of 1000, 750, 500 and 250 μg.mL-1 to determine the minimum inhibitory concentrations. The sample (1) and (3) showed no satisfactory inhibition while (2) showed inhibition at 500 μg/ml.
EXPRESSION, PURIFICATION AND CHARACTERIZATION OF A BIFUNCTIONAL 99-KDA PEPTIDOGLYCAN HYDROLASE FROM PEDIOCOCCUS ACIDILACTICI ATCC 8042
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Background
Lactic acid bacteria inhibit the growth of pathogens and food deleterious microorganisms because they produce substances with antibacterial activity, such as peptidoglycan hydrolases (PGH). *Pediococcus acidilactici* ATCC 8042 has been reported to inhibit pathogenic microorganisms such as *Staphylococcus aureus* through the production of two proteins with lytic activity, which are approximately 110 and 99 kDa. The 99-kDa PGH has high homology to a putative protein reported in *Pediococcus acidilactici* 7_4, where two different lytics domains have been identified but not characterized.

Objectives
The aims of this work were cloning, expression, purification and biochemical characterization this bifunctional enzyme.

Methods
The 99-kDa PGH was cloned and expressed successfully and showed activity against *Micrococcus lysodeikticus*. The protein was then purified using gel filtration chromatography. For the characterization 4-Nitrophenyl-N-acetyl-β-D-glucosamine was used as substrate.

Conclusions
The pure protein showed an optimal pH for antibacterial activity and stability of 6.0 and 5.0 to 7.0, respectively. The optimal temperature for activity was 60°C, and it lost all activity after incubation at 70°C for 1 h. The number of strains susceptible to the recombinant 99-kDa enzyme was lower to those lysed by the mixture of the 110- and 99-kDa PGHs of *P. acidilactici*, which indicates that there might by synergy and interactions between these two enzymes. This is the first peptidoglycan hydrolase from LAB that has been shown to possess two lytic sites. The results of this study will aid in the design of new antibacterial agents from natural origins that can combat foodborne disease and improve hygienic practices in industrial sector.
BIOCHEMICAL CHARACTERIZATION OF A SPORE LACCASE FROM A HALOTOLERANT BACTERIUM, BACILLUS SP.

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Background

Laccases, one of polyphenol oxidases, have many applications in different industries and can be used in bioremediation, organic synthesis, and biosensors.

Objectives

In present study, different bacterial strains were screened to find laccase producing strain. Biochemical characterization and Crystal violet decolorization effect of the spore laccase from a spore producing strain was examined.

Methods

As total 50 bacterial strains isolated from a soil sample of a chromite mine in Iran were screened for laccase production on nutrient agar medium supplemented with 3\% (w/v) NaCl and 0.02\% (v/v) guaiacol. Spores from a spore forming Gram-positive strain showed laccase activity. The strain was identified and pH and temperature optima for the enzyme activity were determined. Effects of inhibitors and metal ions on the enzyme activity were studied. Decolorization of Crystal violet was investigated by spore laccase.

Conclusions

The closest relative of the laccase producing strain was \textit{Bacillus safensis} FO-036b\textsuperscript{(T)}. The spore laccase showed optimal activity at pH 5 and 35°C, had a good tolerance to metal ions, and retained 104\% of its initial activity against 1 mM of NaN\textsubscript{3}. The enzyme decolorized 64\% of 10 mg/L Crystal violet, so it could be a good candidate to bioremediation applications.
To Low Cost Fermentation Processes

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Background

Fermentation processes have been used for over 45 centuries, especially for the production of food, with ethanol and lactic acid as the main biogenic compound. These products have a high added value, because they are used as stimulant and/or because of their food preserving nature. This high added value allows significant costs for the fermentation process.

During the last 30 years fermentation technology has also been applied for the production of chemicals and fuels. These products have to compete with low-cost petrochemicals, putting restrictions on the costs of the fermentation processes. The current state-of-art fermentation technology is often too expensive for competitive production of chemicals and fuels.

In the same period the instruments to modify microorganisms have evolved considerably. The ability to build whole genomes, the application of –omic techniques and genome scale modelling give unprecedented possibilities to design microorganisms for optimal product formation.

Objectives

Instead of adapting the process to the microorganisms to reach the best results, it is now time to develop strategies which adapt the microorganisms to the process.

Methods

Key drivers for process improvement are the reduction of oxygen requirement by redox engineering, the increase of product tolerance and the separation of the product from the aqueous broth by phase separation.

Conclusions
In this paper/presentation we describe our activities to realize this and estimate the cost reduction that can be realized. Itaconic acid and fatty acids will be shown as examples. A cost reduction to a value below that of contemporary ethanol production (800 €/kg) seems feasible.
REACTIVE OXYGEN SPECIES REGULATE LOVASTATIN BIOSYNTHESIS IN ASPERGILLUS TERREUS

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Background

Lovastatin (LOV) is a secondary metabolite, produced by Aspergillus terreus. It has great commercial importance since it lowers cholesterol levels in blood. Studies from our group showed a link between reactive oxygen species (ROS) and LOV biosynthesis in submerged (SmF) and solid-state fermentation (SSF). Our results showed that sod1 gene (oxidative stress defense enzyme) was intensely expressed during rapid growth phase (or trophophase) in LOV fermentations, but it was down regulated in production phase or idiophase. Probably because of this, in that moment ROS levels increased, generating an oxidative state during idiophase (Miranda et al 2013).

Objectives

The objective of the present work was finding evidence that ROS contribute to the regulation of lovastatin biosynthesis.

Methods

ROS were manipulated during LOV fermentations. Exogenous antioxidants were used to eliminate or reduce ROS accumulation during lovastatin fermentations. Its effect on lovastatin production was determined. Also, its effect on the expression of LOV genes (lovE and lovF), and on genes yap1 and srrA (encoding oxidative stress defense transcription factors) was determined by Northern analysis.

Similar (complementary) experiments, increasing ROS by H2O2 addition to LOV fermentations, were also performed.

Conclusions

1. ROS accumulation in idiophase is not only necessary for the normal production of lovastatin, but contributes to the transcriptional regulation of the biosynthetic genes, including the specific regulatory gene lovE.
2. ROS regulation could be mediated by oxidative-stress-response transcription factors: Yap1 might be acting as a negative regulator; while SrrA could play an important role in positively controlling lovastatin production.
IDENTIFICATION AND BIOACTIVE POTENTIAL OF ENDOPHYTIC FUNGI ISOLATED FROM MEDICINAL PLANT WITHANIA SOMNIFERA

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Background

Endophytes are microbes that colonize living, internal tissues of plant without causing any immediate of negative effect. Various studies have shown that endophytes are endowed with great potential for human welfare like having potential in the form of antibiotics, antiviral, anti asthmatic, antioxidant and anticancer compounds. Endophytes from medicinal plants have become a hot topic for metabolite discovery. There is still a lot of potential of endophytes which need to be discovered for pharmaceutical applications. Keeping these things in consideration, the aim of the present study was to isolate fungal endophytes from different accessions of medicinal plant Withania somnifera and to screen the endophytes for the production of various bioactive molecules.

Objectives

To isolate the endophytic microorganisms from Withania somnifera (Ashwagandha) & screen for antimicrobial activity against a wide range of pathogenic microorganisms

Methods

Isolation of endophytes suing the standard methodology published earlier

Conclusions

Out of 7 endophytes, four isolates were identified by ITS amplification and sequencing. All the four isolates showed significant similarity with Alternaria alternata, Alternaria tenuissima strain, Alternaria sp. and Alternaria compacta.

The isolated endophytes were also screened for antimicrobial activity against a wide range of pathogenic microorganisms and after analyzing the results some of the endophytes showed promising antimicrobial activities.

Our results confirm that the isolated endophytes could serve as an alternative source of various bioactive molecules of novel structure and function as endophytes are still largely unexplored.
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SECRETION AND FUNCTIONAL DISPLAY OF FUSION PROTEINS THROUGH THE CURLI BIOGENESIS PATHWAY
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Background
Curli are functional amyloids expressed as fibres on the surface of Enterobacteriaceae. Contrary to the protein misfolding events associated with pathogenic amyloidosis, curli are the result of a dedicated biosynthetic pathway. A specialized transporter in the outer membrane, CsgG, operates in conjunction with the two accessory proteins CsgE and CsgF to secrete curlin subunits to the extracellular surface, where they nucleate into cross-beta strand fibres.

Objectives
Here we investigate the substrate tolerance of the CsgG transporter and the capability of heterologous sequences to be built into curli fibres.

Methods
We genetically fused different proteins to the C-terminus of csgA.

Conclusions
Non-native polypeptides ranging up to at least 260 residues were exported when fused to the curli subunit CsgA. Secretion efficiency depended on the folding properties of the passenger sequences, with substrates exceeding an approximately 2 nm transverse diameter blocking passage through the transport channel. Secretion of smaller passengers was compatible with prior DsbA-mediated disulphide bridge formation in the fusion partner, indicating that CsgG is capable of translocating non-linear polypeptide stretches. Using fusions we further demonstrate the exported or secreted heterologous passenger proteins can attain their native, active fold, establishing curli biogenesis pathway as a platform for the secretion and surface display of small heterologous proteins.
Background

In the recent years, the applicability of actinomycete strains as probiotics for plants, serving as agents of biological control of plant diseases, has been actively investigated.

Objectives

415 strains of extremophile actinomycete strains from the unusual ecosystems of Kazakhstan.

Methods

Antifungal properties of extremophilic actinomycetes were studied against the fungal pathogens of grain cultures (genera *Fusarium* and *Aspergillus*) by agar diffusion method.

Conclusions

104 actinomycetes strains from the extreme ecosystems of Kazakhstan (solonchak, takirs and takir-type soils, solonetz and solod) showed antifungal activity against the fungal pathogens of grain cultures (*Fusarium sp.*, *Aspergillus niger*). 20 strains of extremophilic actinomycetes have been selected, capable of growing at a low content of nutrients in the medium, in the presence of high salt concentrations (∋ 5%) and pH level (∋9.0). They have the ability to inhibit the growth of phytopathogenic fungi (diameter of inhibition zone 20-30 mm) in the different environmental conditions - neutral, in the presence of high NaCl concentrations (5% or more), and high pH values (9-11). This practically valuable feature is especially important for the plant cultivation since many biopreparations developed for neutral ecological niches become useless after salinization or alkalization of the soil. Scientific novelty of the research results lies in the use of biotechnological potential of extremophilic actinomycetes in the development of biological preparations for plant cultivation under various environmental conditions.
Background

A major challenge in the sustainable production of biofuels and biochemicals is efficient enzymatic conversion of plant biomass into monomeric sugars. Most enzyme mixtures are currently produced by a small selection of fungal species (e.g. *Trichoderma reesei*, *Aspergillus niger*). However, the fungal kingdom holds many more fungal species which produce enzyme mixtures with beneficial characteristics such as high (hemi-)cellulase activity and high thermostability. *Sordariales* is one of the few fungal orders with thermophilic isolates, of which many have been associated with the production of thermostable enzymes.

Objectives

The aim of our study is to assess the diversity within *Sordariales* for efficient plant biomass degradation.

Methods

Phylogenetic analysis and growth analysis revealed that optimal growth temperature is a polyphyletic trait within *Sordariales* with separate mesophilic, thermostolerant and thermophilic clades. Four thermophilic clades were clearly distinguished: *Myceliophthora* species, *Thielavia terrestris*, *Chaetomium thermophilum*, and *Mycothermus thermophilus*. Thermophiles within *Myceliophthora* showed the most potential as efficient plant biomass degraders. Especially *M. heterothallica* had good growth on a large range of substrate and was able to produce offspring with a large physiological and genetic variety. Crossing and selection strategies were used to further improve *M. heterothallica* in degrading biomasses such as sugarbeet pulp and spruce. The mechanisms behind improved biomass saccharification were understood by proteomics and genome analysis.

Conclusions
This study showed the strategic strength of combining fungal diversity with specific selection strategies to find enzyme mixtures with interesting industrial properties.
Background

Biotransformation is substrate transformation to desired product by using suitable kind of biocatalysts. Nowadays, it is an alternative tool for the development sustainable technologies for the production of chemicals and drugs. Aminophosphonates are analogues of amino acids in which a carboxylic moiety is replaced by phosphonic functionality or related groups. They are compounds with stable carbon to phosphorus bond and have wide range of biological activities and variety of applications in industry [Żymańczyk-Duda, Phosphorus, Sulfur and Silicon, 2008, 183; Mucha A., J. Med.Chem. 2011, 54]. Biosynthesis of chiral, organophosphorous compounds is still not fully explored field of science.

Objectives

The aim of work was selection of the whole-cell biocatalysts with hydrolytic activity for preparation of phosphonate analogues of aspartic acid. Thus, biocatalytic methods, which allowed obtaining derivatives of aminophosphonic acids via biohydrolysis of lactam ring were elaborated.

Methods

To obtain phosphonic analogue of aspartic acid via biohydrolysis of O,O-dimethyl-4-oxoazetidin-2-ylyphosphonate, whole cells of P. minioluteum were used as a biocatalyst. NMR spectra interpretation allowed postulating possible mechanism of presented microbial conversion as amide bond hydrolysis. Biohydrolysis was only achieved in deionized water as bioconversion medium. Application of another reaction media caused partial splitting of the substrate during biotransformation process. The separation process required reverse phase chromatography to obtain product from biotransformation mixture.

Conclusions

Presented method is simple and quite efficient. Application of P.minioluteum as a biocatalyst resulted in production of desired product with moderate yield. It means, that process is a good starting point for further scaling up the process.
SELECT ANTIMICROBIAL PEPTIDES (AMPS) AS BACTERICIDAL AGENTS FOR EX VIVO STORED HUMAN PLATELETS
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Background
Bacterial contamination of \textit{ex vivo} stored human platelets and the transmittance of sepsis by platelet transfusion represent the highest infectious disease risk in transfusion medicine today.

Objectives
An alternative approach to detection of contaminated units is pathogen reduction technology that directly inactivates or prevents the replication of pathogens present in platelet products. We have developed a novel approach to pathogen reduction, one that promises to be more specific and less damaging to the platelet product and at the same time effective against 6 bacterial species common to transfusion of platelets.

Methods
The methodology is based on treatment of platelets with small synthetic peptides named PD1-PD4 derived from the thrombin-induced human platelet-associated antimicrobial proteins, and repeats of Arg-Trp (RW1-RW5), and their selected combinations. This treatment demonstrated microbicidal activity against \textit{Staphylococcus aureus, Staphylococcus epidermidis, Escherichia coli, Pseudomonas aeruginosa, Klebsiella pneumoniae} and \textit{Bacillus cereus} in the spiked platelet samples. We also demonstrated that AMP treated platelets maintain their \textit{in vitro} properties during 7 days of storage. Preclinical evaluation of peptides PD3, PD4, and RW2-RW5 demonstrated that these AMPs do not adversely affect the human platelet recovery and survival in a SCID mouse model. We have further shown that the peptides are not immunogenic in rabbits, and do not affect platelet performance in \textit{in vitro} tests.

Conclusions
The body of work presented here provides a proof of a concept that AMPs could be incorporated into a novel platelet-sparing pathogen reduction treatment and warrants further study.
Background
The Gram-negative β-proteobacterium Ralstonia eutropha H16 is primarily known for polyhydroxybutyrate (PHB) production and its ability to grow chemolithoautotrophically by using CO$_2$ and H$_2$ as sole carbon and energy sources. R. eutropha H16 has also attracted significant interest for its ability to metabolize heavy metals, to degrade a variety of chloraromatic compounds or chemically related pollutants.

Objectives
The use of R. eutropha H16 as a production organism under chemolithoautotrophic growth conditions is favored. However, inducible expression systems are yet required for gene expression in R. eutropha H16. The aim of this work was to identify inducible expression systems for the use in R. eutropha H16 facilitating gene expression.

Methods
A family of efficient and highly stable plasmid expression vectors was created for the use in R. eutropha H16 by applying various biosynthetic methods.

Conclusions
The use of promoters derived from bacteriophage T5 was described in this work and hereby the J5 promoter proved to be the strongest promoter yet to be applied in R. eutropha H16. Moreover, the implementation of the RP4 partition sequence in plasmid design increased plasmid stability significantly and enables fermentations with marginal plasmid loss of recombinant R. eutropha H16 for at least 96 h. The utility of the new vector family in R. eutropha H16 is demonstrated by providing expression data with different model proteins and consequently further raise the value of this organism as cell factory for biotechnological applications including protein and metabolite production.
Background

Lignocellulosic plant material is degraded by anaerobic fungi (AF) with their rhizoid and various free and cellulosome-bound lignocellulolytic enzymes. AF could thus be used to improve biogas production from fiber-rich residues. AF were isolated from faeces of animals living on a high fiber diet. Screening them for the cellulolytic most active AF with conventional tests are labor intensive and time consuming, a quantitative Real-Time PCR (qPCR) routine for glycosyl hydrolase family 5 (GH5) endoglucanase should be developed as a suitable alternative.

Objectives

New anaerobic fungal isolates should be checked and compared for their cellulolytic potential by parallel determination of GH5 mRNA production and conventional parameters. Thus anaerobic fungi with outstanding cellulolytic abilities shall be identified.

Methods

To identify the best moment for mRNA extraction, growth curves of the isolates were recorded, and mRNA was extracted at several points. Additionally gas pressure was measured to see if mRNA production, growth and gas pressure developed accordingly. Reverse-transcribed cDNA was quantified in qPCR with primer system AF Endo designed to specifically detect GH5 endoglucanase of AF.

Conclusions

Novel AF isolates were achieved from a Cameroon sheep, a Kiang, an alpine goat and a domestic yak. In experiments carried out with two of the isolates the GH5 RT-qPCR system tested successfully. GH5 mRNA production was maximal after 18h with similarly developing growth, gas pressure and mRNA level. Further AF species are being tested for their cellulolytic activity. A future study shall compare gained results with the real life cellulolytic ability of tested AF.
Background
These days, there has been a great interest in lignin as one potential renewable source for conversion into chemicals and materials. Lignin is complex and amorphous polymers, therefore, it needs to be modified to meet properties required for industrial uses.
White rot fungi secreting ligninolytic enzymes are promising for enzymatic modification, because they can degrade lignin selectively and more rapidly than other microbes. Therefore, comprehending of biomodification process by white rot fungi is necessary for industrial application of microbial treated lignin.

Objectives
The biomodification of synthetic lignin by white rot fungus, *Phanerochaete chrysosporium*, was investigated in this study. The fungal secretomes and structural changes of the fungal treated synthetic lignin were examined.

Methods
Synthetic lignin used in this study was synthesized from coniferyl and sinapyl alcohol. This polymer, dehydrogenative polymer (DHP) was used as substrate to elucidate the mechanism of microbial modification of lignin.
Detailed structural analysis of lignin were conducted as follows : phenolic hydroxyl group of DHP was analyzed by aminolysis reaction, and the molecular weight and changes of ether bonds in DHP was analyzed by gel permeation chromatography and nitrobenzene oxidation method, respectively. For examining the effects of fungal secretomes in biomodification of synthetic lignin, protein contents by using protein chip and activities of ligninolytic enzymes were measured, respectively.

Conclusions
Consequently, this study is expected to help to understand the biomodification mechanism of synthetic lignin by *P. chrysosporium*, and to evaluate availability of *P. chrysosporium* in biomodification of lignin for value added application of lignin.
Background

*Ralstonia eutropha* is a Gram-negative, strictly respiratory facultative chemolithoautotrophic bacterium which can use H$_2$ and CO$_2$ as sole sources of energy and carbon in the absence of organic substrates. It has attracted great interest for its ability to degrade a large list of chloroaromatic compounds and chemically related pollutants. Furthermore it was already applied for the production of biodegradable polymer polyhydroxyalkanoates on an industrial scale. *R. eutropha* serves as a model organism for the mechanisms involved in the control of autotrophic carbon dioxide fixation, hydrogen oxidation and denitrification.

Objectives

We are interested in establishing specialized *R. eutropha* based cell factories by genetic engineering. The particular interest is constructing cells efficiently performing oxidoreductase reactions by overexpression of homologous and/or heterologous enzymes. One of the main types of oxidoreductase reactions is performed by dehydrogenases, which have a wide range of possible biotechnological applications.

Methods

A selection of alcohol dehydrogenases as well as short chain dehydrogenases of *R. eutropha* H16 was cloned and expressed in native versions in *E.coli* as well as in *R. eutropha* H16 in order to study basic functional capability of these enzymes. For each of the enzymes cofactor specificity and the substrate range, both for oxidation and reduction way was analysed in a photometric assay by the detection of NAD and thereby specific activity units were calculated.

Conclusions

Two of the tested enzymes showed high selectivity toward s-enantiomers for all tested secondary alcohols with NADP$^+$ as preferable cofactor. Furthermore a difference in enzyme activity depending on the expression host could be observed.
METHANOTROPH-CATALYZED BIOCONVERSION OF METHANE TO METHANOL

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Background

Methane has attracted much attention as the alternative chemical feedstock and conversion of methane to methanol has attracted enormous interest. Indirect chemical conversion of methane to methanol is associated with a number of problems including low energy efficiency and high capital cost.

Objectives

We investigated a batch methane-to-methanol conversion in high yield using Methylosinus trichosporium OB3b whole cells. Cultivation and conversion relevant parameters including air/methane ratio and copper concentration were optimized.

Methods

M. trichosporium OB3b was cultured in a 600 mL joint flask with rubber septum containing 200 mL NMS medium with supply of 7:3 air/methane ratio at 30 °C. Various chemical inhibitors for methanol dehydrogenase were screened and added to accumulate methanol. Sodium formate was added as a reducing power source to maintain methane monoxygenase activity.

Conclusions

In the presence of 100 mM potassium phosphate as MDH inhibitor, 0.4 g/L methanol was accumulated and more than 70% (mol methanol/mol methane) of methane was converted to methanol. Volumetric productivity of 0.049g/L/h was obtained in the batch reaction with M. trichosporium OB3b as the biocatalyst. Biocatalytic conversion using methanotrophic microorganism will offer an energy-efficient method in commercialization of methane-to-methanol.

References

OPTIMIZATION OF CULTURE CONDITIONS FOR L-ASPARAGINASE PRODUCTION BY HALOPHILIC BACTERIUM, VIBRIO SP STRAIN GBFX3
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Background

Increased amino acid requirement of malignant cells is exploited in metabolic antitumor therapy, e.g., enzymotherapies based on asparagine or glutamine deprivation. L-Asparaginase (L-Asparagine amidohydrolase; EC 3.5.1.1) which catalyzes the conversion of L-Asparagine to aspartic acid and ammonia is one of promising enzyme in pharmaceutical industries. A wide variety of microorganisms have L-asparaginase. Halophilic bacteria may contain L-asparaginase with novel immunological properties that can be used in hypersensitive patients because of modified surface structures.

Objectives

The main objective of this study was to optimize the culture conditions for L-asparaginase production by selected halophilic strain.

Methods

The effect of different concentrations of NaCl and glucose ranging from 0-10% and 0-1% respectively and the influence of different carbon sources such as saccharose, maltose, glucose and fructose on the enzyme production was evaluated by varying one factor at a time. The effect of pH (5–9) and temperature (25-35-45 °C) were also studied. After 72h incubation, the samples were collected by centrifugation for the determination of L-asparaginase activity by the nesslerization method.

Conclusions
A halophilc bacterium *Vibrio* sp. Strain GBFx3 which was isolated from Gomishan Lake at Golestan province of Iran was screened for L-asparaginase activity. The highest amount of L-asparaginase production was observed in 0.5% glucose or 0.5% saccharose as sole carbon source, 2.5% NaCl, 34°C and pH 8 which were 0.63, 0.5, 0.4 and 0.43 IU/ml respectively.
DEVELOPMENT OF A POLYFUNCTIONING PROLONGED-ACTION BIOLOGICAL PREPARATION FOR CROP PRODUCTION BASED ON THE RHIZOSPHERE BACTERIAL STRAINS WITH ANTIFUNGAL AND PLANT-GROWTH STIMULATING PROPERTIES

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Background
Associations of the rhizosphere bacterial strains belonging to the genera Pseudomonas and Azotobacter simultaneously possess both antifungal and plant-growth stimulating properties and are the basis for new trends in the development of biological preparations for crop production.

Objectives
Strains Pseudomonas R-7 and Azotobacter A-6 isolated from the rhizosphere of tomato crops cultivated in Kazakhstan.

Methods
Antagonistic properties of the bacteria were studied using agar diffusion method against the pathogens of fungal diseases in tomatoes of the genera Phytophthora, Fusarium, Alternaria, Cladosporium. Growth-stimulating properties of bacteria were studied by inoculation of tomato seeds with a culture liquid of bacteria with a cell titer of n x10⁹.

Conclusions
Strain Pseudomonas R-7 showed antagonism against phytopathogenic fungi: Phytophthora sp. - 19 mm, Fusarium sp. - 16 mm, Alternaria sp. - 23 mm, Cladosporium sp. - 18 mm. When using a strain Azotobacter A-6, the growth inhibition zone for Phytophthora sp. was 22 mm, Fusarium sp. - 24 mm, Alternaria sp. - 23 mm, Cladosporium sp. - 20 mm. Strain Azotobacter A-6 also enhanced the tomato germination capacity by 22.0% as compared with the control, increased the plant height by 15.5%, root length by 26.3%, plant mass by 15.0 %, and number of leaves by 33.3%.

A composition of the new biological preparation for crop production has been formulated based on the strains of rhizosphere bacteria Pseudomonas P-7 and Azotobacter A-6, compatibility of the strains under cultivation and storage examined, optimal ratio of bacterial microorganisms and soil extract in the combined culturing selected, ensuring the continued viability of cells within a scheduled storage period.
Background

Lipases catalyze esterification, interesterification and transesterification reactions and have gained serious attention for the production of sustainable biofuels, motivated by environmental concerns combined with the depletion of fossil fuels.

Objectives

The aim of this study was the isolation of new strains bearing high lipase activity in order to exploit their lipases in biofuel production.

Methods

42 environmental strains, isolated from sludge of a waste treatment facility (Psitallia, Greece), were screened for the presence of lipase activity, using a rhodamine B procedure. One isolate, identified as *Stenotrophomonas maltophilia* (named Psi-1) by means of IGS-based molecular taxonomy, was selected for further analysis. A 1.203 bp amplicon of Psi-1 corresponding to a putative secreted lipase gene (as revealed by bioinformatic analysis) was cloned and overexpressed in a pET29c - *E. coli* BL21(DE3) system. The recombinant protein was purified using Ni-NTA chromatography. Lipase activity was verified by in-gel assays (zymograms) and photometric biochemical assays.

Conclusions

A novel lipase gene from a new *Stenotrophomonas maltophilia* isolate was identified, cloned and overexpressed. The recombinant enzyme was proved to exhibit lipase activity and has a potential as a catalyst for biofuel production.

Acknowledgments
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Background

The use of enzymes as biocatalysts for the preparation of optically pure compounds is becoming a common method especially in the pharmaceutical industry [Nestl B.M., 2011, *Curr Opin Chem Biol*]. Obtaining enantiopure active compounds with a defined configuration appeared to be more desirable, not only because of effectiveness of such pharmaceuticals, but also because of safety requirements [Ogawa J., 2002, *Curr Opin Biotech*]. Immobilized whole-cells are one of the biocatalyst form that can be used in biotransformation processes. This system allows reusing biocatalyst, facilitates downstream processes and enhance the stability of the enzyme. Moreover, use of immobilized catalyst in flow reactor allows carrying out continuous processes.

Objectives

The aim of the study was the kinetic resolution of a racemic mixture of 2-butyryloxy-2-(ethoxy-P-phenylphosphinyl)acetic acid by biotransformation using immobilized whole-cell biocatalyst. *Penicillium oxalicum* has been reported as an active biocatalyst in the hydrolysis of tested compound in shake flask system [Serafin M., 2014, *Chemik*]. Optically pure products can find application as chiral discriminator and building block of defined absolute configuration in synthesis of biologically active compounds such as pharmaceuticals or pesticides.

Methods

The activity of biocatalyst, cells immobilized in two ways, was tested toward racemic mixture of starting compound. Products were analyzed by nuclear magnetic resonance spectroscopy $^{31}$P NMR with the addition of quinine as a chiral solvating agent.

Conclusions

Simple and effective method of whole-cell immobilization was elaborated. Activity of biocatalyst was confirmed by hydrolysis of tested compound with 50% of conversion.
degree and high enantiomeric excess. Developed system allowed to enlarged the scale of the process.
ITACONIC ACID PRODUCTION IN ASPERGILLUS NIGER

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Background
The filamentous fungus Aspergillus niger has a long tradition of safe use in industry in the production of enzymes and organic acids. Furthermore, it can grow on a wide range of substrates under various environmental conditions and has an optimized pathway towards producing organic acids, as indicated by its high production titers of citric acid in submerged fermentations (>300 g/L). These features make it an interesting host for the production of itaconic acid (IA), a high-value building block chemical with a broad application potential in the chemical industry. We have successfully identified the IA production pathway of Aspergillus terreus and engineered it into A. niger. The resulting A. niger strain is able to produce IA at low levels.

Objectives
In order to increase the yield we have tested various medium compositions and fermentation conditions. This resulted in an optimization of the fermentation medium and cultivation conditions, accompanied with an enhancement of yield and titer of IA production. Moreover, recently we have discovered a novel pathway found, to our knowledge, exclusively in A. niger that enables higher productivity, titer and yield of IA. This novel pathway provides a platform for further improvement of organic acid production in A. niger.

Methods
Fungal transformation and fermentation

Conclusions
This novel pathway enabled increased IA acid productivity, yield and titer.
Background
In a marine carbon cycle, the metabolic pathway of agar is not fully understood. Agar is a recalcitrant hetero-polysaccharide. Many agarases are known to fragment agar polymer into disaccharide unit, neoagarobiose, that is split into two monomeric sugars (D-galactose and 3,6-L-anhydrogalactose). Metabolic fate of 3,6-L-anhydrogalactose is still mysterious.

Objectives
We aim to elucidate full repertoire of agarolytic system in nature not only for understanding the marine carbon cycle but also for construct a synthetic agarolytic system for biomass utilization.

Methods
Using experimentally verified functional agarolytic genes as a probe, we searched all available microbial genomes in the public databases and collected all microbial genomes having potential agarolytic activity. In comparative genomics approach, we combined three distinct procedures such as ortholog prediction, gene cluster analysis and transcriptomic analysis (RNAseq) of three genomes, *Saccharophagus degradans*, *Marinimicrobium agarolyticum* and *Vibrio sp. EJY3*, we predicted potential genes related to agar metabolic pathways.

Conclusions
The predicted gene set from comparative genomics was divided into several functional categories: i) metabolic enzymes, ii) regulatory proteins (e.g. transcription factor), iii) signaling proteins and transmembrane proteins. We identified phylogenetically conserved genes and designated those genes as the core gene set. By functional examination, we revealed new genes essential for 3,6-anhydrogalactose and designed a synthetic E. coli system metabolizing agar as a sole carbon source.
Background

Optimized protein production in filamentous fungi requires the availability of fungal strains with low levels of secreted protease activity in order to improve secreted protein levels, including shelf life. Already for several decades research has been carried out to obtain these type of mutants, leading to the isolation of mutants with very favorable characteristics, one being a mutation in a transcriptional regulatory gene, *prtT* (e.g. Punt et al.);, 2008).

Objectives

Based on these results further improved strains have been developed using different selection approaches.

Methods

Controlled fermentation experiments with selected mutant strains revealed different improved characteristics, whereas full genome sequencing was carried out to identify the genetic basis of the mutant phenotypes. Identification of relevant protease-regulatory genes has also been carried out using collections of regulatory gene knock-out strains in *N. crassa* and *A. niger*.

Conclusions

Several examples of the use of selected mutant strains in our research to discover and produce novel hydrolytic enzymes will be presented.
Background
In recent years the direct process of ethanol production from lignocellulosic materials, has been considered greatly due to its efficiency and money-saving manner of manufacturing. Some *Clostridium* species such as *C. thermocellum* sp. and *C. cellulolyticum* sp. has received many attentions because of their extracellular enzyme production ability.

Objectives
The ultimate goal of present study is evaluating the ethanol production ability of *C. cellulolyticum* from untreated bagasse and delignified bagasse as cellulose source. The ability of *C. cellulolyticum* H10 for direct consumption of cellulose, eliminate the hydrolysis processes and lowered the end cost of ethanol production and also decreased the environmental threats of the existing processes.

Methods
In this study the bacterial strain was cultured in modified CM3 culture medium in anaerobic condition and after 20 days the produced ethanol was determined by gas chromatography, then cellulose was replaced with untreated bagasse and the ethanol production was examined. Untreated bagasse was delignified with three different pretreatment methods which include sodium hydroxide, sodium chlorite and acetic/nitric acid and the delignified outputs of all these delignification methods were used as carbon sources for bacterial growth and ethanol production were determined.

Conclusions
Acetic/nitric acid delignification methods showed the highest delignification value with 86.7% lignin content decrease and the highest ethanol per cellulose yield with values of 0.19 (gr ethanol/gr cellulose).
LOW CYTOSOLIC PH AS A SIGNAL FOR CELL SURVIVAL IN H2SO4 STRESSED CELLS
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Background
In the process of bioethanol production yeast biomass is recycled using treatment with dilute sulfuric acid to control the bacterial population. This treatment can lead to loss of cell viability, with consequences on the fermentation yield.

Objectives
To understand and ideally enhance yeast cell survival in these conditions, we analyzed the functional cellular responses of S. cerevisiae to inorganic acid stress.

Methods
Was analyzed growth and intracellular pH (pHi) in low pH conditions, using the pH-sensitive GFP derivative ratiometric pHluorin expressed in the cytoplasm of the strains.

Conclusions
S. cerevisiae mutants in cell wall integrity (CWI) and calcium signaling have reduced growth and viability at a low external pH (pHex) of 2.5. WT yeast showed evident defect in growth only at a pHex of 1.5, showing that a functional cell wall biogenesis is crucial for survival. To our surprise, the CWI mutant showed enhanced viability at pHex of 1.5 compared to pHex 2.5. While at a pHex of 2.5 the pHi was unaffected compared to pHex 5.0, at pHex 1.5 the pHi of a CWI mutant was reduced. This reduction by itself did not lead to loss of viability. Rather, artificial reduction of pHi at pHex 2.5 rescued the CWI and Ca²⁺ signaling mutants. Also, the lethal effect of low pHex on CWI and Ca²⁺ mutants takes place only in growing cells. However, a lowered pHi leads to a reduction in growth rate, and thus protects the cells from death. Likely, cytosolic pH is a signal that directs the growth-stress tolerance trade-off in yeast.
Background
Bacteria must acquire iron by competing with environmental chelation. One mechanism for bacterial iron acquisition utilizes siderophores. These molecules are small-molecule chelating agents that have been isolated from many microorganisms. As a current study, siderophore is used to deliver an antibiotic payload, thorough production of siderophore linked antibiotics e.g., salmycins.

Objectives
In this study we aimed siderophore production from soil bacteria and detection of their antimicrobial activity.

Methods
Bacteria was isolated from soil samples. For detection of siderophore existence in these bacteria, Cas agar medium was used. Identification of siderophore producer bacteria were used 16s rRNA analysis. The crude siderophore was produced and extracted from different soil bacteria. Antimicrobial effect of siderophore was determined on several pathogens (Candida albicans ATCC 10231, Pseudomonas aeruginosa 27853, E.coli 25922, Staphylococcus auerus 25923, Bacillus subtilis 6633, Proteus mirabilis, Enterobacter aerogenes).

Conclusions
As a result of study, we were observed that siderophore from soil bacteria have antimicrobial effect on pathogen microorganisms. Nowadays, these studies are taught as a new method against the growing antibiotic resistance. Mimicry of Iron-uptake dependent mechanism will can be new approach in medicine.
Background
Solvent tolerant strains of the ubiquitous soil bacterium Pseudomonas putida are increasingly important for a wide range of industrial biotechnology applications. Because of its remarkable solvent and stress tolerance and its energy efficiency, P.putida S12 has been described as an important strain for production of value added aromatic compounds and for efficient whole cell biotransformation of HMF to FDCA (1, 2, 3, 4).

Objectives
We have undertaken detailed genome sequence analysis to identify and understand the background of solvent tolerance and energy efficiency in P.putida S12.

Methods
Whole genome sequencing was performed through Illumina HiSeq and PacBio RSII sequencing. Assembly was performed using the CLC Genomic Workbench (BLASR, SSPACE-LongRead scaffolder, Gapfiller version 1.10) and manual closure of remaining gaps. The completed genome sequence was subject to automated annotation by the NCBI Prokaryotic Genome Annotation Pipeline with manual annotation of prominent Pseudomonas features.

Conclusions
The full P.putida S12 genome consists of a 5.8 Mb circular genome and a 557 kb circular megaplasmid. Both the genome and the megaplasmid contain various mobile elements among which multiple copies of the ISS12-type repeat sequence (5). Location of ISS12 repeats and number variation in various strains indicate a role in acquisition and maintenance of solvent tolerance. In addition, gene composition of the megaplasmid indicates an essential role in instigation of solvent tolerance and resistance to other stress conditions. Further comparative analysis of the S12 genome will be presented together with functional analysis of predicted gene and
metabolic functions.

References

Background

In an earlier work we found a ROS build up during lovastatin production phase, which coincided with sod1 gene (oxidative stress defense enzyme) down regulation. Later, we showed that ROS regulate lovastatin biosynthetic genes. The mechanism by which ROS induce these genes is unknown, but it is considered to be through oxidative-stress-response transcription factor(s). Expression profiles suggested that A. terreus Yap1 is a candidate, although probably as a negative regulator. These findings could be used to design new genetic improvement methods.

Objectives

Study the role of Yap1 in the control of ROS and in lovastatin biosynthesis, in liquid submerged fermentation (SmF) and in solid-state fermentation (SSF). Also investigate if the perturbation in the ROS profile (caused by silencing yap1) can increase lovastatin production.

Methods

Silencing vector was constructed by ligation of a fragment of At yap1 gene to pGdpPki-RNAi vector. A. terreus TUB F-514 transformants were characterized. Lovastatin was quantified by HPLC, and ROS concentration by diclorofluoroesein and gene expression by Northen Blot.

Conclusions

1) yap1 silencing in A. terreus also caused decreased expression of gene sod1.

2) ROS build up began before schedule, reaching higher levels than in the parental.
Hence,

3) Gene brlA (regulator of conidiation) expressed earlier, and reached higher spore densities.

4) Also, gene lovE (lovastatin genes specific transcription factor) expression and lovastatin production started earlier and reached higher production levels than the parental in both culture systems. yap1-silenced mutants displayed lovastatin production increases of: 60% in SmF and 70% in SSF.
Background

The limited supply and the negative environmental effects of the use of petroleum-derived fuels and chemicals have stimulated efforts for the development of more environmentally-friendly processes.

Objectives

The bacterial fermentation of carbohydrates is a promising way for the production of green chemicals and biofuels. My project aims at improving the efficiency of the anaerobic conversion of sugars by Clostridium species into C3 alcohols, namely isopropanol.

Methods

Genome sequencing and fermentation performance studies were carried out on multiple Clostridia strains including the natural Isopropanol-Butanol-Ethanol producing strain Clostridium beijerinckii DSM 6423 (NRRL B593). This strain was then used to generate, through N-methyl-N′-nitro-N-nitrosoguanidine (NTG) mutagenesis, 36 strains with altered fermentation profiles. The selective growth was performed in the presence of 3 suicide substrates or a high concentration of isopropanol (> 35g/L). Further improvement of this first set of mutated strains was achieved through genome shuffling which uses recursive protoplast fusion and offers the advantage of entire genome recombination (Gao, Zhao, Zhang, He, & Jin, 2012). Screening of the final strains was based on enhanced isopropanol tolerance, the best of them being able to tolerate concentrations of up to 50g/L.

Conclusions
Some improvement in isopropanol production compared to the wild type strain was attained with strains showing higher solvent yields and/or better selectivity. The best *Clostridium* strains obtained produce less butanol and more isopropanol allowing a more efficient bioprocess.
EVALUATION OF OENOCCUS OENI EXOPOLYSACCHARIDES AS ENDogeneous PROTECTIVE AGENTS FOR MALOLACTIC STARTER PRODUCTION

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Background

The malolactic fermentation (FML) is an essential winemaking step driven by lactic acid bacteria. It takes place after the alcoholic fermentation and mainly consists in the conversion of L-malic to L-lactic acid, with subsequent improvement of the wine microbial stability, aroma and flavor. Oenococcus oeni is the most suitable species for driving FML in wine. O. oeni may come from the indigenous microflora found on grapes or in the cellar, or it may be massively added to wine as a malolactic starter. O. oeni malolactic starters are currently selected for their resistance to wine and their ability to produce polysaccharides is so far not considered. However, this property could be crucial for the protection of the bacteria during starter production and use.

Objectives

Our main objective was to examine whether the stimulation of the production of exopolysaccharides by the bacteria in situ before freeze drying could protect the bacteria and improve the survival rates towards productions steps but also during inoculation in wine.

Methods

The genome sequencing of 50 O. oeni strains permitted us to realize the inventory of the exopolysaccharides genes (RAST, Kaas). The exopolysaccharides analysis included the quantification (colorimetric and analytical methods) and phenotypic observation (optical and electron microscopy) of the produced exopolysaccharides. The production of O. oeni strains in a lyophilized form and their later inoculation in wine (freeze-drying, cells viability).

Conclusions
We observed that the tested strains showed specific survival behaviors, which were perfectly correlated with their EPS biosynthetic ability. The protecting role of the bacterial exopolysaccharides is now to be proposed at industrial level.
Background

Background: The second generation ethanol using agro-industrial waste is a promising alternative biofuel but the challenge is a technological approach to deconstruction the recalcitrant lignocellulose.

Objectives

Objective: The present work aimed to study the effect of pre-treatment of sugar cane bagasse with microwave radiation in the presence of glycerol on chemical composition and the efficiency of subsequent enzymatic hydrolysis.

Methods

Bagasse immersed in glycerol 10% were subjected to 2 min of microwave irradiation and the solid fractions resulting were used in the analysis of fibers, TGA, DTG, DSC, FTIR, X-ray and transmission electron microscopy and the liquid fractions were used to determine sugar and phenol contents. Samples of bagasse treated and untreated (control) were submitted to enzymatic hydrolysis for 24 to 72 h at 55 °C with enzymatic solutions obtained by the cultivation of M. thermophila M.7.7.

Conclusions

Infrared spectra and thermal analysis showed that pre-treatment acted on the lignin and hemicellulose of bagasse. The hydrolysis with the enzyme produced by M. thermophila supplemented with β-glucosidase, afforded 74.0 mg/g and after 72 h the highest yield of reducing sugars was 240.9 mg/g, with β-glucosidase or not. The pre-treatment with microwave and glycerol improve in 40% the enzymatic hydrolyses of the cellulose.
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FUNGAL AND BACTERIAL CELLULOLYTIC ENZYMES TRCEL7B AND CFXYN11A ACTING IN SYNERGY DURING THE HYDROLYSIS OF SUGAR CANE BAGASSE

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Background

Enzymatic bioconversion of lignocellulose to sugar monomers is performed by cellulases and hemicellulases acting sinergically. Synergy among cellulases has been documented [1]; synergy between cellulases and accessory enzymes has been studied in cellulosome systems from Clostridium cellulovorans [2]. Trichoderma reesei and Cellulomonas flavigena are two no related cellulolytic organisms which produce enzymes with reported cellulase and xylanase activities, respectively[3,4]


Objectives

To evaluate synergy between two no complex enzymes, fungal cellulase (TrCel7B) and bacterial xylanase (CfXyn11A), in the sugar release of enzymatic hydrolysis of sugar cane bagasse.

Methods

Full length CfXyn11A xylanase from C. flavigena was expressed in E.coli [4]. Core Tr Cel7B was expressed in T.reesei [3]. Enzymatic bagasse hydrolysis was performed in equimolar ratios of cellulose(C) and xylanase(X): 100C,75C:25X,50C:50X,25C:75X, 100X. Release of sugar monomers was followed by HPLC after acid hydrolysis. Degree of synergy(D.S.) was quantified as the ratio of the specific activities of the enzymes in the hydrolysis mixtures divided by the sum of the individual specific activities at the concentration used.

Conclusions
Synergy between CfXyn11A and TrCel7b during bagasse hydrolysis was observed in glucose and xylose but not in arabinose release. The highest xylose and arabinose release observed at 12 h suggests a debraching role of the xylanase prior to cellulase hydrolysis occurring mainly after 24 h. However, a high D.S. observed in the 50C:50X at 12h, enforce the best performance of enzymes when they act together.
Glucose release

![Graph showing glucose release over time with different conditions represented by various lines and markers.](image-url)
Synergy xylose release 12h

[Graph showing data related to synergy xylose release 12h with U/micromol and degree of synergy indicated]
Background

Yarrowia lipolytica which has been extensively used as a model oleaginous yeast for producing heterologous proteins has capability to accumulate lipids (lipidbody, peroxisome etc) up to 50% of the DCW (dry cell weight). In addition, Y. lipolytica can be easily manipulated in gene deletion and insertion because it possesses a haploid genome and its genome sequence was completed and it is also known as GRAS (generally recognized as safe). Therefore, Y. lipolytica can be a high potential host strain in producing oleaginous (hydrophobic) and high value-added compounds used in nutraceutical and cosmetic materials which are hydrophobic and produced from plants by conventional extraction processes.

Objectives

In this study, we aim to construct basic and potential platform strain suitable for production of nutraceutical and cosmetic materials by using Y. lipolytica.

Methods

We used Y. lipolytica Po1g and constructed an ura3 auxotroph strain, Y. lipolytica (PolgDura3), in order to use URA3 blaster system which is a useful tool to carry out repeat genetic deletion and insertion on the genome. In addition, we constructed a Y. lipolytica (PolgDku70Dura3) in which Ku70 involved in non-homologous recombination was deleted to increase recombination efficiency in this strain.

Conclusions

We confirm that Y. lipolytica (PolgDku70Dura3) showed higher homologous recombination efficiency (>5 folds) than Y. lipolytica (PolgDura3). Next, we are introducing foreign genes involved in biosynthesis of some specific nutraceutical and cosmetic materials into Y. lipolytica (PolgDku70Dura3). This strain and genetic tool will be very useful to construct various customized strains for producing nutraceutical and cosmetic materials.
MODULATION OF OXIDATIVE FOLDING TO IMPROVE RECOMBINANT PROTEIN PRODUCTION IN THE YEAST SACCHAROMYCES CEREVISIAE

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Background
The yeast Saccharomyces cerevisiae is widely used in biotechnology for the production of both bulk chemicals, like ethanol and organic acids, and higher-value compounds such as recombinant proteins, like pro-insulin. Recombinant protein production in S. cerevisiae induces different stress responses, including oxidative stress response. This is especially the case when the overall protein folding rate and the oxidative folding rate, i.e. the formation of di-sulfide bonds, are not properly balanced (1).

Objectives
In the present study we aim to develop metabolic engineering strategies to increase recombinant protein production by specifically adjusting oxidative folding.

Methods
Two industrially relevant proteins were selected based on different overall folding rates: pro-insulin and alpha-amylase. The oxidative folding of these proteins was modulated by A) altering the expression levels of several key players in oxidative folding, e.g. the thiol oxidases Ero1 and Erv2, and B) changing the number of possible di-sulfide bonds, i.e. the number of cysteine residues present.

Conclusions
The effects of these modulations on reactive oxygen species (ROS) levels, unfolded protein response, oxidative state, protein titers and overall physiology are monitored under strictly controlled growth conditions.

Reference
PRODUCTION OF BIO-OIL BY OLEAGINOUS YEAST
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Background

The Dutch government is stimulating the transition to a more biobased economy. Microbial oil can be produced from biomass in a green and sustainable way without competing to food crops by using oleaginous microorganisms such as yeast, fungi, bacteria and microalgae. At the HAN BioCentre a unique robust oil producing yeast strain has been selected called HBC025. The strain grows on a broad range of C5 and C6 sugars. The oil is accumulated by HBC025 in the form of triacylglycerols (TAGs) containing predominantly oleic (18:1), linoleic (18:2) and palmitic (16:0) acid. The produced microbial oil can be used as biodiesel but also in other applications such as paint, roads, floors and cosmetics.

Objectives

Objective is the development of an economically feasible fermentation and extraction process for second generation oil production by HBC025.

Methods

A fed-batch fermentation process was developed on glucose as model substrate. Hydrolyzed paper pulp was obtained from Parenco (Renkum, The Netherlands). Extraction methods tested were homogenization and enzyme assisted auto-lysis.

Conclusions

Maximum productivity on glucose as substrate was 0.5 g bio-oil/l/h, which is comparable to the best lipid producing strains in literature (Ageitos et al. 2011). HBC025 was also able to grow on hydrolyzed paper pulp as substrate with the same growth rate. Calculations showed that the process can be economically feasible only in case components from the yeast biomass are also marketed besides the yeast oil.

References
Background

The methylotrophic *Pichia pastoris* yeast expression system is widely used for production of heterologous proteins. However, till now morphological changes have not been reported in *P. pastoris*. This study reports for the first time, a comprehensive characterization of Granulocyte colony stimulating factor (GCSF) expression in filamentous *P. pastoris*.

Objectives

To investigate the morphological changes in *P. pastoris* during protein production phase.

Methods

Mut* transformants, containing the GCSF-cDNA fused with modified kex2 cleavage site (in the α-factor secretion signal) under the control of the alcohol oxidase 1 promoter, were used in the study. The transformants were cultivated in BMGY (buffered glycerol complex medium) followed by BMMY (buffered methanol complex medium). Extracellular protein was quantitated by gel densitometry from 20-fold concentrated culture supernatant. A combination of methanol level and feeding strategy was developed to increase productivity of GCSF at fermenter level.

Observation:

Reversible Pseudohyphae was observed, resulting in increased surface to volume ratio with an increase in extracellular protein relative to the normal phenotype of spherical cells at same cell dry weight.
Conclusions

*P. pastoris* initiates a striking developmental transition to filamentous form, more so in the Mut* phenotype, that results in more surface to volume ratio, and affects productivity of the culture. To the best of our knowledge, data presented here opens the possibility of a filamentous form of *P. pastoris* for production of heterologous proteins.
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HIGH-LEVEL PRODUCTION OF MONO-COMPONENT AND ENZYME MIXTURES IN MYCELIOPHTHORA THERMOPHILA
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Background

The fungus Myceliophthora thermophila C1 was developed into an efficient and versatile platform for high-level production of industrially relevant enzymes.

Objectives

Through strain development strategies, two strain lineages were obtained that are currently being developed and exploited as production hosts. One lineage (HC) is able to produce and secrete high amounts of enzyme mixtures that contain large amounts of (hemi-) cellulases. The other lineage (LC) is impaired in its cellulase producing capability, resulting in low background-protein production. The LC strain has been further developed for high-level enzyme production. Total protein levels up to 80 g/L has been reached of which ~ 80% correlates to the introduced enzyme.

Methods

By transforming the LC strain with selected C1 genes, a wide collection of strains was obtained, each of which produced mainly one enzyme. This has led to an enzyme library of over 100 functional enzymes of which many have been purified and characterized. The LC strain can also be designed and constructed in such a way that it produces only those enzymes that are functional under dedicated application conditions.

Conclusions

In conclusion, M. thermophila was developed into a high-level protein-production platform. The HC strain is successfully applied to produce enzymes for the production of biofuels and biobased-chemicals. The LC strain is being used to produce single enzymes and defined combinations of enzymes. The obtained C1-enzyme library is a rich source for academic and industrial research. The properties of M. thermophila C1
make this fungus a highly suitable alternative for traditional fungal protein production hosts.
NEW φBT1 SITE-SPECIFIC INTEGRATIVE VECTORS WITH NEUTRAL PHENOTYPE IN STREPTOMYCES

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Background
Integrative plasmids are one of the best options to introduce genes in low-copy-number and a stable form into Streptomyces, but they generate mutations in their integration sites. Phage (PHI)C31, the most common integrative site used in Streptomyces, integrates at different positions (attB and pseudo-attB sites) in the Streptomyces genome generating different mutations and phenotypes. The less common integration site (PHI)BT1, integrates at a unique attB site localized in gene SCO4848 (S. coelicolor genome) or their homologues in other streptomycetes.

Objectives
Constructing an integrative plasmid with neutral phenotype.

Methods
Four plasmids were created modifying the φBT1 integrative vector pMS82: pNG1, in which the SCO4849 was introduced under the control of the promoter region of SCO4849 to restore the phenotype generated by the integration of the plasmid; pNG2, in which the promoter PermE* was introduced into pNG1 to facilitate heterologous gene expression; pNG3 in which the bla gene for ampicillin resistance was included into pNG1 in order to facilitate selection in E. coli; and pNG4, in which the bla gene was introduced into pNG2.

Conclusions
The plasmids pNG1, pNG2, pNG3, and pNG4, are the only integrative vectors designed to produce a neutral phenotype when they are integrated in streptomycetes.
THE INFLUENCE OF LACTIC ACID BACTERIA ADDITIVE ON THE LEVEL OF RESIDUAL WATER-SOLUBLE CARBOHYDRATES IN CORN SILAGE

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Background
Ensiling is a high moisture crop conservation method based on the fermentation of water soluble carbohydrates (WSC) guided by epiphytic microflora, specifically by lactic acid bacteria (LAB). LAB additives (inoculants) in ensiling are used for promotion of optimal fermentation and rapid pH drop thus improving dominant nutrients preservation. However, the impact of inoculation on the level of residual WSC, which in high levels may result in silages aerobic deterioration, is not clear.

Objectives
During the present study, the application of LAB inoculant on residual WSC was investigated.

Methods
The three different yellow corn hybrid (Bc 418b, Bc 678 and Bc exp 6) produced in the same production conditions (31,01 – 38,52% DM) were ensiled in five replications in laboratory scale silos with and without Sil-All®4×4 LAB inoculant in a concentration 1x10⁷ CFU/g of fresh material. The silages were sampled on the 21st and the 60th day. The contents of lactic acid, WSC and pH were monitored.

Conclusions
The analyses showed that the inoculated silages had a significant increase (P<0,05) of lactic acid concentrations (g/kg DM basic) both on the 21st (31,45) and the 60th day (33,28) in contrast to silages without inoculant (21st 22,49; 60th 26,38). The same pattern was observed with pH. At the end of ensiling the difference in WSC content between silage with (8,63 g/kg DM) and without inoculant (7,39 g/kg DM) was not statistically significant (P>0,05). Inoculation of silages with Sil-All®4×4 stimulates lactic acid production and rapid pH drop whereas has no influence on the level of residual water-soluble carbohydrates.
FUNCTIONAL ANALYSIS OF FILIPIN TAILORING GENES FROM STREPTOMYCES FILIPINENSIS

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Background

Streptomyces filipinensis is the industrial producer of filipin, a 28-membered non-glycosylated pentaene macrolide widely used for the detection and the quantitation of cholesterol in biological membranes.

Objectives

Identification of tailoring genes belonging to the biosynthetic cluster for the biosynthesis of filipin, and generation of engineered derivatives for pathway elucidation.

Methods

A region of 13778 base pairs of DNA from the S. filipinensis genome was isolated, sequenced, and characterized by gene replacement and complementation techniques.

Conclusions

This set of genes shows synteny with the homologous pte genes from the filipin cluster of S. avermitilis, and includes two cytochrome P450 monooxygenase encoding genes, filC and filD, which are proposed to catalyze specific hydroxylations of the macrolide ring at C26 and C1´ respectively. Gene deletion and complementation experiments provided evidence for their role during filipin III biosynthesis. Filipin III derivatives were accumulated by the recombinant mutants at high yield. These have been characterized by mass spectrometry following high-performance liquid chromatography purification thus revealing the post-polyketide steps during polyene biosynthesis. Two alternative routes lead to the formation of filipin III from the initial product of polyketide synthase chain assembly and cyclization filipin I, one trough filipin II, and the other one trough 1´-hydroxyfilipin I, both compounds being biologically active.
Background
Lactic acid bacteria (LAB) have been extensively used for thousands of years in food fermentation and nowadays in the industrial production of lactic acid and other metabolites.

Objectives
In this study, the biosynthetic potential of lactic acid bacteria was exploited for biosurfactant (BS) production.

Methods
66 Lactobacilli strains were screened for BS production when growing in different MRS-based media at static conditions. To determine BS production (extracellular and cell-bound), surface tension (ST) of supernatant and phosphate buffered saline (PBS) extract of the strains were measured by tensiometer. To determine critical micellar concentrations (CMC), PBS extracts of the strains remarkably reduced ST were diluted.

Pyrene solubilization assays were further done as a complementary assay to prove the presence of BSs. 1 mg of pyrene was distributed into glass test tubes and was subjected to supernatant and PBS extract of the strains in shaking conditions for 24h. The values were then compared with those of pyrene solubilization at the presence of rhamnolipid in different CMCs.

Conclusions
The results showed there is a significant potential for BS production among the strains screened and 9 out of 66 Lactobacilli strains reduced ST of PBS extract between 19 and 22 units. The values are close to the data reported by Moldes et al. 2007 for Lactobacillus pentosus CECET-4023 (20 units) and Gudina et al. 2011 for Lactobacillus paracasei spp. paracasei A20 (22 units).
Pyrene solubilization assay can be taken into account as a promising method to identify BS-producing strains.

Concentrations and characterization of BSs produced are under investigation.
CHARACTERIZATION OF SCO4439, A D-ALANYL-D-ALANINE CARBOXYPEPTIDASE INVOLVED IN SPORE CELL WALL MATURATION, SPORE RESISTANCE, GERMINATION AND MYCELIAL RESISTANCE TO VANCOMYCIN AND TEICOPLANIN

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Background

Streptomyces are model microbes with complex developmental cycles. The last advances in system biology methodologies allowed the identification of large datasheets of proteins and genes differentially expressed during development whose biological function remains, in many cases, unexplored.

Objectives

Characterization of the biological function of SCO4439, a developmentally associated D-alanyl-D-alanine carboxypeptidase (DD-CPase) overexpressed during the aerial mycelium and sporulation stages.

Methods

Strain: Streptomyces coelicolor M145
SCO4439::Tn5062 mutant was made according to Fernández-Martínez (2011). Antonie Van Leeuwenhoek 99:515-522.

Conclusions

1- SCO4439 is a multidomain protein harbouring a DD-CPase at its carboxyl end, and a putative cytosolic transcriptional regulator domain at the N-terminal end. Both domains are separated by a putative transmembrane region.
2- The DD-CPase activity was demonstrated 'in vitro' to have a preference for DAla-DAla peptides, and to be inhibited by penicillin G.
3- SCO4439 was demonstrated 'in vivo' to be involved in the resistance of the spores to heating and acid, in the spore swelling during germination, and in the resistance of the mycelium to glycopeptide antibiotics (vancomycin and teicoplanin).
4- The DD-CPase domain, together with the putative hydrophobic transmembrane region are highly conserved in Streptomyces, and the presence of both domains is essential to restore the phenotypes observed in the SCO4439::Tn5062 mutant. The conservation of the putative transcriptional regulator domain is much lower, and it is not necessary to restore the phenotypes detected in the SCO4439::Tn5062 mutant.
5- SCO4439 controls PG cross-linking releasing DAla from peptidoglycan lateral chains and reducing the substrate for the transpeptidation.
6- The level of peptidoglycan cross-linking is regulating spore resistance and germination.
AN EFFICIENT FED-BATCH FERMENTATION PROCESS: THE PRODUCTION OF STREPTOKINASE FROM E. COLI
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Background
Streptokinase is a therapeutic protein drug of bacterial origin. Its role in thrombolytic therapy has been well established. It is either produced from \textit{Streptococcus}, its natural producer or from \textit{E. coli} by recombinant means. The production from the former, however, is problematic due to low yield and simultaneous production of toxic endolytic chemicals. At IMTECH, the gene encoding for streptokinase from \textit{Streptococcus equisimilis} was cloned and expressed in \textit{E. coli} BL21 (DE3). Initially the expression in the latter was very low, hence the coding sequence was appropriately modified using molecular biology approaches and as a result of such optimizations, a high expression level of upto 80 mg l\textsuperscript{-1} was obtained in shake flask cultivation.

Objectives
The main objective of this study was to develop a bioprocess that could be used for mass scale production and purification of this high value therapeutic protein.

Methods
To achieve the above objective, a fed-batch fermentation process was developed and optimized. Cultivation parameters like dissolved oxygen concentration (DOC), pH, media constituents and feed rate were carefully optimized and product yield was monitored.

Conclusions
The process optimization resulted in an intracellular expression level of 1120 mg l\textsuperscript{-1} in high cell density fermentation. This represented a 14-fold increase in production levels from flask to bio-reactor. Also the specific expression level \textit{i.e.} gram protein produced per gram wet cell mass remained unchanged. This yield is the highest reported so far for recombinant Streptokinase.
Background
This research investigates the mechanism of lignocellulose degradation by brown rot fungi and potential industrial applications of this system. The brown rot non-enzymatic degradative mechanism is largely unexplored for use in biomimetic industrial/biorefinery applications and understanding the degradative mechanisms of brown rot fungi will lead to better methods of addressing the recalcitrance problems associated with lignin and crystalline cellulose in the pretreatment of biomass in biorefinery applications. A chelator mediated Fenton (CMF) system has evolved to substitute for components of the cellulolytic enzyme machinery in the brown rot fungi, generating an alternative efficient mechanism for depolymerization of biomass. The CMF system is unique among biological systems in being the only reported substrate deconstruction system based on oxygen radical chemistry that permits non-enzymatic deconstruction at a considerable distance (several microns) from the organism.

Objectives
Specific objectives of this research include: 1) Examining the morphology of the fungal sheath in a brown rotted fungal-wood complex to determine how the sheath may aid in the movement of CMF components and enzymes; and 2) Analyzing the structure of lignocellulose in early and late-stages of brown rot fungal degradation to assess how the CMF system modifies the wood cell wall non-enzymatically.

Methods
This research utilized several different techniques including the ORNL Bio-SANS (small angle neutron scattering) beam line to obtain the mechanistic information necessary to better characterize processes involved in non-enzymatic biodegradation of lignocellulose by brown rot fungi.

Conclusions
This research supports a CMF model system being employed by brown rot fungal organisms in lignocellulose deconstruction.
CHARACTERIZATION OF 3-KETOSTEROID-9α-HYDROXYLASES IN RHODOCOCCUS RUBER STRAIN CHOL-4

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Background
Bacterial catabolism of steroid compounds provides a broad range of intermediaries needed for the synthesis of pharmaceutical steroid drugs such as 4-androsten-3,17-dione (AD) and 1,4-androstadiene-3,17-dione (ADD). Rhodococcus ruber strain Chol-4, isolated from a sewage sludge sample, is able to grow in minimal medium supplemented with steroids, showing a large catabolic capacity. The 3-Ketosteroid 9α-Hydroxylase (KSH) is a key enzyme in the general scheme of bacterial steroid catabolism; it initiates the opening of the steroid ring by the 9α-hydroxylation of the C9 carbon of 4-ene-3-oxosteroids (e.g. AD) or 1,4-diene-3-oxosteroids (e.g. ADD), transforming them into 9α-hydroxy-4-androsten-3,17-dione (e.g. 9OHAD) or 9α-hydroxy-1,4-androstadiene-3,17-dione (e.g. 9OHADD), respectively.

Objectives
Study and characterization of 3-ketosteroid-9α-hydroxylases in Rhodococcus ruber strain chol-4

Methods
The ORF finder and the pDRAW32 programs were used to detect the ORFs. Determination of KSHs induced or constitutive transcription, along with the co-transcription of each cluster, was made by RT-PCR. Mutagenesis of both single, double and triple kshA and kshB were made by unmarked gene deletion. Growth studies were analyzed on different substrates.

Conclusions
This work presents a set of data that clarifies KHAs specific roles in strain chol-4. We have characterized three different kshA and one kshB homologous ORFs in strain Chol-4. All of them present constitutive transcription and co-transcription. The mutants have shown differences in growing depending on the substrate.
BACKGROUND

Transposase is an enzyme that binds to the ends of a transposon and catalyzes the movement of the transposon to another part of the genome by a cut and paste mechanism or a replicative transposition mechanism. *Rhizobium* is a genus of Gram-negative soil bacteria, many of which fix nitrogen. Nitrogen fixation is an important part of the nitrogen cycle. Plants cannot use atmospheric nitrogen (N$_2$) they must use nitrogen compounds such as nitrates.

OBJECTIVES

The objective of this research was to determine whether at random transposon mutagenesis could be applied in genetic studies of *Rhizobium japonicum*. One major reason for this is that a range of defined mutations is not available.

METHODS

In present study *R. japonicum* at frequencies sufficient to allow the isolation of large numbers of insertion mutants. The selection of Tn5 mutants was facilitated by the expression, in all the *R. japonicum* strains we have tested, of the Tn5 encoded kanamycin resistance. A number of auxotrophic and symbiotically defective, single, random transposon (Tn5) mutants were obtained in three slow-growing strains of different DNA homology and serogroups and the single fast growing strain of *R. japonicum*.

CONCLUSIONS

In conclusion, the diversity of auxotrophs detected and the isolation of symbiotic mutants suggests that transposon Tn5 can be used as a generalized mutagen to isolate a variety of mutants with defects in symbiotic nitrogen fixation. The analysis of such mutants should prove to be useful in elucidating the biochemical, genetic, and regulatory events involved in the *R. japonicum* which effectively nodulates certain Indian soybean cultivars.
Background

*Ralstonia solanacearum* is one of the most destructive plant pathogens and causes bacterial wilt in about 450 plant species worldwide. During infection, the pathogen primarily colonizes plant vascular system in high density leading to wilt and eventually plant death. Expression of virulence factors by the pathogen is regulated by a quorum sensing system activated 3-hydroxy palmitic acid methyl ester (3OH-PAME).

Objectives

The objective of this study was to determine 3OH-PAME degradation by bacterial strains isolated from eggplant xylem and to determine their wilt prevention and growth promotion ability in eggplant.

Methods

Bacterial strains (n = 6) were earlier isolated from xylem sap of eggplant. Using a bioassay involving indicator *R. solanacearum* strain AW1-3 and High Performance Liquid Chromatography Mass Spectrometry, 3OH-PAME degradation by bacterial isolates was estimated. Strain XB174 degraded 3OH-PAME and was evaluated for wilt prevention and growth promotion in eggplant under greenhouse conditions. XB174 was identified by 16s rRNA gene sequencing and its activity against Tween-20, Tween-80 and tributyrin was determined.

Conclusions

Among the strains screened using bioassay, only XB174 degraded 3OH-PAME to 3OH-palmitic acid. Specific activity of crude extracellular enzyme of XB174 towards 3OH-PAME was 17.78 U mg⁻¹. XB174 prevented wilt (55.0 ± 7% over control) and improved growth (19.2 ± 10% more growth promotion efficacy over control) in eggplant. XB174 is identified as *Acinetobacter* sp., degraded Tween-20, Tween-80 and tributyrin.
The study reveals the quorum quenching activity of *Acinetobacter* strain XB174 against *R. solanacearum*. After additional screening, extracellular enzyme from XB174 may have applications in agriculture and industries.
Background

Quorum sensing is a cell to cell communication system that coordinates gene expression in many bacterial species. It has become an increasingly interesting target for the development of alternative drugs for treating multi-drug resistant \textit{Acinetobacter baumannii} infection. However, the role of quorum sensing system in \textit{A. baumannii} has not been characterized.

Objectives

The aim of this study is to characterize the functions of AbaR, a putative LuxR type receptor, using an isogenic mutant, and to shed some light on to the development of effective alternative drugs targeting AbaR.

Methods

The disruption of abaR in \textit{A. baumannii} lead to significant decrease in biofilm formation. In addition, the pellicle production and swarming motility of the abaR mutant was substantially decreased compared to that of wild-type. The abaR mutant complemented with the intact abaR gene reverted back to that of wild-type capable of producing biofilm and displaying swarming motility. The survival rate of mice infected with the abaR mutant strain was significantly high than that of those infected with the \textit{A. baumannii} wild-type. Also, whole transcriptome analysis was performed in order to compare the gene expression in wild-type and abaR mutant strains. Furthermore, chemical screening was carried out for the possible quorum quenching compounds targeting AbaR.

Conclusions

The data from the current study reveals that AbaR plays an important role in \textit{A. baumannii} pathogenesis and this regulator protein could be a putative target of quorum quenching compounds. Supported by KHIDI (grant number: HI14C0257)
Background

*Staphylococcus aureus* biofilm-associated infections are difficult to treat and novel targets are needed to combat these infections. The QS modulator hamamelitannin increases susceptibility of *S. aureus* biofilms towards vancomycin *in vitro* as well as *in vivo*. However, it is unclear how HAM affects susceptibility.

Objectives

Elucidate the mechanism of action of HAM at the molecular level.

Methods

Two parallel strategies were followed. First, we evaluated the effect of HAM on biofilms of *S. aureus* strains with mutations in the QS systems or in genes involved in biofilm formation and virulence. Secondly, using illumina sequencing we identified genes that were differentially expressed in untreated biofilms and biofilms treated with VAN alone or in combination with HAM. Results obtained with both strategies were further investigated using the appropriate tools.

Conclusions

No loss in HAM activity was observed for most of the mutants. In contrast, HAM did not affect biofilm susceptibility of *S. aureus* strains with mutations in the QS system. Using sequencing, we identified a large number of genes that were differentially regulated after treatment (e.g. genes involved in biosynthesis of lysine, D-alanine, glutamine consuming pathways and virulence). This indicates that HAM reduces the upregulation of peptidoglycan biosynthesis normally observed after treatment with VAN. This possibly leads to the increased susceptibility of *S. aureus* biofilm cells towards VAN. Our results further indicate that combination therapy could positively affect morbidity since the upregulation of virulence factors observed for VAN treatment are not observed when VAN is combined with HAM.
Background
MELiSSA (Micro-Ecological Life Support System Alternative) has been conceived as a 5 compartments microorganisms and higher plants recycling system for long haul space flights. *Rhodospirillum rubrum* S1H colonizes compartment II and grows under light anaerobic conditions (LAN) using acetate as carbon source (MELiSSA conditions). Previous work reported that continuous culture of the bacterium in a photobioreactor lead to thick biofilm formation, leading to bioreactor arrest.

Objectives
The aim of this research is to investigate the relation of the quorum sensing (QS) system and biofilm formation of *R. rubrum* S1H (wild type, WT) under MELiSSA relevant culture conditions.

Methods
In this context we have constructed a mutant strain named M68 that does not produce acyl homoserine lactones (AHLs) signaling molecules. The transcriptomic and proteomic profiles and phenotype of WT and M68 under MELiSSA conditions were compared. In addition, the biofilm development of WT and M68 in a flow cell system under light microaerobic conditions was studied.

Conclusions
*R. rubrum* has a cell-to-cell communication system based on AHLs which regulates 8% (326 genes) of the genome of M68. In *R. rubrum* QS regulates pigmentation, photosynthesis, energy generation, carbon metabolism, motility and biofilm formation. To our knowledge this is the first report where QS is linked to biofilm formation in *R. rubrum* S1H under light microaerobic conditions. Further flow cell experiments under MELiSSA conditions will help us to study biofilm formation and to test substances with known anti-biofouling properties.
Background

In addition to causing various infections, Acinetobacter species are described as nosocomical pathogens. These species are increasingly developing resistance against antibacterial agents due to their capability to adapt to any environment. Acinetobacter species develops these capabilities against their environment by quorum sensing (QS) mechanism which is a bacterial communication or ambient sensing mechanism. Quorum sensing systems needed for iron uptake comprising genes required for siderophore biosynthesis.

Objectives

In this work, effect of signal molecules on siderophore biosynthesis and its relation with QSS in MDR A. baumannii were studied.

Methods

Twenty strains of MDR A. baumannii from different clinical specimens were identified by standard methods. QSS molecules were identified by cross-validation test and siderophore biosynthesis was evaluated on CASagar and thin layer chromatography (TLC). AHL profiles were determined by using Agrobacterium tumefaciens biosensor strains (A136 or NTL1). QSS molecules combined with the biosensor strains were separated by TLC from each other.

Conclusions

According to results of the study, it was found out that all of the MDR A. baumannii strains produced the following miscellaneous QSS molecules in varying amounts: C8-HSL, C10-HSL and C12-HSL and these strains produced hydroxamate- and catechol-type siderophores. Iron uptaking in the environments is thought to be a direct relationship with quorum sensing system. The variations in the amounts and types of signal molecules were observed to affect virulence properties. QSS molecules can be used as target molecules in medical therapy. The study provides new information about the virulence factors for the better understanding of the infection properties.
of *A.baumannii* in the host.
Background
Quorum sensing molecules (QSM) are involved in the regulation of complicated processes helping bacterial population benefit from their cell-density. Prokaryotes and eukaryotes’ co-evolution raises the prospect of the existence of inter-kingdom signalling pathways. The role of hormone-like molecules in cell communication approves QS role in this cross-talk. Bacterial antagonistic activity against fungi is considered as an inter-kingdom talk. Bacillus and Pseudomonas have the ability to inhibit Aspergillus growth and aflatoxins production. Screening of antagonistic bacteria against Aspergillus flavus in vitro, Bacillus subtilis was identified with high antifungal activity.

Objectives
Bacillus licheniformis has industrial application due to its production of antimicrobial compounds and is related to B. subtilis genetically, whose control of competence-sporulation is regulated by a QS mechanism. QS in B. subtilis is regulated by ComX pheromone. As QS genes have been identified in B. licheniformis NCIMB-8874, our study has focused on QSM in this strain.

Methods
To investigate cell-cell communication, the comQX locus was sub-cloned into a shuttle vector which was expressed in E. coli and pheromone was isolated by reverse phase chromatography.

Conclusions
Pheromone as a QSM is potential signal for communicating between kingdoms and could be applied for bio-control purposes. Identification of new antifungal peptides against A. flavus could lead to the development of biotechnological strategies which facilitate control of aflatoxin contamination.
THE FUNCTIONAL UNITS OF QUORUM SENSING (QS) IN AMMONIA-OXIDIZING BACTERIA

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Background

Quorum sensing (QS) is an important form of cell-to-cell communication that regulates gene expression in response to fluctuations in cell density. One of the best-studied examples of quorum sensing is N-acyl homoserine lactones (AHLs)-mediated cell-cell communication in gram-negative bacteria. While it has been able to describe the QS system in some individual microorganisms for some time now, the functional QS system of nitrifying bacteria has not been completely proved to date.

Objectives

Testing the existence and characterization of quorum-sensing signal produced by nitrifying bacteria (like Nitrosomonas europaea, Nitrosospira multiformis), and the functional QS signal synthase, the cognate receptor responding to the synthase-generated signal in these microorganisms.

Methods

The AHLs product was obtained by extracting bacteria culture supernatants with acidified ethyl acetate, and then detected by AHLs biosensor and liquid chromatography-mass spectrometry (LC-MS). To identify gene(s) encoding AHL synthases and transcriptional activators, the genome of related nitrifying bacteria was searched for genes with similarity to all QS-related genes. AHL syntheses gene was expressed in the heterologous host E. coli BL21. Putative AHLs in the recombinant extract were confirmed by comparing the retention times and mass spectra from LC-MS with those of standard AHLs. As a test of the hypothesis that these AHLs were cognate signals of probable receptor, we also examined the solubility of the R protein.

Conclusions
We have shown a LuxI/R type QS signal synthase and regulator in an ammonia-oxidizing strain, and the results provide an opportunity to complete the QS regulatory networks.
Background

Burkholderia glumae, the causal agent of rice panicle blight, contains a single LuxI-R type quorum sensing (QS) system. TofI synthesizes N-octanoyl homoserine lactone (C8-HSL), which is recognized by TofR to regulate various private and public goods. The complex of TofR and C8-HSL activates expression of genes for toxoflavin and flagellar biosynthesis and an IclR type transcriptional regulator gene, qsmR. Among QS-dependent public goods, oxalate is produced to protect cells from ammonia-mediated alkaline toxicity in LB during stationary phase. RNAseq analysis indicated that expression of isocitrate lyase gene (aceA) might depend on QsmR.

Objectives
We aimed to confirm that glyoxylate cycle is controlled by QS and to determine roles of QS-dependent glyoxylate cycle in B. glumae.

Methods
To ascertain control of glyoxylate cycle by QS, we measured expression level of aceA and malate synthase (glcB) genes from the chromosomal Tn3-gusA fusions in each gene in the wild type and the QS mutants. Electrophoretic mobility shift assays were performed using the promoter regions of aceA and glcB and purified QsmR. AceA enzyme activity was determined by measuring the formation of glyoxylate-phenylhydrazone in the presence of phenylhydrazine and isocitrate at 324 nm. Levels of oxalate production of each strain were measured by using oxalate assay kit (Libios).

Conclusions
Glyoxylate cycle is controlled positively by QS and is important for oxalate biosynthesis in B. glumae.
INVOLVEMENT OF RALFURANONE PRODUCTION IN THE VIRULENCE OF RALSTONIA SOLANACEARUM OE1-1

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Background

Ralstonia solanacearum causes a destructive disease called “bacterial wilt” in numerous plant species. Its virulence is controlled by the transcriptional regulator PhcA, the activity of which is, in turn, regulated in a cell-density dependent manner, termed quorum sensing.

Objectives

We herein described the identification and characterization of ralfuranones J–L, new PhcA-regulated secondary metabolites, and the known derivatives, ralfuranones A and B, from R. solanacearum strain OE1-1.

Methods

Their structures were determined by spectroscopic and chemical methods. These ralfuranones were also detected in vascular exudates from host plants infected with OE1-1. Deletion of ralA, which encodes an enzyme for ralfuranone biosynthesis, reduced the virulence of OE1-1 in tomato plants. Virulence was restored by complementation of the ralA gene.

Conclusions

The results suggest that ralfuranones play important roles in the virulence of OE1-1.
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LOSS OF QUORUM SENSING SYSTEM CAUSES OUTER MEMBRANE VESICLE FORMATION IN BURKHOLDERIA GLUMAE
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Background
Burkholderia glumae possesses one LuxI-R type quorum sensing (QS) system. The QS system of B. glumae activates expression of oxalate biosynthesis genes to avoid ammonia-mediated alkaline toxicity as results of amino acid catabolism. However, QS mutants do not produce oxalate, which causes population crash in stationary phase.

Objectives
We aimed to address a question whether QS mutants experience envelop stress to cause vesicle formation.

Methods
Outer membrane vesicles (OMVs) were purified from QS mutants using density-gradient sedimentation techniques. Total proteins were identified from the OMVs by LC-MS/MS analyses. The membrane ultrastructure of QS mutants and their vesiculation were visualized by transmission electron microscopy (TEM) after ultrathin sections.

Conclusions
Total 368 vesicular proteins were identified from the OMV proteomes. Most vesicular proteins were found to be involved in molecular functions associated with transporter, protein secretion, and protein localization. TEM revealed that periplasmic space of QS mutants is swollen due to hyperhydration in exponential phase. Previous metabolome analyses showed that serious imbalance of glutamate in QS mutants, which suggests that QS mutants might experience a turgor pressure problem. We propose that OMVs of B. glumae might be induced to relieve turgor stress caused by imbalanced metabolism.
Background: The bacterial occupation of a surface can be associated with the production and realizing of compounds which can optimize and accelerate this process.

Methods: The exponential phase cells of *Staphylococcus epidermidis* strain GISK 33 were washed twice (0.85% NaCl) and diluted to $10^7$ CFU/ml (PBS, pH 7.2). The cell-free medium filtrates were obtained after the *S.epidermidis* sorption on a polystyrene and glass surface for 60 min. The effect of filtrates media on the bacterial cell adhesion was evaluated. In addition these filtrates were subjected to MALDI-TOF analysis and treatment by trypsin and proteinase K (100 mkg/ml, 37°C, 2 h) with followed by enzymes separation by membrane filtration (10 kDa).

Conclusions: The cell-free medium after *S.epidermidis* adhesion to the polystyrene had a stimulating effect on the adhesion of the same bacterial cells on the polystyrene surface compare to the filtrates after the bacterial contacting with the glass surface. The level of found by MALDI-TOF analysis compounds with low mol. weight 552, 574 and 596 Da in the filtrate from polystyrene which showed the greatest stimulatory effect on bacterial adhesion was higher than the glass filtrate. The peptide nature of these compounds was proved by the reduction of the stimulatory effect on the *S.epidermidis* 33 adhesion to the control level (PBS) after the treatment by proteases. Thus, the evidence of the possibility of autoregulation of *S.epidermidis* cell adhesion by using the low molecular weight peptide compounds was provided.

This work was supported by RFBR (14-04-00687).
Background
The enterotoxin produced by Clostridium perfringens is associated with the sporulation process. The regulation of this process is therefore of interest. We previously described the presence of a low MW, heat- stable compound in the culture fluids of this organism which stimulated the sporulation of homologous and heterologous strains, including enterotoxin-positive strains.

Objectives
Isolate and characterize the presumptive peptide involved in promoting sporulation of Clostridium perfringens

Methods
The peptide was isolated from a defined medium following Sephadex LH-2, SP Sephadex, DEAE Sephadex chromatography followed by HPLC using C8 and C18 columns. Mass spectroscopy, amino acid analysis, and N-terminal sequencing revealed a 1018 Da peptide composed of glutamic acid, glycine, alanine, and an unidentified residue. N-terminal sequencing suggests a branched chain or cyclic structure. The isolatee compound was effective in stimulation sporulation of C. perfringens in a dose-dependent manner at levels <2 nm/l and differs from sporulation-stimulating peptide previously identified from Bacillus subtilis and which was ineffective in promoting sporulation of C. perfringens.

Conclusions
We believe the peptide functions as a quorum-sensing molecule. The identification of a sporulation signal could help identifying the molecular events leading to sporulation and enterotoxin production by this organism.
Background
The discovery of new agent is demanded by the appearance of multidrug resistant bacteria, like super bacteria, for many years. Also, quorum sensing, cell-to-cell communication, is regarded as remarkable target for development of new antibiotics in recent

Objectives
In this study, we investigated antibacterial and anti-quorum sensing (anti-QS) capacity of dietary plants cultivated in Korea and their effect on formation of bacterial biofilms.

Methods
Inhibition of quorum sensing (QS) was investigated using the biosensor bacteria Chromobacterium violaceum and Pseudomonas aeruginosa PA01. The crude extracts of five plants were evaluated on antibacterial activity against five major food pathogen by bi-layer agar well method.

Conclusions
Especially, the extract of Zingiberaceae plant inhibited production of violet pigment in C. violaceum at 10mg/ml and the extracts of Punica granntum L. and Prune mume showed anti-QS activity against C. violaceum. Biofilm formation was measured by crystal violet and resazurin staining and the extract from Zingiberaceae plant had lower minimal inhibitory concentration than that of Citru junos and Punica granntum L. All of the extracts showed antibacterial activity against S. aureus, L. monocytogens, and B. cereus. The extract of Punica granntum L. also had high activity against E. coli and S. Enteritidis. The extract from Zingiberaceae plant had lower minimal inhibitory concentration (MIC) than that of Punica granntum L. against gram positive bacteria. The extract of Zingiberaceae plant had the lowest MIC of 50ug/ml against S. aureus and L. monocytogenes. These results exhibit the potential of five dietary plants to use as food preservatives and new antibacterial agents.
Background

The regulation of biofilm–formation in many pseudomonads is controlled by quorum sensing (QS) and is key to their ability to colonize different environments. However, in the soil and plant-associated \textit{P. fluorescens} SBW25, mutations in diguanylate cyclase-associated genes increase c-di-GMP levels to induce attachment and cellulose expression, resulting in the formation of biofilms at the air-liquid interface of static microcosms, and no involvement of QS-dependent behaviour has been reported.

Objectives

The objective of the study was to examine the ability of SBW25 to quorum sensing.

Methods
Bioinformatics, cultivation assays, TLC and CLSM were used.

Conclusions

A bioinformatics analysis of the SBW25 genome has identified putative N-acyl homoserine lactone (AHL) and α-hydroxy ketone (AHK)-dependent QS pathways, including an AHL/AHK synthase-like protein belonging to the HdtS family (PFLU0050) and a CqsA-like protein (PFLU5614). Furthermore, a putative link can be established between QS and the regulation of c-di-GMP levels in SBW25 based on the TpbA/TpbB system of *P. aeruginosa*. In this preliminary work, bioassays with AHL/AHK reporter strains overlaid onto TLC of liquid culture extracts demonstrate that SBW25 produces detectable levels of AHL and AHKs. Although these quorum compounds have yet to be identified, tests using exogenous dodecanoyl homoserine lactone suggest that in SBW25, biofilm structure, eDNA, and possibly siderophore production, may all be regulated by QS pathways. This work is the first to provide experimental proof that SBW25 is capable of responding to AHL/AHK quorum signals like many other pseudomonads, and to suggest that biofilm-formation may also be AHL-regulated under different environmental conditions.
CELL-CELL COMMUNICATION PROVIDES FITNESS BENEFITS TO POPULATIONS OF LISTERIA MONOCYTOGENES IN THE SOIL ENVIRONMENT

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Background

Listeria monocytogenes is a ubiquitous opportunistic human pathogen detected in many habitats spanning from the farm environment to food industry and the gastrointestinal tract.

Cell-cell communication participates to the adaptation of bacteria to their environment. In the species Listeria monocytogenes, the Agr system is required for full virulence and biofilm formation but its actual role is still poorly understood.

Objectives

To investigate whether the ability to communicate provides a benefit to L. monocytogenes in soil, a complex environment combining biotic and abiotic characteristics.

Methods

Deletion of the gene coding the response regulator or the signal propeptide. Population dynamics of the mutant and/or parental strains in soil microcosms. Both unsterilised and sterilised soils were investigated.

Conclusions

Deletion of the gene coding the regulator or the signal did not affect population dynamics in sterilized soil but survival was altered in biotic soil suggesting that the Agr system was involved to face the complex soil biotic environment. This was confirmed by co-incubation experiments. The fitness of the response negative mutant was lower either in the presence or absence of the parental strain but the fitness of the signal negative mutant depended on the strain with which it was coincubated. Survival of the signal negative mutant was higher when cocultured with the parental strain than when cocultured with the response negative mutant. These results showed that the ability to respond to communication provided a benefit to listerial cells. These results might also indicate that in soil, the Agr system controls private goods rather than public goods.
SCREENING OF QUORUM SENSING INHIBITORS WITH AN E. COLI BIOSENSOR AND ENHANCEMENT OF THEIR QUORUM QUENCHING ACTIVITY BY ENCAPSULATING THEM IN CHITOSAN-BASED NANOCAPSULES

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Background
The quorum sensing (QS) hypothesis explains a type of bacterial cell-cell communication which is mediated by means of autoinducer low molecular weight exocellular compounds. This phenomenon has profound implications in the control of many important bacterial traits (e.g. biofilm formation, secretion of virulence factors, etc.). A typical QS system in gram-negative bacterial involves the production and response to acylated homoserine lactone (AHL) signals that are recognized by the LuxR receptor. Several strategies have been conceived to block or disrupt QS (known as quorum quenching, QQ) and thus offer an alternative strategy to reduce the collective power of bacterial pathogens.

Objectives
In this regards, we have screened a library of total 24 purified compounds and have tested their QQ activity when applied at a concentration of 1mM.

Methods
To this end an E. coli Top 10 biosensor reporter of AHL-mediated QS which constitutively expresses green fluorescent protein (GFP) upon exogenous addition of 3-oxo-hexanoyl homoserine lactone (3OC₆HSL) has been used. A computer-based docking approach was used to elucidate in further detail the interaction between the hit compounds with the TraR protein 3D crystal structure.

Conclusions
The hit compounds (i.e. able to inhibit significantly the production of GFP but without inducing a toxic response) were vanillin, trans-cinnamaldehyde, caffeine, genipin, PQS (Pseudomonas quinolone signal) and MOQ (1H-2-methyl-4-quinolone). We have also tested the effect of loading the hit compounds into chitosan-based nanocapsules on their QQ activity and found that this is a strategy to further control and modulate their QQ bioactivity.
A LARGE SET OF GENES LINKED TO QUORUM SENSING-DEPENDENT REGULATION IDENTIFIED BY A GENOME WIDE TRANSCRIPTOME ANALYSIS OF SINORHIZOBIUM FREDII NGR234
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Background

The alphaproteobacterium Sinorhizobium fredii NGR234 is outstanding and unique among the rhizobia with its ability to form nitrogen-fixing nodules with a wide range of legumes (1). Beside many other striking features, its 6.9 Mbp genome encodes for two N-acyl-homoserine-lactone synthase genes (i.e. traI and ngrI) which are involved in the biosynthesis of two distinct autoinducer I-type molecules (2,3).

Objectives

Here we report on the construction of a NGR234-ΔtraI and a NGR234-Δngrl mutant and their genome wide transcriptome analysis. The high-resolution RNA-seq analysis of early stationary phase cultures in the NGR234-ΔtraI background suggested that up to 316 genes were differentially expressed in the NGR234-ΔtraI mutant vs. the parent strain and 466 in the background of NGR234-Δngrl vs. the parent strain. Accordingly, a common set of 186 genes was regulated by the TraI/R and Ngrl/R regulon including flagella biosynthesis genes and genes linked to EPS succinoglycan biosynthesis. Among the genes that were differentially regulated in NGR234-ΔtraI were those linked to replication of the pNGR234a symbiotic plasmid and cytochrome c oxidases. In the NGR234-Δngrl mutant biotin and pyrroloquinoline quinone biosynthesis genes were differentially expressed as well as the entire cluster of the NGR234 type III secretion system (T3SS-II). Further we also discovered that genes responsible for rhizopine catabolism in NGR234 were strongly repressed in the presence of high levels of N-acyl-homoserine-lactones.

Methods

high-resolution RNA-seq analysis

Conclusions
Together with nodulation assays, our RNA-seq-based findings suggested that QS-dependent gene regulation appears to be of higher relevance during non-symbiotic growth rather than for life within root nodules.
REGULATORY RNA ArISES FROM 3´-UTR OF AHL SENSOR ENCODED RNA AFTER ITS PREPROCESSING IN PECTOBACTERIUM ATROSEPTICUM
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Background
In a broad group of proteobacteria two quorum sensing-related genes, which encode synthase of autoinducers and their sensor, respectively, have convergent topology and overlap by their 3´-ends. It was suggested that the expression of one gene may antagonize the transcription of another one because of the convergent arrangement of these genes.

Objectives
We proposed that the topology of quorum sensing-related genes of plant pathogenic bacterium Pectobacterium atrosepticum, expI and expR, determines the mechanism of regulation of their expression.

Methods
We noticed that expR gene lacks any obvious transcriptional stop signals. Using chain-specific RT-qPCR we found that at the stationary growth phase expR mRNAs having long 3´-untranslated region (UTR) were formed and the expression of oppositely oriented expI gene was simultaneously decreased. It is likely, that RNA products of the expR regulatory gene may inhibit the expression of the autoinducer synthase gene through the interactions with expI mRNA. Using 3´-RACE method we found that at the stationary growth phase the extended expR transcripts underwent preprocessing resulting in their break down into two fragments. The first fragment encompassed the entire length of open reading frame (ORF) of expR gene and the second one included only a long 3´-UTR having a predicted regulatory function.

Conclusions
We found that cis-encoded RNA may participate in the regulation of expression of two convergent functionally related genes. The length of one of two transcripts varies respective to the cell growth phase affecting the expression of oppositely oriented gene. Moreover, cis-encoded regulatory RNAs arises from protein encoded RNAs that undergo preprocessing in their 3´-UTR.
PROTEOME PROFILING OF BURKHOLDERIA PSEUDOMALLEI QUORUM SENSING MUTANTS

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Background
Burkholderia pseudomallei, the causative agent of melioidosis, is a gram-negative soil bacterium in tropical areas. B. pseudomallei employs several N-acyl-homoserine lactone (AHL)-mediated quorum sensing (QS) systems, which activate specific sets of genes as a function of cell density [1]. The genome of B. pseudomallei encodes genes for three QS systems with one luxI and one luxR homologue, respectively, and additionally three orphan luxR homologues [2]. The luxI homologues encode AHL synthases, which produce specific AHLS binding to the respective transcriptional regulator and thus regulating expression of specific genes involved in virulence like biofilm formation, siderophore biosynthesis or swarming motility [3].

Objectives
The aim was to clarify the influence of the different QS systems on virulence factor expression.

Methods
We constructed mutants in the synthase-coding genes. Subproteome fractions of the mutant strains were subjected to mass spectrometry analysis to identify targets of the different QS systems. We used the DIA approach IMS² in combination with the Hi3 approach for quantification of cytosolic proteins and the GeLC MS/MS approach for the analysis of extracellular proteins. Furthermore, the AHL spectrum, synthesized by the different QS systems was determined by analytical thin layer chromatography.

Conclusions
The luxI homologues influence protein expression by up- and downregulation of AHL-dependent proteins in the QS circuitry of B. pseudomallei.

ENZYMATIC PRODUCTION OF MODIFIED STARCHES WITH SLOW DIGESTION PROPERTIES
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Background

In human nutrition, the proper rate of glucose release and absorption from digesting starch may play important roles in body health. Therefore, improving food quality with higher amounts of slow digestible starch is becoming an area of interest for researchers in academia and industry.

Objectives

The objectives of this study is to produce the modified starch with slow digestion properties by starch-modifying enzymes.

Methods

Two branching enzymes (BEs), PH1386 of \textit{Pyrococcus horikoshii} OT3 and Atha_0558 of \textit{Caldicellulosiruptor bescii}, were expressed in \textit{E. coli} and purified by Ni-NTA affinity chromatography. The enzymatic properties of two BEs have been studied by high performance anionic exchange chromatography (HPAEC) analysis. The melting temperature range (Tr) and melting enthalpy (ΔH) representing the degree of retrogradation of modified starches were also studied by differential scanning calorimetry. Finally, the digestion properties of modified starches were examined by pancreatic enzyme and α-glucosidase.

Conclusions

HPAEC analysis of BE-modified starches revealed that PH1386 produces the branch chains with mainly DP 10-12, while Atha_0558 prefers DP 6-7. The proportion of A chains (DP ≤12) of amylopectin was increased and the proportion of B1 (DP 13-36) and B2 chains (DP ≥37) was decreased compared to control. The Tr and ΔH also decreased compared to control, suggesting that retrogradation rate of the BE-modified starches was delayed. The increased resistant starch contents and
decreased rapidly digestible starch contents of BE-modified starches are thought to be due to the increase in α-1,6 linkages and the highly branched structure of starch.
MAXIMIZING BIOTECHNOLOGICAL PRODUCTION OF 3-HYDROXYPROPIONALDEHYDE AS A NATURAL FOOD PRESERVATIVE WHILE MINIMIZING ITS CONVERSION TO TOXIC ACROLEIN

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Background

3-hydroxypropionaldehyde (3-HPA) has strong antimicrobial activity and application potential as a natural food preservative for disinfection of minimally processed vegetables. During glycerol metabolism, Lactobacillus reuteri DSM 20016T can accumulate 3-HPA, which undergoes reversible dimerization and hydration, and can be further converted enzymatically into inactive 1,3-propanediol (1,3-PD), restoring intracellular redox potential. Dehydration of 3-HPA resulting in acrolein, a toxic metabolite, is also possible, however, conditions promoting this transformation have not been fully understood due to the lack of analytical methods to simultaneously quantify 3-HPA as well as acrolein.

Objectives

The aim of this study was to maximize the capacity for biotechnological processing of glycerol to 3-HPA using L. reuteri, while accounting for the accumulation of acrolein after production and storage to evaluate toxicological risk.

Methods

Strategies to improve 3-HPA production included the addition of redox-active compounds during glycerol fermentation to prevent conversion to 1,3-PD, the usage of immobilized cells resulting in high densities of viable cells as well as the optimization of process parameters in batch and fed-batch processes. Special attention was paid to cultivation conditions of L. reuteri prior to glycerol conversion. Acrolein and 3-HPA were simultaneously quantified using a newly established analytical method employing ion-chromatography with pulsed amperometric detection.

Conclusions

Redox-active compounds, process conditions and pre-treatment of L. reuteri cultures influenced 3-HPA yield as well as ratio of 3-HPA to 1,3-PD. Temperature and pH
optimization prevented formation of acrolein during biotechnological production and storage, promoting the safety of 3-HPA when applied as natural food preservative.
COMPARATIVE STUDY OF FERMENTATION PROCESSES BY FREE AND IMMOBILIZED GRAPEVINE INDIGENOUS STRAINS FOR THE PRODUCTION OF HIGH QUALITY WINES

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Background
Vinification is a complex microbiological process. Immobilized microorganisms constitute an alternative approach aiming to lower the overall cost of production and improve wine quality.

Objectives
This study focuses on the immobilization of indigenous Saccharomyces and non-Saccharomyces strains for use in wine production.

Methods
Saccharomyces cerevisiae strains are known for their ability to produce high ethanol levels, while non-Saccharomyces species contribute to the enhancement of the wine flavor resulting in high quality wines. Two indigenous environmental yeast strains isolated from a Greek agricultural region (Zitsa, Epirus), Saccharomyces cerevisiae Z622 and Metschnikowia pulcherrima var Zitsae ZY6 were used as starter cultures in a sequential fashion for wine fermentation. Delignified cellulose is used as an immobilization support meeting all requirements and demands for use in food production and wine making.

Conclusions
Both yeast strains were successfully immobilized maintaining their metabolic activity during fermentation. Immobilized cells showed a better overall metabolic activity compared to control free cells.

Acknowledgments
This project is co-financed by the European Union (European Regional Development Fund — ERDF), through the operational programs for “competitiveness and entrepreneurship” and regions in transition “Cooperation 2011 — Partnerships of Production and Research Institutions in Focused Research and Technology Sectors”, of the National Strategic Reference Framework (NSRF) 2007–2013, and the Hellenic Ministry of Education, Lifelong Learning and Religious Affairs — General Secretariat for Research and Technology
Background
Lactobacilli play an important role in the production of fermented sausages, due to their antimicrobial activity and their effect on the organoleptic properties of meat products. *Lb. sakei* is gaining relevance because it produces bacteriocins with anti-listerial activity. However, the use of bacteriocins may be limited due to their action against phylogenetically related species and ineffectiveness against some pathogenic and undesirable spoilage bacteria. Another group of proteins with antibacterial activity are peptidoglycan hydrolases, which so far have not been reported for this bacterial species and probably play a role in food safety and in the organoleptic properties of meat products; because they prevent the growth of pathogens and spoilage bacteria, including heterofermentative lactic acid bacteria (LAB).

Objectives
The aims of this work were to identify lactic acid bacteria isolated from fermented meat, as well as the proteins responsible for their extracellular antibacterial activity against different pathogenic and LAB strains.

Methods
Isolated lactobacilli were identified by 16S rDNA sequencing. Antimicrobial and lytic activities were evaluated in the culture supernatant by agar diffusion tests and zymography, using in both cases different target microorganisms. A 79-kDa protein was identified which showed antimicrobial activity. Protein identification was performed by LC/MALDI TOF/TOF and the data showed a high correspondence with a putative N-acetylmuramoyl-L-alanine amidase.

Conclusions
A *Lb. sakei* strain isolated from fermented meat which has displayed external antibacterial activity has been cultured and studied for the first time. The aforesaid activity is probably related to an protein with peptidoglycan hydrolase activity. This may have a potential use in food preservation, considering that this activity was obtained from a bacteria isolated from a meat product.
LEVANSUCRASE OF PSEUDOMonas SYRINGAE AS PRODUCER OF NOVEL PREBIOTIC FRUCTANS: A STRUCTURE-FUNCTION STUDY OF THE ENZYME

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Background

Gut microbiota has numerous roles in human life and its significance has thus far been underestimated. Therefore, more detailed studies on diversity and metabolism of gut microbiota are needed.

Objectives

Gut microbiota can be shaped by prebiotics. Levan-type (β 2,6-linked) fructans can be considered as potential prebiotics [1]. We have synthesized levan and levan-type fructooligosaccharides by heterologously expressed levansucrase Lsc3 of Pseudomonas syringae pv. tomato. These substrates should affect gut microbiota as were perfectly fermented by a gut generalist B. thetaiotaomicron [2]. Here we focus on structure-function relationships of the Lsc3 protein and its biotechnological potential.

Methods

The His-tagged Lsc3 protein was site-directedly mutated, biochemical methods were used to characterize the mutant proteins and 3D structure modelling was applied to interpret the results.

Conclusions

Levansucrase Lsc3 has high polymerizing activity (up to 80%) and extremely high stability confirming its biotechnological potential for the synthesis of fructans. Several novel catalysis-related positions for levansucrases were revealed and their predicted location on a 3D model of Lsc3 will be presented.

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References:


Background

*Lactococcus lactis* is industrially employed to manufacture fermented dairy products. Spray drying is the cheapest preservation method for *L. lactis* starter cultures, but during this process cultures encounter heat and oxidative stress, resulting in low survival. Viability of starter cultures is essential for their role in dairy acidification, supporting the need to understand and improve their robustness.

Objectives

We have measured the ability of 39 *L. lactis* strains to survive industrially relevant stresses. This set of strains contained different *L. lactis* strains from dairy as well as plant origin. The observed 4-log variation in heat and oxidative stress survival was compared with genomic content, resulting in the identification of genes associated with robustness. Presence and activity of genes can play an important role in the observed diversity in robustness.

Methods

Therefore, four *L. lactis* strains with varying robustness phenotypes were fermented under twelve different conditions, varying in temperature, salt concentration, pH, and oxygen level. Cells were harvested at exponential phase of growth for transcriptome analysis and survival measurements.

Conclusions

The varying growth conditions resulted in up to 4-log differences in robustness towards heat and oxidative stress. Moreover, clear differences in gene expression profiles were observed. Correlation of robustness phenotypes and gene expression levels revealed transcriptome signatures for oxidative and heat stress survival. For strain MG1363 this included the *metC-cysK* operon, involved in methionine and cysteine metabolism, which triggered us to grow this strain in the absence of
cysteine, resulting in elevated expression levels of the metC-cysK operon and concomitant enhanced robustness towards oxidative stress.
IMPROVEMENT OF SECRETORY EXPRESSION FOR THE SWEET-TASTING PROTEIN BRAZZEIN IN YEAST

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Background
Brazzein is an intensely sweet-tasting protein with four disulfide bridges. In previous study, we constructed the secretory expression system of recombinant brazzein in yeast Kluyveromyces lactis.

Objectives
In this expression system, the control of protein disulfide-bond formation in the endoplasmic reticulum (ER) is often a bottleneck for secretory protein production. The major pathway for protein disulfide-bond formation in endoplasmic reticulum (ER) includes the conserved ER-membrane protein Ero1p and protein disulfide isomerase (PDI).

Methods
In the present study, the PDI gene was introduced in the yeast Kluyveromyces lactis to improve the secretory efficiency of brazzein. The expression of Ero1p was also induced by treatment of the cells with dithiothreitol. The culture condition of the yeast transformants for high yield secretion of the recombinant des-pE1M-brazzein was in YPGal medium for 96 hours at 30 °C.

Conclusions
The amount of misfolded or unfold recombinant des-pE1M-brazzein remaining inside the cell decreased and the amount of the secretory recombinant des-pE1M-brazzein having an intrinsic sweetness increased approximately 1.7-fold than that of the previous expression system. These results demonstrate that the modified K. lactis expression system could be applicable to mass production of the recombinant brazzein with attributes useful in the food industry.
IDENTIFICATION OF SULPHITE RESISTANCE WINE YEASTS 
SACCHAROMYCES CEREVISIAE FROM A POOL OF SELECTED STRAINS
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Background

Sulphite is frequently added in winemaking, hence sulphite resistance mechanisms have been studied extensively in Saccharomyces cerevisiae. Research turned out that yeasts possess plasma membrane protein encoded by the SSU1 gene (lies on chromosome XVI), which excretes sulphite from yeast cells. Some highly sulphite-resistance wine strains have an SSU1 allele – SSU1-R - on chromosome VIII. We tried to elucidate, how long adaptation to high sulphite concentrations affects to the evolution of yeast strains.

Objectives

The aim of experiment was to determine presence of SSU1 and SSU1-R genes and their expression in selected Saccharomyces cerevisiae strains.

Methods

After 10 years of storage with SO₂ (concentration ~ 400 mg/ml) the pool of 12 strains S. cerevisiae, include 11 sulphite strains and negative control strain, were tested for the presence of the SSU1 or SSU1-R gene. We analysed them by using PCR assays, gel electrophoresis and DNA sequencing. Obtained sequences were aligned, in CLUSTAL W tool, with known sequences from databases (BLAST and SGD). Gene expression was tested during alcoholic fermentation for few strains by real-time PCR.

Conclusions

The presence of the SSU1 gene was confirmed in 7 strains (include control strain) and SSU1-R gene in 3 strains. None of those genes was detected in 2 strains. The results indicate that possession of the SSU1 or its allele is independent of the long term storage in must with SO₂. It is well-known that evolution in yeast cells occur dynamic. In our research it was confirmed by the presence of SSU1 in negative control strain.
Background:

Citric acid is the most important and widely used organic acid produced by fermentation. Mineral composition of the fermentation medium has a critical role in citric acid production.

Objectives:

The aim of this study was to investigate the influences of different minerals on growth and citric acid production of *Yarrowia lipolytica* NBRC 1658 in a glucose medium.

Methods:

The mineral salts; FeSO₄, CuSO₄, MnSO₄ and ZnSO₄ were added to the fermentation medium separately with concentration ranges of 0.01-0.1, 0.001-0.02, 0.005-0.05 and 0.002-0.008 g/L, respectively. Experiments were performed in a batch system. Biomass concentration was determined spectrophotometrically. Concentration of citric acid was measured by pyridine-acetic anhydride method.

Conclusions:

In this study it was obtained that by the addition of FeSO₄ into the media, the specific growth rate of the yeast increased and although CuSO₄ addition had a positive effect on the decrease of the duration of the lag phase, it had an adverse effect on the citric acid production as well. The maximum specific growth rate of the yeast determined as 0.053 h⁻¹ by using 0.02 g/L of MnSO₄ in the glucose based medium. Although ZnSO₄ supported the growth of the yeast, citric acid concentration reduced and reached to a maximum value of 13.4 g/L. It was also concluded that citric acid production decreased by the supplement of the salts examined within the concentration ranges above, while certain concentrations of FeSO₄, MnSO₄ and ZnSO₄ had profound effects on the growth of the yeast.
ENHANCEMENT OF THE BIOAVAILABILITY OF EXTRACTABLE BERRY PHENOLICS BY SOLID STATE FERMENTATION

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Background
Increasing interest can now be observed for the utilization of plant-wastes rich in extractable phenols derived from fruit processing. Using these residues, free phenolics with high antioxidant potential can be liberated by solid-state fermentation, via the deglycosylation action of beta-glucosidases of the fermenting fungi. Thus, the bioavailability of the extractable phenols can be enhanced since aglycons get across the cell membrane easily.

Objectives
In our previous studies, isolates of Rhizomucor miehei and Mucor corticolas showed intensive extracellular beta-glucosidase activity on plant-derived residues. Here, we analyzed the ability of these zygomycetes to generate free phenolics under jostaberry-based solid-state fermentation.

Methods
Solid pomace medium was supplemented with soy flour as nitrogen source. During incubation samples were taken every second or third day and extracted with distilled water, ethanol:water 50:50 or HCl:methanol 10:90 solutions. Besides beta-glucosidase activity, total phenolics and total anthocyanins were determined. Antioxidant activity tests including radical scavenging capacity and ferric reducing capacity assays were also performed.

Conclusions
The R. miehei and M. corticolas bioconversion of jostaberry pomace leads to enhanced total phenolic content. Positive correlation between total phenolic content and beta-glucosidase activity was found in both systems. Ferric reducing antioxidant power was slightly increased during the first phase of fermentation; however, anthocyanin content showed significant drop after 3-5 days. Results indicate that R. miehei and M. corticolas fermentations are applicable methods for liberation and enrichment of health-relevant extractable phenolics from jostaberry pomace. This research was supported by the Hungarian Research Fund (OTKA PD 112234).
INFLUENCE OF STARTER INOCULUM ON DYNAMIC GROWTH OF STREPTOVERTICILLIUM MOBARAENSE IN O3 MEDIUM.

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Background

Streptoverticillium mobaraense KKP 2013 strain obtained from the Culture Collection of Industrial Microorganisms is Gram-positive Actinomycetes commonly occurring in natural environment. Under certain conditions, it has the ability to produce the enzyme MTG, protein-glutamine gamma-glutamyltransferase, EC 2.3.2.13, commonly called transglutaminase. Transglutaminase catalyzes the formation of a covalent bond between the proteins, it allows also crosslinking reactions, namely the production of new intra- or intermolecular bonds between the proteins and peptides. MTG due to its characteristics is widely used in the production of meats, dairy and bakery products.

Objectives

In the process for producing transglutaminase one of the important steps is to obtain an appropriate number of microorganisms, which allows to obtain the highest possible quantity of active enzyme.

Methods

In our study, we tried to determine the growth rate of strain KKP 2013 S. mobaraense in the proliferating medium O3. In the precise number of microorganisms on the breeding stage, we used the method of pour plate technique on PCA medium, according to PN-EN ISO 4833-1: 2013-2014.

Conclusions

We established the optimal volume of medium in relation to the number of bacteria in the starting inoculum, which will produce the highest activity of the resulting enzyme.
BACKGROUND

While inimical food preservation approaches often result in heavily damaged and stressed populations of foodborne pathogens and spoilage microorganisms, very little is known about the actual molecular and genetic events and dynamics that govern the (sub)lethal injury and subsequent resuscitation or death of these cells. Nevertheless, insights into these phenomena might be decisive for a proper understanding of the resulting behavior and evolvability of the surviving subpopulation.

OBJECTIVES

In this study, we set out to monitor and dissect (sub)lethal injury and resuscitation phenomena in populations and cells of *Escherichia coli* that are stressed by heat or high hydrostatic pressure treatment.

METHODS

Directed evolution, genetics and time-lapse fluorescence microscopy.

CONCLUSIONS

Based on adaptive mutations, genetics and live cell biology, different subcellular processes and structures have been delineated and monitored that play a role in the heterogeneous injury and resuscitation dynamics of high temperature or pressure stressed *E. coli* cells and populations, thereby revealing a surprising impact on the spatial dynamics of nucleoids and protein aggregation inside stressed cells.
INHIBITION OF S. AUREUS A, B, C AND D ENTEROTOXINS BY SUBINHIBITORY DOES OF TERPENOID PHENOLS FROM PLANTS ESSENTIAL OILS

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Background

*Staphylococcus aureus* is a pathogen of major concern for clinical infection and foodborne illness and has remarkable invasiveness and toxin-mediated virulence. Studies have shown that plant essential oils can influence production of toxins, avoiding overuse of antibiotics and the development of bacterial resistance.

Objectives

This study aimed to verify the influence of major terpenoid phenols compounds from plants essential oils in subinhibitory doses on enterotoxins A, B, C and D produced by *Staphylococcus aureus*.

Methods

Five phenolic compounds (eugenol, geraniol, cinnamaldehyde, citronellol and terpineol) were tested. Amounts corresponding to 60% and 80% of Minimum Inhibitory Concentration (MIC⁹₀) were added to culture medium Tryptic Soy Broth (TSB). Each treatment was inoculated with a suspension of *S. aureus* producer of each enterotoxin A, B, C and D and incubated (37°C/24h). TSB without compounds was used as positive control. After incubation each treatment was centrifuged (9000g/4°C/30min) and supernatant was used to detect enterotoxin production by Reverse Passive Latex Agglutination. Interpretation of results was performed according to manufacturer's instructions kit. Agglutination was characterized as: strong (+++); moderate (++); weak (+) and absence (-) of enterotoxin production. Assays were performed in duplicate. Kruskal-Wallis nonparametric test was performed using scores related to enterotoxin production.

Conclusions

Some phenolic compounds from plants essential oils tested demonstrated great capacity to inhibit enterotoxins produced by *S. aureus* and, consequently decreasing its virulence. Therefore, it may be a promising way to prevent diseases caused by enterotoxigenic *S. aureus* and to contribute for reduction of antimicrobial drugs consumption.
CHARACTERIZATION OF THE DAIRY STREPTOCOCCUS THERMOPHILUS ACA-DC 29 STRAIN THROUGH COMPARATIVE GENOMICS

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Background
Although the Streptococcus genus includes mainly pathogenic species, Streptococcus thermophilus is a widely used dairy starter culture of great economic importance for the food industry. S. thermophilus has been adapted to milk probably through a degenerative evolution process that has led to the loss of typical streptococcal pathogenic traits.

Objectives
The genome sequence of the yogurt isolate S. thermophilus ACA-DC 29 was analyzed for assessing the technological potential of this strain. Comparative genomics analysis was also performed between the genome of ACA-DC 29 and the existing complete genome sequences of S. thermophilus.

Methods
The genome sequence of ACA-DC 29 was annotated using online annotation tools. Full chromosome alignments were calculated with Progressive Mauve. The pangenome, the core genome and the unique genes were predicted with the GView Server. The genomic islands, the CRISPRs and the antimicrobial peptides were predicted with IslandViewer, CRISPRcompar and BAGEL3, respectively.

Conclusions
The analysis of the S. thermophilus ACA-DC 29 genome sequence revealed the absence of pathogenic features. Genes related to the adaptation to milk were identified. Full chromosome alignments showed a high degree of synteny among the different strains. The pangenome of the eight strains comprised of approximately 2,300 genes. Concerning the ACA-DC 29 strain, approximately 250 unique genes involved in various biological processes were also identified. Further analysis indicated that several of them may have been acquired through horizontal gene transfer. Five potential antimicrobial peptides and two CRISPR systems, which may confer resistance against phages, were also predicted.
GREEK TRADITIONAL DAIRY AND MEAT PRODUCTS: A BIOLOGICAL RESERVOIR FOR NEW PROBIOTIC STRAINS

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Background

Probiotics provide health benefits to the host upon ingestion. Lactic acid bacteria are frequently used as probiotics. Important qualifying characteristics of probiotics strains are the acid and bile resistance, the absence of pathogenic traits, the elevated adhesion ability, the production of antimicrobial substances and the inhibition of pathogens. Traditional fermented foods constitute a promising source for discovering new probiotic strains.

Objectives

Lactic acid bacteria isolated from Greek traditional dairy (Yogurt and Feta, Kasseri, Xynotyri, Graviera, Kopanisti, Formaella, Galotyri, Kefalotyri cheeses) and meat (Lountza and Salami) products, were screened for their probiotic potential.

Methods

The isolated strains were evaluated for survival to low pH and in the presence of bile salts. The safety of selected strains was assessed by studying their haemolytic activity and their susceptibility to commonly used antibiotics. Antimicrobial activity against pathogens, was also examined. Finally, the ability of the strains to adhere to collagen-coated 96-well microplates and to the human colorectal adenocarcinoma cell lines HT-29 and Caco-2 in vitro, was investigated.

Conclusions

Of the 128 isolated strains, thirty were selected for further study due to their robust performance under the adverse conditions of the gastrointestinal tract. None of the strains exhibited haemolytic activity while variable antibiotic resistance was detected. Two Streptococcus thermophilus strains presented inhibitory activity against Streptococcus anginosus LMG 14502T and Streptococcus mutans LMG 14558T. Three Lactobacillus plantarum strains were found to adhere better to both cell lines,
as well as to the collagen-coated microplates. The utilization of *in vitro* tests enabled the selection of strains with promising probiotic features.
NATIONWIDE OUTBREAK OF LISTERIOSIS DUE TO READY-TO-EAT SALAD IN SWITZERLAND

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Background
Listeria monocytogenes is an important foodborne pathogen with a significant impact on public health and economy worldwide. Human infections are rare, but L. monocytogenes has the potential to cause serious and life-threatening disease. Clinical conditions primarily comprise septicemia, meningitis, meningoencephalitis and abortion.

Objectives
To describe a nationwide outbreak of listeriosis having occurred during 2013 to 2014 in Switzerland.

Methods
Microbiological methods (serotyping, MLST, PFGE) and epidemiological investigations by telephone interviews were used.

Conclusions
From 26 October 2013 to 23 April 2014, 32 cases of listeriosis infected with an L. monocytogenes strain serovar 4b, sequence type 4 and belonging to a single distinct PFGE pulsotype were registered in patients from several cantons of Switzerland. L. monocytogenes was detected in blood (75%), CSF (16%), ascites (6%) and in joint fluid (3%) samples. By the end of March 2014, a retail company reported an L. monocytogenes contamination of ready-to-eat salads to the authorities after detecting the pathogen through its in-house routine quality control. Product and environmental samples collected during subsequent investigations yielded isolates, matching the outbreak strain, thus confirming that ready-to-eat salad from this company was most likely the outbreak source. The cause for the product contamination was related to a design-inherent hygienic problem of one specific product-feeding belt. Complementary patient interviews also identified ready-to-eat green salads bought at one retailer as the likely outbreak source.
Background
Listeria species are closely related to each other, however, only L. monocytogenes and L. ivanovii exhibit pathogenic features, (human cases of L. ivanovii infection being quite rare). Rapid, accurate identification of Listeria strains is essential for appropriate management and timely intervention for infection control. The accuracy and speed of data acquisition by MALDI-TOF MS makes this an important tool for biological public health hazards, food processing, quality control, and disease diagnoses.

Objectives
Since Listeria species are closely related and have similar profile spectra, the objective was to reliably identify the different Listeria species by MALDI-TOF MS through establishment of a reliable library of mass spectral Listeria fingerprints from reference strains. Therefore the database was enhanced by 52 Listeria strain references, from 9 species.

Methods
Listeria strains were grown on Columbia Agar with 5 % Sheep Blood agar (BD, Heidelberg, Germany) at 37 °C. Biomass was collected after one day, and then processed by ethanol / formic acid standard extraction method. Mass spectra were acquired by using microflex LT (Bruker Daltonik, Bremen, Germany) and identified with MALDI Biotyper Compass software.

Conclusions
With the new, optimized references database, it was possible to identify all tested Listeria strains from 9 species. This was proven by analysis of more than 1200 mass spectra.

Although log (score) value differences were small between Listeria species, the correct species was always the best match, no false identification occurred. In consequence, the important identification of the pathogenic Listeria species is possible with easy, rapid and reliable low-cost MALDI-TOF MS method.
INCIDENCE OF ASPERGILLUS PRODUCER OF OCHRATOXIN A IN THE TROPICAL VITICULTURE IN THE NORTHEAST OF BRAZIL
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Background
The biodiversity of filamentous fungi is one of the most important contributions to the occurrence of mycotoxins in agricultural products. The species belonging to the genus Aspergillus has been blamed for the presence of mycotoxins in wines. The incidence of these genera in grapes will depend on climatic conditions, grape variety, cultivation form and agricultural practices.

Objectives
This study aimed to identify Aspergillus species isolated from wine grapes at harvest, from organic and conventional vineyards, in the São Francisco Valley.

Methods
Varieties evaluated were Touriga Nacional and Ruby Cabernet, and Tempranillo. For the isolation of fungi direct plating was carried out in DRBC culture medium, 25°C/7 days. The isolates were identified using standard culture media. The determination of toxigenic potential was performed by Thin Layer Chromatography.

Conclusions
There was no presence of Aspergillus in the Tempranillo. All A.carbonarius were producers of ochratoxin A. The presence of these species can spoil the grapes. All species identified are naturally present in the vineyards.
Background
The natural microbiological contamination of fruit and coffee beans, are due to the presence of bacteria, yeasts and filamentous fungi, including fungi species of the genus Aspergillus ochratoxin-producing (OTA) has great relevance for coffee security.

Objectives
The objective of this study was to identify ochratoxigenics fungi and quantify the presence of OTA in conventional and organic cultivation of coffee beans in the southern region of Minas Gerais (Brazil) and compare the concentrations in both systems.

Methods
We analyzed 30 samples of coffee beans (Coffea arabica L.), 20 samples of conventional coffee and 10 organic coffee samples. The incidence of OTA in the samples of coffee beans was performed by high performance liquid chromatography (HPLC).

Conclusions
Samples were identified 480 fungi of the genus Aspergillus Section Nigri and Circumdati. The main producing species Aspergillus ochraceus was OTA (83% of the samples). For the OTA in the grain, only one sample collected organic coffee ground, presented OTA, 1.12 ug / kg. Considering these results, it can be stated that the presence of Aspergillus ochraceus OTA producer does not imply contamination of grain by mycotoxins and two coffee cultivation systems have the same risk of contamination.
RATIONAL SELECTION OF YEASTS BASED ON THEIR PECTINOLYTIC ACTIVITIES AND ITS INCIDENCE ON TECHNOLOGICAL AND SENSORIAL ASPECTS OF WINE QUALITY

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Background

Pectinase enzymes have shown a considerable influence in both, sensitive and technological properties of wines. They can help to improve clarification and filterability process, releasing more color and flavor compounds entrapped in the grape skin and facilitating the liberation of phenolic compounds. The addition of commercial enzyme preparations, with filamentous fungi as its main source, can be quite expensive for industry.

Objectives

This work aims to find yeasts that, because of their native pectinases, can be applied on combined fermentations with *Saccharomyces cerevisiae* obtaining significant benefits over traditional wine fermentations.

Methods

785 yeast strains isolated from wineries were identified and tested for several enzymatic activities of recognized interest for enology industry. The impact of *Metschnikowia pulcherrima* as a source of pectinolytic enzymes during wine fermentation was analyzed by measuring its influence on filterability, turbidity and the increase on color, anthocyanin and polyphenol content of wines fermented in combination with *Saccharomyces cerevisiae*. Further metabolites with enological interest were analyzed during the entire fermentation period.

Conclusions

Positive results were obtained in all expected parameters when *M. pulcherrima* was used by comparing wines fermented with *S. cerevisiae* alone and combined with *Kluyveromyces thermotolerans*, even working better than commercial enzymes preparations in most parameters. Additionally, *M. pulcherrima* selected strain was used in a semi-industrial scale combined with three different *S. cerevisiae* strains, obtaining meaningful results on sensorial parameters like total polyphenol index, color intensity and turbidity of wines and on technological properties as wine filterability.
DETECTION AND TYPING OF LACTOBACILLUS PARABUCHNERI, A POTENTIAL SPOILAGE ORGANISM, IN MILK AND CHEESE

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Background

Histamine can be formed by microorganisms in fermented products such as cheese. High concentrations of this compound can trigger symptoms in consumers who have a histamine intolerance. Microbial decarboxylation of histidine is probably the major cause of histamine formation in cheese. We isolated histamine-producing bacteria from various raw-milk cheeses containing histamine levels higher than 50 mg kg⁻¹. Partial sequence analysis of the tuf gene showed that all these bacteria belong to Lactobacillus parabuchneri which is closely related to Lactobacillus buchneri, a bacterium associated with spoilage in plant-based fermented food and beverages but which is also used in silage fermentation.

Objectives

The aims were to develop a real-time PCR for the specific and quantitative detection of L. parabuchneri in milk and cheese as well as a genotyping methods for strain discrimination and biodiversity estimation.

Methods

We sequenced the genome of L. parabuchneri FAM21731 and twelve other strains.

The draft genome sequence was compared to publicly available genome sequences of L. buchneri and other related species to identify a gene sequence which seems to be unique for L. parabuchneri. Sequences were submitted to Tandem repeats finder and CRISPRFinder to assess hypervariable sequences and their potential for strain discrimination.

Conclusions

Results show that the real-time PCR system is specific for the detection and quantification of L. parabuchneri in dairy products and that the multiplex PCR based on hypervariable sequences can discriminate strains.
The use of these methods is of interest since it can be employed to locate potential sources of *L. parabuchneri* contamination on farms and in dairy plants.
DISCRIMINATION OF VIABILITY STATUS OF SALMONELLA TYPHIMURIUM CELLS INDUCED BY DISTINCT INACTIVATION METHODS USING A COMBINED FLOW CYTOMETRY, MALDI-TOF-MS AND MDA APPROACH

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Background
Food chain contamination with Salmonella Typhimurium is one of the leading causes of foodborne illness worldwide. Rapid and reliable methods for the detection of foodborne pathogens and also discrimination profiles concerning its cells viability status are deeply required and of high importance to assure foodborne pathogens control in the food chain.

Objectives
The goal of this study is to ascertain the Salmonella Typhimurium viability status (viable, viable non-culturable and non-viable) induced by distinct inactivation methods through a combined Flow Cytometry, MALDI-TOF-MS and multivariate data analysis (MDA) approach.

Methods
Different inactivation treatments, such as batch pasteurization (65°C; 30 minutes), sodium hypochlorite (5% or 0.006% commercial bleach), commercial available disinfectant solution (AMUKINA), ethanol (100%) and methanol (100%) were tested in Salmonella Typhimurium LT2. Cell viability assessment was evaluated by CFU’s counts and Flow Citometry (Accuri C6) with LIVE(TO)/DEAD(PI) dyes. In addition, footprint patterns of different viability states were obtained by MALDI-TOF-MS using HCCA as a matrix. Data were analyzed by MDA.

Conclusions
All the inactivation methods tested resulted in a total death of Salmonella Typhimurium LT2 population, with the exception of bleach (0.006%) and pasteurization, the latter used in industrial facilities. These treatments induced cells viable non-culturable state, which is of concern, since under favorable conditions Salmonella Typhimurium could restore viability and eventually its pathogenicity potential. Moreover, we obtained discriminatory peptide/proteins profiles associated with different viability states of Salmonella Typhimurium, which combined with MDA could support the use of this approach to detect microbiological contaminants applicable for quality control purposes.
FEMS-3129
Food microbiology

INFLUENCE OF RAW MILK’S THERMODURIC DETERIORATING MICROBIOTA IN THE PASTEURIZED MILK’S SHELF LIFE
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Background
The refrigerated storage of raw milk at the mill has advantages such as cost savings with the sample collection and reducing the loss of raw material. However, inadequate procedures during production and milk collection are considered the main sources of contamination by spoilage and pathogenic microorganisms, resulting in economic and public health problems.

Objectives
The work aims to enumerate the thermotolerant microbiota found in raw milk and in the milk after pasteurization, correlating it with the shelf life of the product.

Methods
Nine raw milk samples were collected directly from the extension tanks, nearby Castro – PR. After LT LT pasteurization, a portion of raw milk and pasteurized milk were diluted in peptone saline solution in the concentrations: integral, 10\textsuperscript{-1}, 10\textsuperscript{-2} and 10\textsuperscript{-3}, sown in depth amid PCA inverted and incubated at 35\textdegree C for 48 hours. Petrifilm plates ECTM for the enumeration of total coliforms and E. coli were also used. An aliquot was plated in depth for thermoduric counting in the same as described for the days when milk was pasteurized. The milk used for plating thermoduric was also inoculated in Petrifilm ECTM for controlling the progression of coliforms and E. coli.

Conclusions
Four samples showed a reduction in the count of thermoduric microbiota between pasteurization and 25 days of experiment. Two samples obtained less than one log cycle of microbial growth, while three samples showed growth of thermoduric microorganisms, more than one log cycle in the same sampling period.
FEMS-3133
Food microbiology

FENOLIC ACIDS EFFECTS ON PROBIOTIC BACTERIA GROWTH AND SURVIVAL
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Background
The consumption of probiotics for colonization and maintenance of the intestinal microbiota, regulation of immune mechanisms and decrease of lactose intolerance is strongly credited through data already obtained by investigation. It is also a widespread antioxidative activity of phenolic acids the prevention of diseases related to oxidative stress such as cancer and cardiovascular diseases.

Objectives
Analyse de groth behavior and tolerance of \textit{Lactobacillus plantarum} and \textit{Lactobacillus casei} when exposed to diferent fenolic acids-

Methods
We examined the behavior of two probiotic bacteria \textit{Lactobacillus plantarum} and \textit{Lactobacillus casei} when exposed to four different concentrations of six different phenolic acids: caffeic, ferulic, p-coumaric, hydroxybenzoic, vanillic and protocatechuich. Over 48h, analyzes were conducted by using a spectrophotometer.

Conclusions
In presence of acids hydroxybenzoic and protocatechuich, the absorbance increased slightly in the deceleration phase at a concentration of 50 mg/L and 100 mg/L, and decreases to a concentration of 200 mg/L compared to the values absorbance of the control. While for \textit{L. casei}, was observed for all acids tested, the absorbance values at a concentration greater than 50 mg/L, and values approximately equal to a concentration of 200 mg/L, compared to the control absorbance values. For protocatechuich acid, ferulic acid and p-coumaric acid has been possible to verify that the absorbance values were higher than controls at a concentration of 100 mg/L . In all cases, it was found that 200 mg/L showed an effect similar to the control except hydroxybenzoic acid.
LISTERIA MONOCYTOGENES NMLR: ITS TRANSCRIPTOME AND ROLE IN STRESS RESPONSE
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Background

NmlR, a transcription regulator in MerR family, is involved in oxidative and nitrosative stress responses in Neisseria gonorrhoeae and Haemophilus influenzae. A homolog is also found in a foodborne pathogen Listeria monocytogenes (lm).

Objectives
In this study, in order to study the role of NmlRlm in L. monocytogenes, a null nmlRlm strain was constructed. Transcriptomes of 10403S wild type (WT) and its isogenic null nmlRlm mutant (MT) strains grown to stationary phase were determined. Phenotypes of these strains in response to different stress conditions were also assessed.

Methods
RNAseq experiments were performed on Ion Torrent platform and the transcriptomes of WT and MT during stationary phase were identified. WT and MT strains were exposed to acid stress and oxidative stress conditions. Role of NmlRlm in virulence-associated phenotype was also evaluated in intracellular growth assay using U937 human macrophage-like cell line. Cell sizes of WT and MT were measured using transmission electron microscopy.

Conclusions
RNAseq result reveals that NmlRlm negatively regulates 46 genes and positively regulates 28 genes. NmlRlm-dependent genes are grouped into 28 operons, eight of which overlap HrcA regulon, another negative regulator in L. monocytogenes. Phenotypic characterization revealed that MT strain survived significantly less than WT under acid stress (pH 2.5) and oxidative stress (3% hydrogen peroxide). Null mutation in nmlRlm also resulted in significant decrease in cell length and impaired intracellular growth in U937. Our findings indicate that NmlRlm is not only involved in oxidative stress response but also contributes to acid persistence and intracellular growth via either direct regulation or co-regulation with other regulators.
Background

*Lactobacillus kefiranofaciens* M1, a novel probiotic strain isolated from Taiwanese kefir grains, has been demonstrated to possess anti-allergic, anti-asthmatic, anti-colicitis and immunomodulatory effects in our previous in vitro and in vivo studies. These findings support that this strain has the potential to be applied in probiotic products. *L. kefiranofaciens* M1 must survive after processing, production and gastric passage to exert beneficial effects. Thus, the stress tolerance of *L. kefiranofaciens* M1 is important for its survivability.

Objectives

This study was investigated the adaptation and tolerance of *L. kefiranofaciens* M1 to various environmental stresses.

Methods

*L. kefiranofaciens* M1 was adapted to sublethal heat (37°C), cold (25°C), acid (pH 5), bile salts (0.05%), salts (0.1 M), ethanol (4%) and hydrogen peroxide (100 ppm) for 1 h and then challenged with lethal heat (52°C), cold (-20°C), acid (pH 3), bile salts (0.2%), salts (3 M), ethanol (20%) and hydrogen peroxide (1000 ppm), respectively.

Conclusions

The results showed that adaptation to ethanol, hydrogen peroxide, salts and bile salts increased the tolerance of *L. kefiranofaciens* M1 to heat, cold, bile salts and ethanol stresses. Heat adaptation enhanced the tolerance to heat, bile salts and hydrogen peroxide. Acid adaptation caused an increased resistance to cold, acid, bile salts and hydrogen peroxide. Cold adaptation also induced cross-protection against cold, bile salts, salts and ethanol. However, ethanol- and cold-adapted cells decreased their survival after exposure to hydrogen peroxide. The occurrence of stress tolerance response in *L. kefiranofaciens* M1 varied depending on the type and condition of stress.
TRADITIONAL SOUR MILK FERMENTATION USING A POTENTIAL PROBIOTIC LACTOBACILLUS PLANTARUM STRAIN

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Background

Probiotic foods receive market interest as health-promoting, functional foods.

Objectives

To evaluate the performance of a *Lactobacillus plantarum* strain with probiotic potential as co-starter culture in sour milk fermentation and its ability to give a final product with desirable sensory characteristics.

Methods

UHT milk was inoculated with *Streptococcus thermophilus* and *Lactobacillus delbrueckii* subsp. *bulgaricus* without (control case) or with *Lb. plantarum* and incubated in appropriate conditions (42°C, 6h). Fermented samples were stored at 4 and 12°C until the end of shelf life of product. Microbiological analysis was performed in parallel with pH and titratable acidity measurements as well as with sensory analysis of the product. The presence of the potential probiotic strain was determined using PFGE.

Conclusions

On first day of storage, the population levels of all microbial groups exceeded 8 log cfu/ml for control and probiotic samples. During storage, the population levels were slightly elevated on probiotic samples compared to the control ones, whereas they were found to be above 8 log cfu/ml for all cases until the end of shelf life. The physicochemical properties (pH, titratable acidity) and the sensory characteristics of the potential probiotic product were similar to those of the control. According to PFGE the *Lb. plantarum* strain was recovered in high percentages during the products shelf life at both temperatures.

Acknowledgment: This work has been co-financed by the European Regional Development Fund (ERDF) of the EU and by National Resources under the Operational Program Competitiveness and Entrepreneurship (EPAN II), Action "COOPERATION 2011", Project "ProbioDairyMeat".
GREEK FUNCTIONAL YOGHURT: ENHANCING THE PRODUCTION PROCESS USING A SELECTED LACTOBACILLUS STRAIN WITH PROBIOTIC POTENTIAL
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Background
Probiotic food products are in general fermented foods, with many studies reporting that the best matrices to deliver probiotics are dairy products, such as fermented milks and yogurt.

Objectives
To evaluate the performance of a Lactobacillus plantarum strain of dairy origin with probiotic potential as co-starter culture in Greek set-type yoghurt fermentation.

Methods
Low-pasteurized milk was inoculated with Streptococcus thermophilus and Lactobacillus delbrueckii subsp. bulgaricus (control) or with the former starters plus Lb. plantarum (probiotic) and incubated in 42°C for 5h. Subsequently, samples were stored at 4 and 12°C until the end of shelf life of the product. Microbiological analysis was performed in parallel with physicochemical and sensory analysis of the product. The presence of Lb. plantarum was determined using Pulsed Field Gel Electrophoresis (PFGE).

Conclusions
The initial levels of all microbial groups were above 7 log cfu/g for control and probiotic samples. During storage, the population levels of all counts were slightly elevated on probiotic samples compared to the control ones, whereas they exceeded the 7 log cfu/g at all cases. Probiotic yoghurt produced with Lb. plantarum strain exhibited a better sensory profile, with a rich traditional taste, but with similar physicochemical properties to the control. According to PFGE, the Lb. plantarum strain was recovered in high percentages during the products shelf life at both temperatures.

Acknowledgment: This work has been co-financed by the European Regional Development Fund (ERDF) of the EU and by National Resources under the Operational Program Competitiveness and Entrepreneurship (EPAN II), Action "COOPERATION 2011", Project "ProbioDairyMeat".
IN VITRO ANTIBACTERICIAL ACTIVITY AND COMPETITIVE INHIBITION EFFECT ON CACO-2 CELLS OF PROBIOTIC LACTOBACILLI ISOLATED FROM SARDINIAN DAIRY PRODUCTS

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Background

The research of novel formulations with newly selected probiotic strains is important to satisfy the increasing request of the market and to obtain functional products with health-conferring properties. The most studied probiotic bacterial strains belong to the genus Lactobacillus, an important group of Lactic Acid Bacteria with functional and technological properties. Antagonistic activity toward potentially pathogenic microorganisms and adhesion to gut tissue are some of the main requirements when selecting successful probiotic strains.

Objectives

The aim of this work was to evaluate the antibacterial and anti-adhesion activity of Lactobacillus plantarum strains isolated from ewe’s raw milk and their ability to in vitro colonize the intestinal epithelium. These strains were previously selected as potential probiotics because of their ability to survive at low pH values and physiological bile concentrations.

Methods

For detection of antagonistic activity, agar spot test and a well diffusion assay were performed toward Escherichia coli O157:H7, Salmonella Typhimurium and Listeria monocytogenes. The in vitro anti-adhesion effect was measured by a competitive inhibition assay on Caco-2 cells.

Conclusions

The results showed our strains having an excellent antibacterial activity against foodborne bacterial pathogens and anti-adhesion effect toward E. coli O157:H7 and L. monocytogenes, under the condition tested. Therefore, they showed interesting
probiotic characteristics. An *in vivo* study would be useful for a deeper evaluation of their properties, and their potential use in the development of novel functional food. This work has been supported by Regione Autonoma Sardegna (RAS, L.R. 7/2007).
COOPERATION BETWEEN SPECIES IN THE YOGURT CONSORTIUM IS AFFECTED BY THE MODULATION OF INTRACELLULAR PH THAT IS DRIVEN BY STREPTOCOCCUS THERMOPHILUS UREASE ACTIVITY

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Background

The proto-cooperation between Streptococcus thermophilus and Lactobacillus delbrueckii subsp. bulgaricus in the yogurt consortium enhances the growth rate and size of each population. In contrast, the independent growth of the two species in milk leads to a slower growth rate and a smaller population size.

Objectives

In this study, we evaluated how urease activity of S. thermophilus might affect the intracellular pH of L. delbrueckii, modulating its bioenergetics during milk fermentation.

Methods

Urease-mediated intracellular alkalization of S. thermophilus and L. delbrueckii was evaluated by flow cytometry using cFSE as pH-dependent fluorescent probe. Lactose consumption and lactic acid production was followed in vivo by ¹³C-NMR analysis. D-L lactic acid production in milk was measured enzymatically. For the evaluation of the pH-dependent glycolysis efficiency, glucose and lactic acid were measured by HPLC.

Conclusions

We observed that intracellular alkalization caused by urea hydrolysis or the addition of ammonia to milk boosted lactic acid production in S. thermophilus and in L. delbrueckii when the species were grown separately or in combination. Therefore, we propose that urease activity acts as an altruistic cooperative trait, which is costly for urease-positive individuals but provides a local benefit because other individuals can take advantage of urease-modulated pH. Compared to the interactions that are known to occur between S. thermophilus and L. delbrueckii in the yogurt consortium, the modulation of the bioenergetic efficiency due to the intracellular pH alkalization represents a new type of cooperation that directly affects the kinetic parameters of enzymes involved in homolactic fermentation in both the species.
ESCHERICHIA COLI STRAIN DIVERSITY: SELECTING ISOLATES FOR USE AS PATHOGEN SURROGATES

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Background

*Escherichia coli* (*E. coli*) is commonly used as a surrogate for pathogens in research to identify sources of agricultural contamination and to characterize how pathogens persist on plant surfaces. However, *E. coli* strains are highly diverse, exhibiting differences in physical, chemical and biological properties which contribute to fitness in secondary habitats.

Objectives

An important step in selecting *E. coli* for use as pathogen surrogates is to identify strains with characteristics (including adhesion, motility and biofilm formation) that contribute to their occurrence and survival in a manner similar to that of pathogens.

Methods

To this end, strain-level differences in genotype (adhesions (*iha, agn43, eaeA* and *fimH, kpsMTII*) and siderophores (*iroN*E.coli*, chuA*)) and phenotype (biofilm formation, curli expression, and growth rate) were evaluated for isolates from livestock (swine, poultry, dairy) and water sources. Selected *E.coli* isolates (n=18 of 1,300), a common *E. coli* control strain (ATCC 25922), and *Salmonella* (ATCC 13311) were used in soil studies to evaluate adhesion to plastic, sandy loam or clay soils.

Conclusions

Average adhesion of environmental isolates to plastic was two-fold higher than that of the control strain. *Salmonella* and one of the environmental isolates had adhesion rates seven-fold higher than the control strain. That isolate had extremely high adhesion to clay (91.3 ± 2.7%) and sandy loam (91.7 ± 0.1%) soils, but had none of the genes commonly associated with adherence or biofilm formation. These results suggest that *E. coli* strain choice should be an important consideration in its use as a pathogen surrogate.
Background

Raw fruits and some vegetables possess intrinsic chemical and physical features that make them particularly hostile environments for bacteria. To cope with environmental conditions, microorganisms may adopt sophisticated adaptation mechanisms. The diversity of plant environments and of bacterial enzyme activities makes the microbial adaptation to plant niches markedly heterogeneous. *Lactobacillus plantarum* is a highly heterogeneous and versatile lactic acid bacteria frequently found or used in vegetables and fruits fermentation.

Objectives

The aim of the study was to determine biological relevance of differentially expressed genes in *L. plantarum* C2 during fermentation of plant substrates, aiming to provide new insights into transcriptional response and niche adaptation.

Methods

*L. plantarum* C2 was grown and stored in carrot or pineapple juices to mimic the chemical composition of the respective raw matrices. MRS broth was used as the control medium for optimal growth. Whole-transcriptome analysis based on customized microarray profiles has been used to determine altered transcription patterns in *L. plantarum* C2. These were compared with substrate utilization data gathered from high throughput phenotypic microarrays.

Conclusions

Plant substrates exerted a transcriptional pressure and induced specific molecular and metabolic responses in *L. plantarum* C2. RNA and phenotypic microarray analyses revealed altered transcription patterns of genes encoding functions involved in primary metabolism, membrane transport, cofactors and vitamins metabolism, translation regulation, nucleotide metabolism, and fatty acid biosynthesis. Findings contribute to the description of bacterial transcriptional adaptation to niches, and provide a more solid basis for selection the most suitable starters for fermentation of targeted matrices.
IN VITRO ASSESSMENT OF ANTIBACTERIAL ACTIVITY OF RESINS FROM
SOME WOODY PLANT BUDS AND SYNERGISTIC EFFECT BETWEEN
STANDARD PHENOLIC COMPOUNDS

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Background
With emerging of new and reemerging of old infectious diseases, urgent need for new antimicrobial agents arises. Resins are complex mixtures of phenolic and isoprenoid compounds secreted by plants to provide protection against predators and pathogenic microorganisms.

Objectives
The present study was aimed at investigating the in vitro antimicrobial activity of resins from some woody plants on the selected bacterial strains. To elucidate origin of antimicrobial activity, potential synergistic effect between standard phenolic compounds was investigated, also.

Methods
The samples of resins were collected from different regions in Serbia and dissolved in methanol. Well diffusion and broth microdilution methods were implemented for assessing antimicrobial activity.

Conclusions
Gram-positive bacteria exhibited strong susceptibility to samples from Populus sp. while being resistant on samples from other origin. The most sensitive strain was B. subtilis, towards samples collected from Populus nigra and Prunus avium (MIC was 0.05 mg/ml). The strains of S. aureus, MRSA and L. monocytogenes showed similar sensitivity to poplar samples with MIC values mostly under 1 mg/ml. L. monocytogenes was also susceptible to cherry buds samples. Sample U19 from white poplar had significant antimicrobial activity on all tested strains, with lowest detected MIC values. Synergistic effect of gallic acid, quercetin and caffeic acid on B. subtilis; gallic, caffeic, p-coumaric acid and naringenin on E. faecalis; and gallic, caffeic acid and naringenin on S. flexneri was observed. In conclusion, antimicrobial activity of resins is probably outcome of action of phenolic compounds.
A COMPARISON OF LISTERIA SPP. PREVALENCE BETWEEN THE RAW FRESHWATER FISH FROM LAKE AND RETAIL MARKET IN LATVIA

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Background
Listeriosis is a foodborne infection caused by Listeria monocytogenes, which occurs due to ingestion of pathogen containing foodstuffs. Listeria spp. and L. monocytogenes are widespread in environment, but raw and processed fish products were found to be frequently contaminated with Listeria spp. Therefore fish are suspected to be a possible source for Listeria spp. including L. monocytogenes transmission to final food products.

Objectives
The objectives of this study were to determine and compare the prevalence of Listeria spp. in raw freshwater fish from retail market and fish captured from lake in Latvia.

Methods
A total of 51 raw freshwater fish samples were collected from lake (n = 11) and retail market (n = 39) in Latvia. A total amount of 25 g of fish skin, gut and muscle tissues of each fish was used for testing. Testing was performed in accordance to the International Organization for Standardization method (ISO 11290-1: 1996).

Conclusions
The fish samples from lake were found to be Listeria spp. negative. In contrast, 69% of retail fish were found to be contaminated with Listeria spp. There were no significant differences between the prevalence of L. innocua and L. monocytogenes (p>0.05), while the prevalence of L. ivanovii and L. welshimeri was significantly lower than the prevalence of L. monocytogenes and L. innocua (p<0.05). The results
indicate that fish contamination with *Listeria* spp. may occur during fish handling, and the high prevalence of *L. monocytogenes* in raw fish at retail level can pose a threat for public health.
Background

The foodborne disease caused by *Salmonella* Typhimurium is a major health problem worldwide.

Objectives

The aims of this study were to genotype *S*. Typhimurium isolated in Brazil and to verify its antimicrobial resistance profiles.

Methods

A total of 92 *S*. Typhimurium strains, isolated from humans (43) and food (49), between 1983 and 2013 in Brazil, were typed by PFGE, ERIC-PCR and MLVA. Additionally, their antimicrobial resistance was evaluated. Results: The 92 *S*. Typhimurium strains were grouped in two clusters, by PFGE, PFGE-A and PFGE-B (subdivided in PFGE-B1 and PFGE-B2); by MLVA in two clusters, MLVA-A and MLVA-B (subdivided in MLVA-B1 and MLVA-B2). By ERIC-PCR, in three clusters, ERIC-A, ERIC-B and ERIC-C. The strains isolated from humans before the mid-1990s were allocated in the PFGE-A, PFGE-B1, PFGE-B2, MLVA-A, MLVA-B1, MLVA-B2, ERIC-A and ERIC-B. The strains isolated from humans after mid-1990s were distributed in the PFGE-B1, MLVA-B1, MLVA-B2 and ERIC-A. The strains isolated from food were distributed in the PFGE-A, PFGE-B1, MLVA-A, MLVA-B1, MLVA-B2, ERIC-A, ERIC-B and ERIC-C. Twenty-three (25%) strains were multidrug resistance.

Conclusions

The results suggest that the studied strains isolated from humans before the mid-1990s were genetically more diverse, which might indicate that selection of a more adapted *S*. Typhimurium subtype occurred after *S*. Enteritidis became the most prevalent serovar in Brazil. Regarding, the food strains the results suggest the current circulation of more than one subtype. The occurrence of multi-drug resistant strains isolated from food is an alert for the possible risk for human consumption.
MOLECULAR CHARACTERIZATION AND RESISTANCE PROFILE OF CAMPYLOBACTER COLI STRAINS ISOLATED FROM DIFFERENT SOURCES IN BRAZIL
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Background
Campylobacter coli is an important causative agent of human diarrheal diseases worldwide. However, in Brazil is not frequently studied.

Objectives
This study aimed to genotype and to investigate the presence of virulence genes and the resistance profile in C. coli strains.

Methods
A total of 58 C. coli strains isolated from humans(10), animals(14), the environment(18) and food(9), between 1995-2011 in Brazil, were genotyped by PFGE. The presence of 16 virulence genes was searched by PCR. The resistance profile was obtained by MIC for erytromycin, ciprofloxacin, tetracycline and doxacycline. All the strains presented the genes flaA, cadF and sodB. The cdtB, flhA, dnaJ and pldA genes were observed in 20, 15, 10 and 6 strains, respectively. The ciaB, iamA, cdtA, cdtC, docA, virB11, wlan, racR and crsA genes were not detected. PFGE grouped the strains in two main clusters with more than 45.4% of similarity. PFGE-A and PFGE-B clusters comprised 42 and 16 strains, respectively. In both clusters, strains of clinical and non-clinical sources were grouped in subclusters with a similarity value of more than 80%. The resistance profile showed that 19 strains, mainly of non-clinical sources, were resistant to at least one antimicrobial agent.

Conclusions
The PFGE results confirmed the heterogeneity of the C. coli strains studied. However, the high similarity (>80%) among some strains of different origins suggests a possible contamination between clinical and non-clinical sources in Brazil. Because of these, the existence of resistant strains becomes a concern. The presence of important virulence genes indicates the pathogenic potential of those strains.
ANTIBIOTIC RESISTANCE PROFILES AND PREVALENCE OF ENTEROTOXIN GENES OF STAPHYLOCOCCUS AUREUS FROM FOOD HANDLERS

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Background

\textit{Staphylococcus} spp. are common members of the normal human flora. However, some \textit{Staphylococcus} strains are recognized as human pathogens, in particular due to the production of several virulence factors and enterotoxins particularly relevant in food toxinfections.

Objectives

Since many of the food toxinfections by \textit{S. aureus} are typically associated with cross-contamination, detection of \textit{Staphylococcus aureus} was performed on food handlers.

Methods

Hand swabs from 167 food handlers were analysed for the presence of \textit{Staphylococcus aureus} using Baird Parker agar and coagulase test, and genotypically confirmed by RT-PCR. A total of 26 strains were analysed using RT-PCR for the presence of virulence and enterotoxin genes, namely, \textit{sea, seb, sec, sed, seg, sei, tsst1 and pvl}. The same strains were phenotypically characterized in terms of antibiotic resistance using the disk diffusion method and antimicrobial agents from 15 different categories.

Conclusions

More than 11\% of the samples were positive for \textit{S. aureus}. A low prevalence of antibiotic resistant strains was found, with more than 55\% of the strains being sensitive to all of the antimicrobial agents tested. However, a high prevalence of resistance to macrolides was found, with 42\% of the strains showing resistance to erytromycin. At least one of the virulence or enterotoxin genes was detected in 54\% of the strains and the \textit{seg} gene was detected in 42\% of the strains.
POTENTIAL GENETIC TARGETS FOR CONTROLLING CAMPYLOBACTER JEJUNI

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Background

Essential genes have been suggested to provide useful information for the development of new specific anti-infective agents in C. jejuni, as the major cause of bacterial gastroenteritis in humans worldwide (Stahl & Stintzi, 2011). To determine genetic regions that are present or highly prevalent in all C. jejuni strains could be also of interest as potential genetic targets for this purpose.

Objectives

The aim of this study was to identify by microarray comparative genomic hybridization (MCGH) potential genetic targets for controlling C. jejuni.

Methods

The prevalence of 1652 genes among 120 geographically diverse isolates obtained from different sources (human, chicken cattle, water and wild birds) was determined by MCGH using the BµG@S CJv3.0.0 microarray. As MCGH does not confirm the absence or divergence of the tested genes, the results were compared with those obtained by both MCGH and genomic sequencing in previous studies (Stabler et al., 2013; van Tonder et al., 2014).

Conclusions

MCGH analysis detected 1366 genes (83%) with prevalence 90% in the C. jejuni population studied. These genes included 816 genes (60%) recently proposed as core (van Tonder et al., 2014), and 229 genes (28%) proposed as essential gene candidates (Stahl & Stintzi, 2011; Metris et al., 2011). A final list of 22 genes, including core and essential genes, was highlighted. All these genes encode products required for cell growth, and are therefore potential targets to consider for antimicrobial intervention strategies in C. jejuni.
THE EFFECT OF OSMOTIC DEHYDRATION PROCESS IN SUGAR BEET MOLLASES ON MICROBIOLOGICAL PROFILE OF CHICKEN MEAT

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Background

Osmotic dehydration is a water removal process, based on soaking food (fruit, vegetable, meat and fish) in a hypertonic solution. Recent research has shown that use of sugar beet molasses as a hypertonic solution improves osmotic dehydration process from technological, nutritional and microbiological aspect.

Objectives

Effect of osmotic dehydration process on microbiological profile of chicken meat was investigated in order to determine the usefulness of this technique as pre-treatment for further treatment of chicken meat.

Methods

Fresh breast chicken meat was cut into cubes of 1x1x1 cm and contaminated with *Escherichia coli*, *Listeria monocytogenes* and *Salmonella* spp in laboratory conditions prior the process of osmotic dehydration. The process was conducted during 5 hours at 44°C in sugar beet molasses as an osmotic medium. All microorganisms were tested according to ISO methodology: *Salmonella* spp EN ISO 6579:2002, *Listeria monocytogenes* EN ISO 11290-2:1998, *Escherichia coli* ISO 16649-2:2001, *Enterobacteriaceae* ISO 21528-2:2004 and total number of microorganisms EN ISO 4833-1:2013.

Conclusions

The results of the microbiological profile of fresh, contaminated and osmotically dehydrated chicken meat have shown that the numbers of all examined microorganisms have significantly statistically reduced after the process.

From presented results it can be concluded that process of osmotic dehydration in molasses significantly improves microbiological profile of treated chicken meat and it is suitable pretreatment for further chicken meat processing, providing microbiologically safe intermediate product.
USE OF COMPARATIVE GENOMICS AND THIN LAYER CHROMATOGRAPHY TO IDENTIFY SECONDARY METABOLITES RESPONSIBLE FOR ANTI-GRAM NEGATIVE ACTIVITY IN BACILLUS AMYLOLIQUEFACIENS SUBSP. PLANTARUM

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Background

Strong consumer demand for safe and high-quality products has continued to drive food and pet food producers to seek novel solutions targeting Gram-negative bacterial contamination, such as *Escherichia coli* and *Salmonella* spp.

Objectives

Since *Bacillus* spp. are known for their ability to produce antimicrobial compounds, culture supernatants of *Bacillus amyloliquefaciens* strains were evaluated and characterized for their antimicrobial properties. Furthermore, genome sequences were to be generated to provide insights to the mode of action.

Methods

Six *B. amyloliquefaciens* strains were used for the experiments. Titration of activity was conducted by measuring the halos in an agar well diffusion assay, employing an extensive range of bacteria and fungi. The effect of pH, heat and enzymes on the activity of the supernatants was expressed as a percentage of residual activity compared to non-treated samples. Draft genomes were generated by 454 FLX Titanium for each of the *Bacillus* strains and mined for the presence of secondary metabolites, which was furthermore confirmed with TLC-bioautography profiles of methanolic extracts.

Conclusions

All supernatants exhibited activity against important food related bacteria and fungi, including Gram negatives. Generally, considerable activity was retained after heating and at alkaline pH but not under acidic conditions. Whereas different enzymes had a variable effect on the antimicrobial activity. genomic data indicated that the *Bacillus* strains harboured gene clusters encoding proteins involved in the
production of an array of secondary metabolites, including several non-ribosomal peptide synthetases, polyketides, and ribosomally synthesized peptides, which could be responsible for the \textit{in vitro} antimicrobial properties observed.
Background
According to the Register of Infectious Diseases, supplied by the National Public Health Institute (THL), in 2013 Campylobacter sp. was the leading cause of human bacterial infection in Finland with 4059 cases registered (incidence 76 cases/100000 inhabitants). However, the real number of cases is assumed to be considerably higher than the registered cases numbers. The prevalence of Campylobacter sp. in animal-derived foods is affected by its prevalence in the production farms and in the animals. The contamination of the final product is also dependent on procedures carried out in abattoirs and during the various stages of the food production chain. The handling of food contaminated with Campylobacter sp. in domestic or industrial kitchens may also lead to cross-contamination, either directly from raw meat to products that will not undergo further cooking or indirectly via work surfaces, hands or utensils.

Objectives
Estimating the prevalence and concentration of Campylobacter in fresh (chicken, turkey) meat (sampled at retail), as well as presenting a novel Bayesian model to assess the probability of infection and illness.

Methods
Bayesian modeling of prevalence and concentration using Openbugs software based on results from detection and quantification of campylobacters (chicken: 608 samples from 226 batches) and (turkey: 558 samples from 185 batches) using modified NMKL 119:2007 and ISO 10272-2:2006.

Conclusions
Risk of Campylobacter exposure from Finnish fresh poultry meat is mainly due to undercooking or cross-contamination especially during summertime. The model can be used to assess e.g. relative risks, with quantification of uncertainty based on the data.
EFFECT OF ELECTRON BEAM IRRADIATION IN MINAS FRESCAL CHEESE ARTIFICIALLY CONTAMINATED WITH ENTEROHAEMORRHAGIC ESCHERICHIA COLI (EHEC) O157:H7
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Background
The enterohemorrhagic Escherichia coli (EHEC) O157:H7 has been known to cause food-borne outbreaks. Cheeses are among the main food products incriminated in these outbreaks. Characterized as a perishable food, the Minas frescal cheese (MFC) has potential risk for O157:H7 transmission. As a result, the development of new technologies for its conservation has been studied. The use of irradiation has proved to be one of the most convenient options.

Objectives
The objective of the present study was to evaluate the effect of electron beam irradiation (doses of 1.0, 1.5 and 2.0 kGy) in MFC artificially contaminated with O157:H7.

Methods
Samples were produced to microbiological analyzes, one non-contaminated and non-irradiated, one contaminated and non-irradiated and three contaminated and irradiated. And to thiobarbituric acid reactive substances concentration (TBARS) analysis, one non-irradiated and non-contaminated and three non-contaminated and irradiated.

Conclusions
Artificially contaminated and irradiated MFC samples presented a shelf life of ten storage days at 4°C. The dose of 1.5 kGy reduced 2 log the number of O157:H7 and the doses of 1.5 and 2.0 kGy eliminated O157:H7 from the artificially contaminated samples. The TBARS assay was conducted to evaluate if the effect of irradiation could influence in the stability of the lipid fraction present in the cheese. All irradiated cheeses showed TBARS values, over the 40 day storage period, lower than in the non-irradiated sample. Results presented here demonstrate electron beam irradiation of MFC artificially contaminated with O157:H7 is a viable solution and a promising alternative to ensure the safety and quality of this food product.
BEHAVIOR OF SHIGA TOXIN-PRODUCING ESCHERICHIA COLI (STEC) O157:H7 AND O113:H21 UNDER THE EFFECT OF DIFFERENT LEVELS OF PH AND TEMPERATURE

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Background
Shiga toxin-producing Escherichia coli (STEC) cause hemorrhagic colitis, hemolytic uremic syndrome and bloody diarrhea.

Objectives
This work aimed to evaluate the effect of two individual factors (pH and temperature), and their interaction, on the multiplication and survival of STEC (O157:H7 and O113:H21) using statistics as a tool for development of the experimental design and the analysis of results.

Methods
Approximately 10⁸ CFU per ml were inoculated into Trypticase Soy Broth at pH conditions (4.0, 4.5, 5.5, 6.5 and 7.0) and temperature (6, 10, 20, 30 and 35°C). The experimental design was prepared by a central composite design, generated by the software Statistica 7 (StatSoft, OK, USA), and given 11 trials including three central points and four axial points. Assays were performed in 22 h.

Conclusions
Were generated equations that model the bacterial behavior from a multiple linear regression model. In the study with the RJ 581 strain (O157:H7), variables, individually and combined, showed linear significant effect on the response, the effect of the variable pH being the most relevant, with R² equal to 0.94. Explaining the optimal conditions for the multiplication of the RJ 581 strain values are closer to pH 7.0 and 35°C. In the study with the RJ 702 strain (O113:H21), we observed that the independent and combined variables had no significant effect on the response. The R² of the model was 0.4, showing a low suitability of experimental design to RJ 702 strain.
LACTIC ACID BACTERIA AS ANTAGONIST AGENTS TO FOODBORNE PATHOGENS ON FRESH-CUT ‘GOLDEN DELICIOUS’ APPLES

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Background

Food safety is a major concern in fresh-cut industry. Fresh-cut produce can become contaminated with foodborne pathogens during manipulation. Application of natural antimicrobial substances provides new opportunities for the control of pathogenic bacteria. Lactic acid bacteria (LAB) are known to have antagonistic effect against sensitive bacteria species/strains by the secretion of substances with antimicrobial activity.

Objectives

The main objective of this work was to do a screening of 37 LAB isolates for antimicrobial activity on minimally processed ‘Golden delicious’ apples against the foodborne pathogens Escherichia coli O157:H7 NCTC 12900, Salmonella enterica subsp. enterica Michigan ATCC BAA-709, Cronobacter sakazakii ATCC 51329 and Staphylococcus aureus ATCC 25923.

Methods

LAB studied were isolated from fresh-cut fruit purchased in the market. One g of fresh-cut apple was inoculated with the LAB followed by the inoculation of the pathogen at a concentration of $10^8$ cfu/mL. Samples were incubated at 30 °C for 48 h and after the recovery of the pathogens were made on selective/differential mediums. Fresh-cut apples inoculated with only the pathogen were used as control.

Conclusions

From the 37 LAB isolates 18 were capable to reduce all the pathogens more than 2 log cfu/g and 5 achieved a reduction of more than 3 log cfu/g for all the pathogens. S. enterica and C. sakazakii showed reduction with all the isolates studied and E. coli and S. aureus showed reduction with 36 of the 37 isolates. Results obtained support the potential use of LAB as biocontrol agents against foodborne pathogens in fresh-cut fruit.
FERMENTATION PROPERTIES AND POTENTIAL PREBIOTIC ACTIVITY OF A HIGH PURITY GOS ON IN VITRO GUT MICROBIOTA PARAMETERS IN HEALTHY INDIVIDUALS

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Background

Most in vitro and in vivo studies involving prebiotic oligosaccharides have been carried out using inulin and its fructooligosaccharide (IFS) derivatives, as well as various galactooligosaccharides (GOS). It has been shown that these food ingredients have the ability to improve selectively the growth of bifidobacteria and, consequently, lead to important changes in the gut microbiota composition that may confer health benefits to the host. Bimuno®, (Clasado Ltd., Buckinghamshire, UK) is a 50% mixture containing galactooligosaccharides (B-GOS) having multiple biological health activities within the colonic environment, beyond the stimulation of bifidobacteria and lactobacilli at genus level and it has been tested in different in vitro and in vivo studies.

Objectives

The present study aimed to determine the in vitro potential of a new high GOS content version of Bimuno (65% GOS content), comparing to B-GOS used as positive control, in a pH and volume controlled dose response batch culture experiments. Three different doses of the high purity GOS (2.75, 1.38 and 0.92 g) were tested.

Methods

Changes in the gut microbiota during a time course were identified by fluorescence in situ hybridisation (FISH), whilst the small-molecular weight metabolomic profiles and short chain fatty acids (SCFAs) were determined by NMR analysis and Gas Chromatography (GC), respectively.

Conclusions

The High purity GOS showed positive modulation of the microbiota composition after 4h fermentation at the lowest dose compared to B-GOS. Moreover, the administration of the specific GOS induced a significant increase in acetate as the major SCFAs synthesized compared to propionate and butyrate concentrations.
COMMON AND DIVERGENT FEATURES OF THE HEAT-RESISTANT SPOILAGE PROTEASE PRODUCING MICROBIOTA IN MILK FROM TWO DIFFERENT UHT-MILK PRODUCING COUNTRIES, BRAZIL AND BELGIUM

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Background

The majority of the bacteria from raw milk are inactivated by heat treatments commonly used in dairy industry (pasteurization or ultra-high temperature (UHT)). However, these treatments do not destroy heat-resistant spoilage enzymes such as proteases produced by certain psychrotrophic bacteria before processing.

Objectives

The first objective was to identify the main proteolytic micro-organisms in raw milk from Belgium and Brazil, two countries with a large UHT-milk production but under different primary production conditions which can influence the microbiota composition of raw milk. Secondly, the diversity of the protease enzymes was investigated in order to characterize the target of future spoilage detection assays.

Methods

Raw milk samples were stored at simulated conditions of farm storage and transport according to Brazilian and Belgian regulations, respectively. The highly proteolytic strains were polyphasically identified. Their protease enzymes were characterized for heat resistance, proteolytic activity in milk, casein zymogram analysis with MALDI-TOF identification of protein bands, and for gene sequence diversity.

Conclusions

The Pseudomonas fluorescens group together with Ps. fragi and Ps. lundensis were dominant in Belgian raw milk, while in Brazil, almost the same Pseudomonas spp. together with Serratia liquefaciens were dominant, the latter being an indication of less hygienic conditions during milking. Belgian isolates showed overall a significant higher proteolytic activity than most Brazilian isolates. The proteolytic activity from both sources was resistant to UHT conditions with the one from S. liquefaciens being the most heat resistant. It seems that the spoilage protease from S. liquefaciens is
unrelated to the heterogeneous *Pseudomonas* *AprX* protease.
POLYPHASIC IDENTIFICATION OF THE DOMINANT MICROBIOTA OF PEELED BROWN SHRIMP (CRANGON CRANGON) STORED UNDER MODIFIED ATMOSPHERE PACKAGING AT 4 °C WITHOUT PRESERVATIVES

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Background

Brown shrimp are, like many seafood products, prone to rapid spoilage. The main factor limiting shelf life is microbiological activity. Specifically, for brown shrimp without preservatives, the microbiota is dominated by the genera *Pseudoalteromonas* and *Psychrobacter* when stored under iced aerobic conditions, but the spoilage bacteria of shrimp when cool stored under modified atmosphere (MAP) conditions without preservatives, a product which is gaining popularity among consumers, are still unknown.

Objectives

The main objective was to evaluate the variation throughout the year and during storage in the microbial community of MAP packaged cooked and machine peeled shrimp (*Crangon crangon*) without preservatives and the description of the dominant spoilage microbiota after 7 days of shelf life at 4 °C.

Methods

Three series of shelf life experiments resulting from three sampling periods of cooked shrimps throughout the year were conducted with mechanically peeled shrimp stored in a modified atmosphere (40% CO₂ and 60% N₂). Culture-dependent and – independent methods, notably (GTG)₅ clustering followed by 16S rRNA gene sequencing of isolates and 16S rRNA gene based DGGE in combination with metagenomics, were used to monitor the bacterial population in brown shrimp without preservatives.

Conclusions

According to culture dependent identification the microbiota of brown shrimp was dominated by *Arthrobacter bergerei*, *Shewanella putrefaciens*, *Aliivibrio* spp., *Psychrobacter* spp., *Brochothrix thermosphacta* and *Vagococcus salmoninarum*. Using the culture independent approach the dominating genus after 7 days of storage was *Carnobacterium*. Most of the micro-organisms identified through the complementary approach are known to contribute to spoilage, but for some (e.g. *V.*
*salmoninarum* the spoilage potential is yet unknown.
DNAJ GENE AS A MOLECULAR DISCRIMINATOR WITHIN THE LACTOBACILLUS CASEI GROUP
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Background
The current taxonomy of the Lactobacillus casei group is comprises three closely related species: L. casei, L. paracasei and L. rhamnosus. Some strains of these species have probiotic features and are now widely used in the food industries. However, neither phenotypic nor the most frequently applied genotypic marker (16S ribosomal DNA) provides sufficient resolution for accurate identification of L. casei group.

Objectives
The aim of this study was to use the dnaJ gene as a molecular marker for species-level discrimination and identification within the L. casei group.

Methods
The degenerate primers (LcasDnaj-F1/R1) were designed for dnaJ gene amplification and sequencing. The relationships between the members of L. casei group were conducted based on sequence similarity and phylogenetic analysis. The species-specific primers were designed by analysing the highly variable regions of the dnaJ gene.

Conclusions
Within the L. casei group, the nucleotide sequence similarity of dnaJ gene was significantly lower than that of 16S rRNA gene, and all examined strains could be clearly distinguished. In addition, the species-specific primers were developed, which were then employed for two-plex minisequencing analysis, and were shown to be specific for L. paracasei and L. rhamnosus. Our data indicate that the phylogenetic relationships in the L. casei group can be resolved using dnaJ gene sequencing, and the species of L. paracasei and L. rhamnosus can be identified using novel species-specific minisequencing assay.
INFLUENCE OF PH ON GENE EXPRESSION IN LACTOBACILLUS PARABUCHNERI FAM21731

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Background

The formation of biogenic amines in cheese can occur as a result of the presence of bacteria possessing amino acid decarboxylase activity. One of the most important biogenic amines in cheese is histamine, since it can cause serious human health problems upon ingestion.

Objectives

To better understand the factors contributing to histamine accumulation, we isolated histidine decarboxylase positive lactic acid bacteria from various raw-milk cheeses with elevated histamine levels. All isolates belonged to the species Lactobacillus parabuchneri. Various factors, such as starter culture, proteolysis during ripening, storage temperature, salt concentration and pH can affect histamine formation in cheese. For cheeses made from raw milk, a thermal treatment of the milk prior to cheese making to reduce the numbers of histamine producing L. parabuchneri is not an option. Other strategies could be developed to prevent accumulation of biogenic amines if the metabolism of these bacteria is known in more detail.

Methods

RNA from L. parabuchneri FAM21731 grown at various pH was isolated and sequenced on an Ion Torrent PGM sequencer.

Conclusions

Whole transcriptome analysis of L. parabuchneri was established using next generation sequencing technology. The methodology helps us to better understand how cheese making technology and choice of starter culture influence the metabolism of histamine producing L. parabuchneri strains.
EXPRESSION PROFILING OF PEDIOCOCUS ACIDILACTICI FAM18098 WITH A FOCUS ON THREONINE AND SERINE CATABOLISM

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Background

Proteolysis and amino acid catabolism are the most important events taking place during cheese ripening. Microorganisms play a crucial role in these biochemical changes. Among various lactic acid bacteria *Pediococcus acidilactici* is often found in cheese at the end of ripening. Little is known if this species contributes to protein breakdown and flavor development in cheese. When *Pediococcus acidilactici* FAM18098 was used as adjunct culture in cheese making, we observed that it degraded arginine, serine and threonine and synthesized ornithine, alanine and 2-aminobutyrate during cheese ripening.

Objectives

*P. acidilactici* FAM18098 did not degrade serine and threonine under laboratory conditions using MRS broth, a broth usually used for lactic acid bacteria in the laboratory. By changing the composition of media, we found a medium in which this strain showed an amino acid metabolism similar to the one observed in cheese. By analyzing the transcriptome of *P. acidilactici* FAM18098 we want to better understand the transcriptional regulation of genes involved in amino acid metabolism and to find experimental conditions that are similar to the ones of cheese.

Methods

RNA from *P. acidilactici* FAM18098 grown in various media was isolated and sequenced on an Ion Torrent PGM sequencer.

Conclusions

We established a protocol to analyze and compare whole transcriptomes of *P. acidilactici* with next generation sequencing technology. With this methodology we studied genes that are involved in amino acid metabolism under conditions that probably mimic a cheese environment.
Background
The food contact surfaces are subject to contamination by pathogens that could lead to cross-contamination by transfer events to others food products. However, the European regulation n° 1935/2004 of 27th October 2004 (Anonymous, 2004) specifies that materials intended for safe food contact must not interfere with foodstuff characteristics.

As a traditional and natural material, wooden boards are traditionally used as as a “technological tool” during cheeses ripening process. In France, wood is authorized for food contact by the French Arrêté November 1945 (Anonymous, 1945) and the information note of DGCCRF 2012-93(Anonymous, 2012).

Objectives
The aim of this study was to determine the behavior of wooden surfaces in direct food contact, contaminated by a well-known risk along the production chain of dairy products: *Listeria monocytogenes*.

Methods
For this purpose, a protocol was defined and new spruce boards were inoculated by *L. monocytogenes* solution at a concentration of $10^5$ CFU/cm$^2$ and the microbial transfer to pressed non-cooked cheeses was studied. Factors such as cheese time contact, wood moisture content and age of the cheeses were tested.

A comparison of transfer quantification, with other materials surfaces: glass plates and plastic sheets with inclined meshes, used in cheese production, was also realized among the same conditions.

Conclusions
The results showed for all tested surfaces a transfer yield below 3% (CFU/cm$^2$) in the first hour. No differences were found for older cheeses or for higher wood moisture
content. In conclusion, wooden shelves are, as much as, safety for food contact than plastic or glass surfaces.
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BIOFIM FORMATION AND VIRULENCE GENOTYPES OF ARCOBACTER BUTZLERI ISOLATED FROM FOOD PRODUCTS

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Background

Manipulation or consumption of raw or poorly cooked foods of animal origin is an important source of human infection with the emerging food and waterborne pathogen *Arcobacter butzleri*. The ability of this bacterium to form biofilm favors the transmission and its adherence to food and food-contact surfaces possibly favors cross-contamination. Preventing cross-contamination is a key factor in Food Safety. Besides, studying the virulence properties of any potentially pathogenic foodborne bacteria is essential for the consumer safety.

Objectives

The aim of this study was to determine the biofilm formation ability of 42 *A. butzleri* isolates obtained from different food products and to study the prevalence of ten putative virulence genes among adherent isolates.

Methods

Biofilm formation was assayed by microtiter plate method (Teh et al., 2010) and categorized according to Naves et al. (2008). Putative virulence genes were studied by PCR (Douidah et al., 2012; Karadas et al., 2013).

Conclusions

Eight isolates were categorized as weakly adherent and one isolate as strongly, under the tested conditions. Adherence was not related with a specific food product. The genes *cadF, ciaB, cj1349, mviN, pldA* and *tlya* were detected in all adherent isolates; whilst *iroE* (4/9), *hecA* (1/9) and *hecB* (2/9) were detected only in few. *irgA* was not detected.

The occurrence of adherent *A. butzleri* isolates possessing virulence markers in food products of animal origin is an important factor to consider for the foodborne illness risk assessment.
Background

Campylobacter jejuni is a major cause of food-borne diarrheal illness in humans worldwide. Despite being a well known food and water-borne zoonosis, establishing the source of campylobacteriosis is still complicated (Hepworth et al., 2011).

Objectives

The aim of this study was to find genetic markers for clonal complex (CC) and source attribution in C. jejuni.

Methods

Multilocus sequence typing (MLST) and microarray-based genomic hybridization (MCGH) were applied to 69 C. jejuni isolates from different sources (wild birds, surface and wastewater, and human and bovine feces). Oligonucleotide DNA microarrays (BuG@S CJv3.0.0) were used to compare C. jejuni isolates, with C. jejuni NCTC 11168 and RM1221 as reference strains.

Conclusions

The population was genetically diverse. MLST differentiated the isolates into 38 sequence types (STs), of which 29 were included in seven different CCs. Ten new alleles were identified, which resulted in the assignment of 12 new STs.

MCGH confirmed most of the previously described variable and/or plasticity regions (Parker et al., 2006; Hepworth et al., 2011; Stabler et al., 2013). Combining MLST and MCGH results allowed us to detect several potential genetic markers associated with both, specific CCs and certain sources of isolation. More accurately, the genes cas2, cas1, Cj0659c-Cj0660c, Cj0887c, Cj1041-Cj1042, hsdM and hsdR related with the CCs ST-21, ST-42, ST-177 and ST-677; and the genes glpT, ldh and Cj1562 related with isolates from wild birds.
GENETIC DIVERSITY AND HORIZONTAL GENE TRANSFER IN FOODBORNE ARCObACTER CRYAEROPHILUS ISOLATES
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Background

Arcobacter species have been recognized as potential food- and waterborne pathogens, and are implicated in human enteritis. The wide genetic diversity reported among Arcobacter species reflects the continuous evolving nature of these pathogens.

Objectives

The aim of this work was to determine the genetic diversity and virulence gene content of 10 Arcobacter cryaerophilus isolates obtained from clams, mussels and raw cow milk obtained in Vitoria, Spain.

Methods

Genetic diversity was studied by multilocus sequence typing (MLST). The obtained genotypes were analyzed using the MEGA 5.1 software, and possible recombination events were evaluated in silico using the RDP3 software. Recombination events were considered when detected by at least 3 methods. The presence of nine putative virulence genes was determined by PCR.

Conclusions

A total of 9 sequence types (ST) not previously described were identified among the 10 isolates. All the isolates harbored virulence genes. The most frequent were ciaB and mviN (100%), followed by tlyA (40%) and hecA (30%). Due to the incongruent phylogenetic relationships observed for glnA and glyA genes, recombination analysis was performed. This analysis detected potential recombination events in the ST413, ST415 and ST416 isolates, probably derived from interspecies recombination between A. cryaerophilus and A. skirrowii.
From these results, we conclude that MLST typing showed high strain diversity and that recombination should be considered as a potentially relevant mechanism generating genetic diversity in *A. cryaerophilus*. 
FURTHER EVIDENCE FOR STAPHYLOCOCCAL FOOD POISONING OUTBREAKS CAUSED BY SEG AND SEI

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Background
Staphylococcal food poisoning represents the most prevalent foodborne intoxication worldwide. It is caused by oral intake of enterotoxins preformed by Staphylococcus aureus in food. The relevance of enterotoxins SEG and SEI in outbreaks of staphylococcal food poisoning is controversially discussed. Although SEG and SEI elicit emesis in a monkey feeding assay, there has been no conclusive proof of their emetic activity in humans.

Objectives
In this study, we present novel evidence suggesting that SEG and SEI can cause staphylococcal food poisoning and describe the special challenges associated with investigating outbreaks linked to SEG and SEI.

Methods
We analyzed all outbreaks registered with the Swiss Federal Office of Public Health, in which only Staphylococcus aureus strains harboring seg and sei linked to typical signs of staphylococcal food poisoning were isolated (n = 3).

Conclusions
The outbreaks were caused by consumption of raw goat cheese, potato salad, and semi-hard goat cheese, and were linked to strains assigned to CC45 (agr type I), CC30 (agr type III), and CC9 (agr type II), respectively. Investigation of the outbreaks was particularly challenging as, in contrast to classical staphylococcal enterotoxins, there are currently no suitable methods for detection of SEG and SEI in food and feces. Our data provides strong evidence suggesting that SEG and SEI can cause staphylococcal food poisoning in humans. Further research will be necessary to determine the individual role of SEG and SEI in outbreaks.
FOOD POISONING OUTBREAK AMONG CHILDREN AND STAFF AT SWISS BOARDING SCHOOL DUE TO RAW MILK CHEESE CONTAMINATED WITH STAPHYLOCOCCUS AUREUS OF GENOTYPE B

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Background

In 2014, an outbreak of staphylococcal food poisoning occurred at a Swiss boarding school due to consumption of Tomme, a soft cheese produced from raw cow milk. All 14 persons that had ingested the cheese fell ill, among them ten children.

Objectives

We present a food poisoning outbreak caused by raw milk cheese contaminated with a \textit{Staphylococcus (S.) aureus} strain of genotype B.

Methods

The Tomme cheese was tested for presence of staphylococcal enterotoxins and \textit{S. aureus} strains isolated from the cheese were characterized by \textit{spa} typing and a DNA microarray.

Conclusions

The cheese exhibited low levels of staphylococcal enterotoxin A (> 6 ng SEA/g cheese) and high levels of staphylococcal enterotoxin D (> 200 ng SED/g cheese). A total of \(10^7\) CFU coagulase-positive \textit{Staphylococci} per gram cheese were detected, with three different \textit{S. aureus} strains being present at levels higher than \(10^6\) CFU/g. A strain exhibiting \textit{sea} and \textit{sed} was identified as the source of the outbreak. The strain was assigned to \textit{spa} type t711 and CC8 and exhibited genetic criteria consistent with the characteristics of a genotype B strain. This genotype comprises bovine \textit{S. aureus} exclusively associated with very high within-herd prevalence of mastitis and has been described as a major contaminant in Swiss raw milk cheese. It is highly likely that the raw milk used for production of the Tomme was strongly contaminated with \textit{S. aureus} and that levels further increased due to growth of the organism and physical concentration effects during the cheese making process.
TEMPORAL EXPRESSION OF THE STAPHYLOCOCCAL ENTEROTOXIN D GENE UNDER NA CL STRESS CONDITIONS ENCOUNTERED DURING FOOD PRODUCTION AND PRESERVATION
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Background
Staphylococcus aureus represents one of most osmotolerant food-borne pathogens. While its growth is repressed by competing bacteria, the organism exhibits a growth advantage at increased salt concentrations. Staphylococcal enterotoxin D (SED) leads to vomiting and diarrhea upon consumption. To date, the effect of NaCl on both sed expression and its regulatory control are unclear.

Objectives
We aimed to determine the impact of NaCl stress on sed expression and the influence of agr, sarA, and sigB on sed expression under NaCl stress.

Methods
Using qPCR, temporal expression of sed in LB and LB with 4.5% NaCl was compared, as well as sed expression between wild type strains and isogenic Δagr, ΔsarA, and ΔsigB mutants.

Conclusions
In general, NaCl stress led to decreased sed expression. However, one strain exhibited a trend towards increased sed expression under NaCl stress. One ΔsarA mutant each showed decreased sed expression in the early stationary and increased sed expression in the stationary growth phase under NaCl stress. No significant effect of agr on sed expression was detected, and only one ΔsigB mutant showed a significant decrease in sed expression in the early stationary phase under NaCl stress. These findings suggest high strain-specific variation in sed expression and its regulation under NaCl stress.
TRACKING CHANGES OF BACTERIAL COMMUNITY COMPOSITION IN A LARGE INTESTINAL MODEL SYSTEM BY QPCR
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Background
In vitro digestion models are simplified artificial systems to simulate digestion processes in humans/animals. Our digestion system aimed to study structural changes, digestibility and bioavailability of food components and behaviour of microbes. The model consisted of four stages, imitating the environment of mouth, stomach, small intestine and large intestine.

Objectives
Selective agar plates ensured reliable enumeration of bacterial colonies, but cultivation took 2-3 days, especially in case of the slowly growing species (e.g. *Bifidobacterium*), therefore, qPCR technique was applied for enumeration of bacteria.

Methods
Microbial activity was investigated in the large intestinal phase. Bacterial community consisted of two probiotic (*Bifidobacterium* and *Lactobacillus*) and four neutral/potential pathogenic species (*Clostridium*, *Bacteroides*, *Enterococcus*, *Escherichia*), found frequently in the human large intestine. The effect of food components (including prebiotic carbohydrates) on proliferation and survival of bacteria was evaluated on selective agar plates and by qPCR, using newly designed species-specific primers in the latter case.

Conclusions
The use of species specific primers for qPCR-based quantitation was successful, the number of each bacteria was determined precisely. Differences between the plate count and qPCR techniques were within an order of magnitude range in most cases. However, selective agar plates are still widely used for enumeration of bacteria in laboratories, other methods like qPCR are used more and more frequently, due to their speed and reliability. Our experiments showed that carefully selected primers were able to ensure the specificity for quantitation of bacteria from mixed cultures, in the presence of interfering compounds, such as digestion juice.
EFFECT OF OZONATED AND CHLORINATED WATER ON MICROBIOLOGICAL LOAD AND SENSORY PROPERTIES OF FRESH LETTUCE (LACTUCA SATIVA) AND BROCCOLI (BRASSICA)

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Background

Ozonated and chlorinated water used as potential antimicrobials agents in food industry. Since 2001, when US Food and Drug Administration (FDA) announced the permission of Ozon's usage as a suitable antimicrobial agent for food, many investigations have been applied (Singh et al. 2002, Trinetta et al. 2011).

Objectives

The objectives of this experimental work are to determine the effectiveness of ozonated (33.3 mg/l) and chlorinated (50 mg/l) water on the reduction of microbiological load on two vegetable species. The project's target is to estimate the load of four pathogen microorganisms (Escherichia coli, Listeria monocytogenes, Salmonella spp, Enterobacteriaceae) at the different market points and highlight if those specific vegetables are safe for the consumers. One more target is to remark any changes at the qualitative and sensory characteristics. Furthermore, was examined the microbiological load of vegetables when they have been washed with tap, chlorinated and ozonated water.

Methods

The methods which used for the bacteria are Health Protection Agency (HPA), ISO 11290-1:1996/Amd 1:2004, ELOT EN ISO 6579:2003/TC1:2004, Statutory Instrument SI 2383, 1989 and BS 4285:3.7. The part of the sensory analysis consists of a specific panel with ten people, who tasted and evaluated vegetables, after treated and washed with three treatments.

Conclusions
According to the results was proved that the region of origin probably is important for the surcharge of the microbiological load, but this is not affected by the sampling point. Ozonated water has the best performance for all samples for the microbiological control and sensory analysis. In general, broccoli have less Enterobacteriaceae load than lettuce.
DETERMINATION OF KILLER ACTIVITIES OF THE YEAST STRAINS ISOLATED FROM NATURAL FERMENTATION MEDIA OF GEMLIK CULTIVAR BLACK OLIVES

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Background

Yeasts are common microorganisms present in spontaneous table olive fermentation. Killer yeasts secrete protein toxins that are lethal to sensitive strains. *Wickerhamomyces anomalus*, *Kluyveromyces marxianus*, *Pichia guilliermondii*, and *Saccharomyces cerevisiae* strains isolated from olive brines have been shown to produce killer toxins with a broad spectrum of activity against spoilage microorganisms of table olives. The killer characteristics of the yeasts have been applied to combat undesirable yeasts during the production of table olive.

Objectives

The aim of this study was to determine killer activities of the yeast strains isolated during natural fermentation process of ‘Gemlik’ cultivar olives grown in Akhisar and Iznik regions of Turkey. The effect of regional differences on killer yeast profile of Gemlik olives was also investigated.

Methods

A total of 54 endogenic yeast strains were investigated for killer activity. These were belonging to 7 genera as; *Candida*, *Aureobasidium*, *Debaryomyces*, *Cryptococcus*, *Kloeckera*, *Pichia* and *Clavispora*. Killer activity was detected by agar diffusion assay. In the method, two killers; *S.cerevisiae* NCYC 232 (K₁), *S.cerevisiae* NCYC 738 (K₂) and a sensitive strain; *S.cerevisiae* NCYC 1006 were used as reference yeasts.

Conclusions

From 54 strains, 40 were found as killer and 6 were killer-sensitive strains. Additionally, 8 strains were found to have neutral character. All *Debaryomyces hansenii*, *Pichia anomala*, *Clavispora lusitaniae* and *Kloeckera apiculata* strains were found as killer. No sensitive strain was found among the tested isolates. In conclusion, killer behavior of the endogenic yeast strains of Gemlik cultivar olives of Akhisar and Iznik regions was found as common characteristics.
INVESTIGATION OF BRUCELLA SPP. AND BRUCELLA DNA IN RAW MILK OBTAINED FROM TRAKYA REGION, EDİRNE, TURKEY

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Background

Brucellosis is an important zoonotic disease transmitted to human by consuming contaminated milk and milk products. The prevention from Brucellosis is provided by live-attenuated vaccines for animals. Although Trakya is the pilot region in Turkey, for vaccination of animals, Brucellosis is endemoeidemic in this region. Thus, many dairy companies supply their milk from the cows grown in this region; screening of milks for Brucella contamination becomes very important.

Objectives

Our aim was to investigate Brucella spp. in milk, procured from Edirne and compare the conventional and molecular methods in terms of screening Brucella spp. in milk.

Methods

Milk samples from 99 cows at 12 different barns in 5 different villages of Edirne were collected. Bacteriological analyses and Q-PCR were applied to all samples. For samples that were culture negative and Q-PCR positive, a Q-PCR based method was evolved to differentiate the virulent and the vaccine strains.

Conclusions

In 2 of the 99 milk samples, Brucella spp. was isolated by bacteriological methods. After Q-PCR, B. ovis and B. melitensis were not detected, however, in 16 of the 99 samples B.abortus was detected. After the Q-PCR based method evolved to differentiate virulent and vaccine strains, only the 2 bacteriologically positive samples were detected to be positive.

As a result, it is reported that; 2.02% of the samples were detected to contain Brucella, both with the bacteriological method and PCR. It is also determined that, to obtain true positive results in Brucella spp. screening studies for milk, differentiating the virulent and vaccine strain should not be disregarded.
PRODUCTION OF ACE-INHIBITORY PEPTIDES BY LACTIC ACID BACTERIA ISOLATED FROM TRADITIONAL GREEK DAIRY PRODUCTS.

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Background

Fermented dairy products are generally considered to be beneficial for human health as they contain live lactic acid bacteria frequently exhibiting biofunctional features. Here we present 55 lactic acid bacteria isolated from three traditional Greek yogurt samples and five fermented milk samples where their ability to produce bioactive peptides with inhibitory activity against the angiotensin-converting enzyme (ACE) was evaluated.

Objectives

The aim of this study is the potential of several lactic acid bacteria strains to be used as health promoting starter cultures with interesting technological features.

Methods

Strain typing was performed by rep-PCR, while 16S rDNA sequencing was used for the identification at the species level. The angiotensin-converting enzyme inhibitory (ACE-I) activity of the isolates was evaluated using a spectrophotometric assay with N-Hippuryl-His-Leu hydrate as substrate. Finally, a semi-preparative HPLC analysis was conducted using an ACE-5C18 column and positive fractions were subjected to MS analysis.

Conclusions

During this work 55 microorganisms have been isolated from traditional Greek yogurt and fermented milk samples. Strain typing by rep-PCR showed that the 33 isolated bacilli corresponded to 10 and the 22 cocci to 12 strain groups, respectively. The
strains did not show neither strong acidification capacity, nor peptidolytic activity and citrate metabolism, so their use as starter cultures does not seem suitable. Among them, many strains possessed interesting technological features as proteolysis and lipolysis and few exhibited high ACE-I activity. Purification of the ACE-I peptides was performed by RP-HPLC. These results indicate their potential use as adjunct or probiotic cultures.
EVALUATION OF CINNAMON AND MARJORAM ESSENTIAL OILS FOR THE CONTROL OF MYCOTOXIGENIC FUSARIUM SPECIES

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Background

F. culmorum, and F. graminearum are the most common causes of Fusarium ear blight of corn, wheat and other grains. Both of them produce the trichothecenes mycotoxins and F. graminearum also produces zearalenone.

Objectives

The aim of this study was to evaluate the efficacy of cinnamon and marjoram essential oil (EOs) to control the growth of Fusarium species in vitro and to observe the changes of morphological characteristics of the fungi by SEM images after treatment with the oils.

Methods

Colony growth inhibition effect of the EO vapors was investigated on the Fusarium species by the reversed Petri dish method using 0.070 mg/l, 0.14 mg/l, 0.28 mg/l and 0.56 mg/l EO/dish. The antifungal effect was measured by determination of growth-rate (mm/day) and antifungal index (%). MIC (minimal inhibitory concentration) and MFC (minimal fungicidal concentration) values were determined by agar dilution method. Morphological characteristics of the hyphae and micro conidia of F. culmorum were observed using a scanning electron microscope.

Conclusions

Cinnamon EO vapor at 0.28 mg/l concentration caused total growth inhibition of all investigated species. Using marjoram EO vapor total inhibition was not detected, but growth-rates and colony diameters were reduced in each case. Agar dilution method was more effective to inhibit the growth of F. culmorum (MIC value =2.5 mg/ml). SEM imaging of F. culmorum showed that EO treated hyphae were thinner. Rupture of the cell wall and leakage of the cytoplasm contents were also observed. This study showed that EOs have potential antifungal effect against mycotoxigenic Fusarium species.
Background
In fermented foods, the presence of biogenic amines (BAs), which were produced by microbial decarboxylase activity of microorganism, might serve as a useful indicator of food poisoning. Although BAs are also frequently found in ganjang, representative Korean traditional soy-sauce, there is no information about the BAs-producing microorganism in ganjang.

Objectives
In this study, to investigate microorganism responsible for BAs production in ganjang, metabolite and bacterial community analysis were performed during entire fermentation process of ganjang.

Methods
Ganjang with a NaCl concentration of approximately 18% (w/v) was prepared in one batch using traditional manufacturing method. The same amounts of ganjang were periodically sampled and their pH, NaCl concentration, and cell number were measured. $^1$H NMR and PCR-DGGE were applied for analysis of metabolites and bacterial succession during the ganjang fermentation, respectively. In order to investigate the correlations among the ganjang samples, bacterial communities, and metabolites, a multivariate statistical redundancy analysis was performed using the vegan package of the R programming environment.

Conclusions
Metabolite profiling and bacterial community analysis using a $^1$H NMR and PCR-DGGE showed that a large amount of putrescine detected after 74 days of fermentation, which time begins to predominate the members of Chromohalobacter. A statistical analysis based on metabolite profiling and bacterial succession data clearly shows that members of Chromohalobacter are involved in the production of putrescine during ganjang fermentation. This study will allow for the successful understanding of the BAs-producing microorganism in ganjang.
CHARACTERISTICS OF CONTAMINANT MICROBES IN COMPOUND FEEDS AND THE GROWTH INHIBITION EFFECT BY PROBIOTIC B. SUBTILIS

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Background

During long term storage of animal compound feed, contaminated microbes can grow with providing a problem of feed safety.

Objectives

The aim of this study was to investigate contaminant microbes and the effects of probiotic B. subtilis to inhibit them in animal feeds.

Methods

During storage of feed at room temperature, the changes of pH and titrable acidity were monitored with total microbial counts. Bacterial and fungal strains were isolated and tested for antibiotics resistance. Contaminated microbes were further identified by the sequencing of 16S and 18S rDNAs.

Probiotic B. subtilis was inoculated into both non-sterile and sterile feeds and tested for the growth inhibition of contaminated microbes during storage of feed at room temperature with pH change and probiotic viability.

Conclusions

Most contaminated microbes were originated from soil and plants. Sterile and non-sterile compound feeds displayed a different pattern of pH change. B. subtilis showed a normal growth and inhibited the cell growth of contaminant microbes.
EVALUATION OF MICROBIAL CONTAMINATION LEVELS AT THE PRE- AND POST-GERMINATION STAGES ON SPROUTS.

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Background
The consumption of raw sprouted seeds has led to a large number of outbreaks of foodborne illness in a great number of countries.

Objectives
The objective of this study was to investigate and evaluate microbial contamination levels of several kinds of sprouts in pre- and post-germination stage.

Methods
Fourteen kind of sprout seeds were purchased and analyzed. Quantitative analyses comprised aerobic plate counts (APCs) and the measurement of coliforms and Bacillus cereus levels, whereas qualitative analyses involved assessing the levels of Escherichia coli and major foodborne pathogens (E. coli O157:H7, Listeria monocytogenes, Salmonella spp. and Staphylococcus aureus).

Conclusions
The APCs (2.3-4.07 log CFU g\(^{-1}\)) for sprouts seeds increased by approximately 3-4 log CFU g\(^{-1}\) during germinating, reaching 5.61-8.94 log CFU g\(^{-1}\). Similarly, increasing trends were noted in the level of B. cereus (N.D.-2.62 log CFU g\(^{-1}\) at the seed stage, increasing to N.D.-3.14 log CFU g\(^{-1}\) by the germinated stage). E. coli, E. coli O157:H7, Salmonella spp. and L. monocytogenes were not detected in the sprouts analyzed in this study. The present study provides comprehensive information regarding the microbiological safety of seeds and sprouts during manufacturing.
Background

Antioxidants are compounds with role in food preservation, as well as in the prevention and treatment of some degenerative diseases. Despite their high amounts in plants, there is a growing interest for less conventional sources. Cyanobacteria, as a particular producers of bioactive compounds, represent an important source of antioxidants, which in terms of chemical structure include diverse compounds.

Objectives

In the present study, the antioxidant capacity of three filamentous cyanobacterial strains belonging to the Anabaena, Nostoc and Spirulina genera have been tested. Furthermore, the content of C-phycocyanin, a pigment known as antioxidant compound was determined.

Methods

The antioxidant capacity was tested using DPPH and FRAP assays. In DPPH assay, strains Spirulina S1 and Anabaena C5 showed similar IC_{50} values of 118.47 µg/ml and 120.26 µg/ml respectively. In FRAP assay, the highest antioxidant activity was observed in Spirulina strain S1, ranging from 452.41 to 637.38 mg of ascorbic acid (AA) equivalent/gram of dry extract. The lowest antioxidant capacity expressed strain Nostoc 2S9B in both assays (IC_{25} of 21.93 µg/ml, and 142.21-350.18 mg of AA equivalent/gram of dry extract).

The C-phycocyanin content was determined spectrophotometrically. The highest value of 38.63 µg/ml was detected in strain Spirulina S1, while lower contents were obtained in strains Nostoc 2S9B and Anabaena C5 (6.57 µg/ml and 9.34 µg/ml respectively).

Conclusions
Since *Spirulina* strain S1 has expressed the highest antioxidant capacity in both assays, it could be further investigated as a potential source of antioxidants. High C-phyocyanin content found in this strain could be associated with its antioxidant activity.
IDENTIFICATION OF BIOLOGICAL HAZARDS IN SPICES AND HERBS

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Background
Securing the food chains from primary production to consumer-ready food against major deliberate, accidental or natural contaminations is directly related to the safety of food products. Spices and herbs are natural products of almost all processed food that can be contaminated with several microorganisms and toxins. The identification of those condiments would be difficult, as consumers and experts often focus on major food ingredients instead of minor components. Securing of spices and herbs commodity chains is the key issue within the EU FP7 project SPICED.

Objectives
Characterization of the heterogeneous matrices and their respective production and supply chains in context with relevant biological hazards and the improvement of the knowledge on biological hazard properties as well as on-site and high throughput diagnostic methods for appropriate detection are main tasks.

Methods
Within the SPICED project we mainly focusses on the toxins Ricin, which is the toxic component from castor beans of Ricinus communis, and the Enterotoxin-type B produced by the gram-positive bacteria Staphylococcus aureus (SEB). Their qualitative and quantitative detection in spices and herbs including the development of sample preparation methods were performed. For this purpose different types of spices and herbs were spiked with the toxins, stored at room temperature and analyzed e.g. with immunological based methods. Fieldable technologies and methods for the rapid on-site detection of toxins in the different were also examined.

Conclusions
The intention is to present the recent results on the detection and sample preparation of the toxins from the spices and herbs with laboratory and field methods.
EFFECT OF THERMALLY PROCESSED RICE KOJI EXTRACTS ON CHRONOLOGICAL LIFE SPAN OF SACCHAROMYCES CEREVISIAE ON HIGH-SUGAR FERMENTATION
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Background

Yeast cell growth and viability on high ethanol concentrations become limiting factors in fermentation productivity and ethanol yield. That is why measures to improve ethanol tolerance in yeast have been expected.

Objectives
This study focused on koji as a material for sake brewing which provides nutrients for yeast cell growth. Our goal is to develop strategies for extending the life span of yeast by revealing the influence of heat-processed koji extracts on the survival of yeast under ethanol stress.

Methods

Rice koji extracts were prepared by water extracts of dried rice koji heated at 60°C for 5 hrs. The koji extract was autoclaved for different times at 121°C. The availability of thermally processed rice koji extracts was investigated by analysis of ethanol tolerance and survivals on high-sugar fermentation in S. cerevisiae K-701 while making a comparison between heated and non-heated koji extracts.

Conclusions
Addition of heat-processed *koji* extracts to high-sugar medium led to an improvement of chronological life span in *S. cerevisiae* K-701. The longer the heating time of *koji* extracts was, the higher the population of *S. cerevisiae* was during the late stationary phase. The Maillard reaction products which occurred in the heat-processed *koji* extracts had an explicit effect on the survivals of yeast under lethal ethanol stress. These show that heat-processed rice *koji* extracts were served as protectants of both ethanol and oxidative stresses when yeast cells became more oxidized in the stationary phase at higher ethanol concentrations.
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STUDY FOR INCREASING THE ANTIOXIDANT COMPOUNDS BY BACTERIA ISOLATED FROM FERMENTED FOOD
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Introduction
Oxidative stress arising from an imbalance in the human antioxidant status, reactive oxygen species vs. defense and repair mechanisms, is mainly responsible for the development of pathological disease. Phenolic compounds in safflower seeds are receiving much attention as potential therapeutic agents against several pathological diseases. The antioxidant properties of safflower seeds is matairesinol 4'-o-β-D-glucose, 8'-hydroxyarctigenin 4'-o-β-D-glucose, matairesinol, 8'-hydroxyarctigenin, N-feruloylserotonin 5-o-β-D glucoside, N-(p-coumaroyl)-serotonin-5-o-β-D-glucoside, N-feruloylserotonin, N-(p-coumaroyl)serotonin, luteolin 7-o-β-D-glucoside, luteolin, acacetin 7-o-β-D-glucuronide, and acacetin.

Objective.
In our experiment, bacteria isolated from ginger fermented food were used to make phenolic compound from aglycon. It makes to increase antioxidant activity of safflower seeds.

Methods
Fermented samples were got from one of the member of Korea royal cuisine institute. Fermented sample was inoculated in tryptic soy broth media and incubated 28°C for 48h. After bacteria grow, bacteria were centrifuged 6000rpm at 4°C for 20 min. Bacteria were dissolved 60ml mineral salt media (MSM) (20g/L) and mixed with 30g safflower seed. Each bottle was incubated 28°C for one week.

For phenolic compound extraction, each safflower seed mixed with bacteria was sonicated in hexane for 30min twice and filtered. The defatted seed residue was extracted twice with 70% EtOH under sonication, filtered and evaporated under reduced pressure. The extract was used for HPLC and GC-MS and DPPH activity.

Conclusion
This study was to evaluate the antioxidant activity in safflower seeds after fermentation. Bacteria that improved antioxidant activity in safflower seeds after fermentation was identified Sphingomonas sp using 16s rRNA sequencing. Safflower seed was improved antioxidant activity from 7.11% to 51% after 5 days. In HPLC analysis, Major peak of safflower seed extracts after fermentation was changed after 5 days under aerobic condition at 30°C. Furthermore analysis, we will conduct to investigate which compound in the safflower seed will be changed to phenolic compound for increasing antioxidant activity.
EFFECTS OF SEED TREATMENT BY COLD ATMOSPHERIC PLASMA ON CUCUMBER SEEDLING GROWTH
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Background
Cold atmospheric plasma can have different effects on seed germination and seedling growth depending on the plasma frequency, the power and its environmental conditions.

Objectives
Cold plasmas are already well-known for their improvement effect on plant growth. However, the mechanisms of this ability are unclear.

Methods
Non-thermal atmospheric-pressure DBD plasma in He was applied with different exposure times on cucumber seed. Changes in the seed surface with different exposure time were examined by scanning electron microscope (SEM). Direct and indirect influence on cucumber seed of the cold plasma is compared in terms of seedling growth.

Conclusions
Germination and early seedling growth of cucumber were improved by the plasma treatment for 6 second. In contrast, the seeds exposed for 10 minutes were not germinated. In 6 sec treatment and no treatment control, gold nanoparticles were observed along the cell wall. There were no particles observed in the 10 min treatment. It suggests that no tissue damage is observed visually or microscopically following 6 sec and control, but the rough surface of seeds were smooth and damaged at long exposure time (10 min). We observed that the indirect method had more enhanced effect on growth than direct method. This infers to an important conclusion that the effect of plasma on improved growth of seedlings is due to various plasma components that can exist inside or outside the plasma region not mechanical power such as an abrasion of seed surface.
METABOLICALLY ACTIVE BACTERIA IN LYMPHATIC TISSUES OF PIGS AND ITS SPREAD DURING SLAUGHTER
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Background
The occurrence of potential food-borne pathogenic organisms in lymphatic tissues of farm animals has practical relevance for carcass contamination. A transmission of pathogens, which can occur from harvesting until the last step in the meat-processing chain, remains the preeminent difficulty in slaughter house hygiene.

Objectives
The aims of this study were i.) to evaluate the contamination degree of porcine cervical musculature during slaughter, ii.) to compare the bacterial microbial community detected by DNA-based amplicon sequencing with RNA-based amplicon sequencing and thus measuring the fraction of metabolically active bacteria in lymphatic tissues.

Methods
Mandibular lymph nodes, tonsils and scrapings from cervical musculature were sampled from eight healthy slaughter pigs derived from different farmers. RNA was isolated from lymph node cortex regions and tonsils (n=16) and DNA was isolated from cervical musculature (n=8). Total RNA was transcribed into cDNA and all samples were sequenced with Roche/454 using 16S rRNA gene amplicons.

Conclusions
Organisms relevant for food safety, e.g. *Erysipelothrix, Listeria, Escherichia* and *Pseudomonas*, could be detected in lymphatic tissues. RNA-based amplicon sequencing revealed a highly diverse, metabolically active bacterial microbiome in mandibular lymph nodes and tonsils; consisting of 348 and 390 operational taxonomic units (OTUs) respectively (30% shared OTUs). The functional metagenome prediction (PICRUST) and subsequent assignments of orthologues to COG categories revealed amino acid transport and metabolism-related orthologues being increased in lymph nodes compared to tonsils. Both, mandibular lymph nodes and tonsils of slaughter pigs harbor a replication-competent bacterial microbiome indicating a possible contamination source during and after slaughter.
Background
Food safety is an area of growing concern due to the increasing demand for microbiologically safe products. Additionally, disinfection techniques must evolve to cope with the development of antimicrobial resistance. An efficient disinfection strategy must consider the type of microbial contaminant. Therefore, the knowledge on the microorganisms present in an industrial process is crucial to define the best strategy for their control.

Objectives
The aims of the present work were to isolate and characterize the resident microflora present in a minimally processed vegetables (MPV) plant.

Methods
The microorganisms were isolated from a MPV plant process surfaces by cotton swabbing, from the air and from the vegetable surfaces. The isolates were identified by 16S rRNA gene sequencing with 4 primers: 27F, 518F, 800R e 1492R. The isolates were also characterized for their production of proteases, gelatinases and siderophores, quorum-sensing inhibition and biofilm formation ability.

Conclusions
From 50 different isolates 46% belong to the Pseudomonas genera and 22 were from different species. Some microorganisms persisted along the food chain, which means that the cleaning process should be targeted for the more resistant microorganisms. Most of the microbial isolates are capable of producing virulence and food spoilage molecules. Moreover, Pseudomonas were the genera with a higher biofilm formation ability, being the predominant microflora and showing recalcitrant properties along the process chain.
Background

Biogenic amines (BA) are low-molecular nitrogenous organic bases, which are formed in foodstuffs by microbial decarboxylation of the precursor amino acids and are potentially toxic to human health. Cheeses are among the foods most commonly associated with the presence of BA (1). Concentration of BA results from a balance between amino acid decarboxylating and amine oxidising activities.

Objectives

To correlate the content of BA of twenty traditional Apulian or Sicilian (Southern Italy) cheeses with several technology and microbiological features such as time of ripening, pH, concentration of precursor free amino acids (FAA), and occurrence of decarboxylase-positive lactic acid bacteria.

Methods

Cheeses were analysed through plate counting and HPLC, and lactic acid bacteria isolated from cheeses were assayed (by decarboxylase medium) for their capacity to generate BA. Principal Component Analysis was performed to find the effect of different parameters on the distribution of the cheeses.

Conclusions

Although short-ripened cheeses did not show significant BA concentrations, the only BA showing high positive correlation with time of ripening was histamine. Concentration of histidine and, especially, percentage of histidine-decarboxylase bacteria presumably affected histamine concentration. High pH values were negatively correlated to the concentration of tyramine, putrescine, and cadaverine (Fig. 1). Fifty percent of the cheeses contained at least one BA at potentially toxic concentrations. Unambiguous and ever-valid relations among parameters and BA are difficult to determine, because BA are the result of combined and varied factors.
Factor 1: 30.55%

Factor 2: 22.26%

Plot showing the relationship between different variables such as putrescine, tyramine, type of rennet, starter, cadaverine, orn+, orn, histamine, his, lys, ty+, his+, ty, time of opening, and pH.
DYNAMICS OF BACTERIAL COMMUNITIES DURING THE RIPENING PROCESS OF CROATIAN RAW EWE’S MILK CHEESE
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Background

Croatian raw ewe’s milk cheeses are hard cheeses that are produced by traditional techniques without pasteurization and application of starter cultures, and they are characterized by an aging process of 90-120 days. This aging time is rather long and the low pH and water activity (aw) of ripened cheese usually does not support the growth of pathogens. However, due to the commercial pressure, the ripening time has been shortened and it has become market practice to sell the cheese as soon as possible.

Objectives

A close monitoring of bacterial communities and a reliable identification of indigenous microflora is crucial in order to maintain the quality and safety of artisan cheese.

Methods

Cheese samples of three cheese types (0, 45 and 90 days of ripening) were collected from 6 cheese makers. Total DNA was extracted and microbial diversity was investigated based on fingerprinting in combination with next generation sequencing of 16S rRNA gene amplicons.

Conclusions

Overall up to 213 OTUs could be assigned. Twenty of the major OTUs were present in all samples and include mostly LAB, mainly Lactococcus and Enterococcus species. Abundance and diversity of these genera differed to a large extent between the 3 investigated cheese types and in response to the ripening process. Also a large number of non LAB genera could be identified based on phylogenetic alignments including mainly Enterobacteriaceae and Staphylococcaceae. Some species belonging to these two families could be clearly assigned to species which are known as potential human pathogens. However, during cheese ripening their abundance was reduced.
Background
Sausages from game meat produced by traditional procedures by small scale producers in Croatia do not include the application of starter cultures. Such artisan products are increasingly appreciated because of their sensory properties and authenticity, although considerations regarding their microbiological safety are existent.

Objectives
An in depth analysis and characterization of the indigenous microflora of traditionally produced wild boar meat sausages from Croatia is needed in order to estimate microbiological hazards and to preserve an indigenous microbial pool.

Methods
Traditionally produced wild boar sausages were collected from three farms during ripening (0, 2, 4, 7, 10, 20 and 40 days) and analyzed for spoilage and pathogenic microflora (*L. monocytogenes*, *Salmonella* spp., total *Enterobacteriaceae*, coliforms, yeasts and molds, *B. cereus* group and coagulase-positive staphylococci) as well as for beneficial microbes e.g. lactic acid bacteria (LAB) and coagulase-negative staphylococci (CNS). Genomic DNA was extracted from 720 LAB and CNS isolates. After RAPD and rep-PCR based grouping identification was done by PCR and partly 16S rRNA gene sequencing.

Conclusions
A remarkable diversity was found among the investigated LAB and CNS populations of which several isolates relevant for sausage ripening and product quality were identified as e.g. *Lactobacillus sakei*, *Leuconostoc mesenteroides*, *Staphylococcus xylosus*, *Staphylococcus saprophyticus* and *Staphylococcus warneri*. Although some spoilage and pathogenic microflora were present at the initial stages of sausages’ ripening, they were absent in the ripened “ready to eat” sausage.
REDUCING THE RISK OF BABY LEAF SURFACE BACTERIA WITH IRRIGATION RESTRICTIONS

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Background

Baby leaf crops like Spinach (\textit{Spinacia oleracea}), Chard (\textit{Beta vulgaris}) and Rocket (\textit{Diplotaxis tenuifolia}) are grown on huge open space flat fields in Sweden. It is hard to stop birds and wild animals to visit the field with related risks of E. coli infections. But as a grower, one can make sure the irrigation water doesn’t act as a bacteria vector.

Objectives

The main objective of this study was to reduce the risk of E. coli bacteria reaching baby leaf crops during crop growth in field, thereby reducing the risk of food borne diseases further down the delivery chain.

Methods

Leaves were grown with more or less constant optimal wet conditions in the root zone (irrigation water given up to field capacity every other day) or with restricted irrigation (drought conditions) in the final part of the growth period. 24 hours prior to harvest plants were sprayed with a known quantity of E. coli. Harvested leaves were put in a stomacher and the leaf rinsates plated on agar and the colonies counted and compared to non-sprayed leaves.

Conclusions

Irrigation restriction only had minor impact on E. coli colonization on the leaf surface. The yield and quality was significantly reduced and the content of bioactive compounds affected. The main conclusion is that drought stress during the final part of baby leaf production is not to recommend and as a way to reduce the risk of food borne diseases caused by baby leaf salads.
Background
The presence of coliform bacteria is routinely assessed to establish the microbiological safety of water supplies and raw or processed foods. Coliforms are a group of lactose-fermenting Enterobacteriaceae, which most likely acquired the lacZ gene by horizontal transfer and therefore constitute a polyphyletic group. Among this group of bacteria is Escherichia coli, the pathogen that is most frequently associated with foodborne disease outbreaks and is often identified by β-glucuronidase enzymatic activity or by the redundant detection of uidA by PCR.

Objectives
Because a significant fraction of essential E. coli genes are preserved throughout the bacterial kingdom, alternative oligonucleotide primers for specific E. coli detection are not easily identified.

Methods
Here two strategies were used to design oligonucleotide primers with differing levels of specificity for the simultaneous detection of total coliforms and E. coli by multiplex PCR. A lacZ sequence and an orphan gene were chosen as targets for amplification.

Conclusions
A comparison with previously described primers indicates an increase in identification efficacy when tested with laboratory collection and lactose-fermenting strains isolated from dairy samples. While lacZ amplicons were found in a wide range of lactose-fermenting strains, amplification using the selected orphan gene was highly specific for E. coli. Additionally, the detection of this target sequence is non-redundant with enzymatic methods.
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Food microbiology

EFFECT OF OXYGEN CONCENTRATION AND GAS PRESSURE ON THE GROWTH OF ARTHROSPIRA SP. PCC 8005
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Background
The MELiSSA project of the European Space Agency, ESA, aims to create a bio-regenerative life support system for long term space travels. One of its bioreactors contains Arthrospira sp. PCC 8005, an edible cyanobacteria that can remove CO₂ and provide O₂. Normal operating conditions will likely enhance oxygen concentration and gas pressure buildup.

Objectives
The objective of this study is to elucidate the impact of oxygen concentration and gas pressure on the duration of the lag phase, the specific growth rate and the maximum number of attained generations in a batch culture of Arthrospira sp. PCC 8005.

Methods
Cultures of Arthrospira sp. PCC 8005 were grown in 100 ml closed septum vials with Zarrouk-UBP medium, at 30 ⁰C with 32 µE m⁻² s⁻¹ of irradiance and shaken at 130 rpm. Growth was monitored measuring OD₇₅₀nm. Produced oxygen was accumulated. Oxygen tests were done with an initial 100% oxygen gas phase, in comparison to 100% argon, both at initial 1 bar. Pressure tests were done at 1, 2 and 3 bars of starting absolute pressure in 100% argon. Growth parameters were calculated using a modified Gompertz function. Statistical analysis was performed with 95% confidence interval T-Test.

Conclusions
The oxygen concentration has only a limited but statistically significant effect on the growth rate. Growth tests at elevated pressures of up to 3 bar had no effect on neither growth parameter. Test results at elevated pressures with 100% oxygen pressures, are expected to prove and further quantify the inhibitory effect of oxygen on the growth.
DETECTION OF ITURIN AND FENGYCIN BY MALDI-TOF IN FOOD MATRIX OF PUBA, A STAPLE BRAZILIAN FERMENTED FOOD
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Background
Puba or carimã is a Brazilian staple food obtained by spontaneous submerged fermentation of cassava (Manihot esculenta, Crantz) roots. Traditional fermentation of cassava is predominantly performed by lactic acid bacteria, but yeasts and Bacillus spp. were also found in our sample.

Objectives
To investigate the production of lipopeptides by Bacillus sp. P5 isolated from puba in the culture medium and in puba matrix by MALDI-TOF.

Methods
In a previous study, 16S rRNA of Bacillus sp. P5, isolated from puba, was sequenced. The phylogenetic analyses showed recovery in a node with 92% of support with B. amyloliquefaciens. The culture supernatant was subjected to extraction with n-butanol (Landy medium) purification by reversed-phase HPLC (C18 column) and MALDI-TOF mass spectrometry analysis. Surfactins, iturins and fengycins were detected. To investigate the production of lipopeptides by Bacillus sp. P5 isolated from puba in the culture medium and in puba matrix by MALDI-TOF. An aqueous suspension of puba was extracted with butanol and concentrated in ZipTip® C18. Molecular mass was determined by MALDI-TOF. Data were acquired in the mass range m/z 1,000-4,000 for the butanol extract. The results are shown in Figure 1. Two clusters were found: iturin and fengycin (Fgy) (1A). Expansion of the spectrogram of Fgy indicated protonated ion and sodium and potassium adducts (1B), and in (C) MS/MS spectrum of precursor ion of m/z 1478.5 (Fgy C_{17}) which generated fingerprints of fgy A.

**Conclusions**

In conclusion, puba is manufactured in an unhygienic environment, and iturin and fengycin exhibit biocontrol properties that are probably important for the safety of this product.
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BACTERIAL ASSESSMENT OF FOOD - HANDLERS IN SARI CITY AND ITS SUBURB, MAZANDARAN PROVINCE, NORTH OF IRAN.
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Background
Diarrheal diseases, mostly caused by food borne or waterborne microbial pathogens, are leading causes of illness and deaths in developing countries, killing an estimated 1.9 million people annually at the global level.

Objectives
This study was designed to determine the prevalence of carriers of pathogenic bacteria among the food-handlers employed at food service facilities in Sari city and its suburbs, Mazandaran Province, Iran

Methods
Of the total subjects examined, 62.2 % were found to be carriers of some pathogenic bacteria.

The pathogens isolated and identified were the bacteria Staphylococcus aureus, shigella sonnei and pseudomonas aeruginosa.

Fast food makers were the greatest prevalence of bacteria in respect of their fingernail contents (86.6 %, \( p=0.04 \)) followed by butchers (76.4%), storekeepers (73%), bakers (58.3%) and restaurant workers (51.5 %). Staphylococcus aureus was the predominant bacteria isolated from nail-washing samples (45.9%), followed by Escherichia coli (28.4%), coliform (18.2%) and Pseudomonas aeruginosa (6.5%),

Illiterate food–handlers had the highest percentage of positive cultures from nail-washing specimens (86.8%, \( p= 0.0005 \)) and the lowest rate of infestation was among university educated subjects (33.3 %).

The rate of of Staphylococcus aureus nasal carrier among food-handlers was 18.6 % with the highest rate among store-keepers (31.7%) followed by bakers (24.3%), restaurant workers (19.5%), butchers (14.6%) and fast-food makers (9.7%). Shigella sonneii were isolated from stool samples of two food-handlers (0.9%).

Conclusions
The finding emphasized the importance of food-handlers as potential sources of infections. A medical check–up program with health education could improve worker’s health status.
Background

Lactic acid bacteria (LAB) are living organisms that play a major role in many food and dairy products as a probiotics. Donkey’s milk is being used as a source of human nutrition, especially for infants with allergy to cow milk proteins, due to its antimicrobial components, many defense factors and probiotic properties. Few studies have so far dealt with the microbiota in donkey’s milk, specifically lactic acid bacteria (LAB) diversity.

Objectives

This study was undertaken to identify the microflora of LAB in donkey’s milk in IRAN.

Methods

The culture-dependent microbial and 16S rRNA sequencing techniques were used to identify lactic acid bacteria species in donkey milk. A total of 250 LABs from donkey milk were assessed by culture-dependent microbial tests, including culture at different temperatures, pH and Multiple antibiotic resistance indexing.

Conclusions

*Enterococcus faecalis* (55%) and *Streptococcus devriesei* (45%) were the most prevalent species in donkey milk. No other LAB belonging to the most technically important genera was identified. Biochemical and sugar fermenting analyses confirmed the results of 16S rRNA sequencing. Both species identified were sensitive to nearly all antibiotic tested, including vancomycin. Few strains were found to have >99% sequence similarity with *Streptococcus devriesei*, indicating the presence of this LAB in donkey milk. This is the first study to investigate the LAB microbiota present in donkey’s milk in IRAN.
BIOGEOGRAPHY OF NON-SACCHAROMYCES YEASTS ASSOCIATED WITH SPONTANEOUSLY FERMENTED MUSTS FROM DIFFERENT VINEYARDS AND WINE PRODUCING REGIONS IN GREECE

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Background

Non-Saccharomyces (wild) yeasts may confer diverse chemical composition and improved sensory properties to wines. Yet, our knowledge on population structure of these species is limited.

Objectives

Species diversity and population structure of 486 wild yeasts associated with spontaneously fermented musts from two geographically separated regions in Greece, Peza and Nemea, were assessed. The efficiency of different fingerprinting methods in resolving yeast populations was evaluated.

Methods

Species identification was conducted by PCR-RFLP analysis. For molecular typing the tandem repeat-tRNA (TRtRNA)-PCR and RAPD analysis with primers (GTG)₃, R₅ or RF₂ were applied. Molecular patterns and vineyard populations were clustered by Principal Coordinates Analysis and UPGMA, respectively. Differences of populations were evaluated by analysis of molecular variance (AMOVA).

Conclusions

Ten populations of wild species were identified and further genotyped by TRtRNA-PCR with the primer ISSR-MB (Lachancea thermotolerans, Metschnikowia pulcherrima and Hanseniaspora osmophila), and RAPD with the primer RF₂ (Candida zemplinina), (GTG)₃ (Hanseniaspora uvarum and Hyphopichia pseudoburtonii) or R₅ (Issatchenkia orientalis, Pichia anomala, Torulaspora delbrueckii, and Hanseniaspora guilliermondii). Except for I. orientalis, the percentage of biodiversity of yeast populations was higher in Nemea than in Peza. No particular grouping of banding patterns according to the sampling point or to the vineyard of origin was observed.
However, AMOVA showed that populations of *C. zemplinina* and *L. thermotolerans* differed significantly between the two regions. Results point to the presence of region specific sub-populations of non-*Saccharomyces* yeasts species that could enhance the Greek wines distinctiveness.

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Background
According to the microbial terroir concept, the use of indigenous yeasts as starters in winemaking may add to the uniqueness of regional wines.

Objectives
Genetically different yeast strains from spontaneously fermented musts from Santorini island, Mantinea plateau and Nemea region were characterized phenotypically in order to select strains with desirable enological properties to be used as starters.

Methods
Strains belonging to Candida zemplinina, Issatchenkia orientalis, Hanseniaspora quilliermondii, H. opuntiae, Lachancea thermotolerans, Saccharomyces cerevisiae and Torulaspora delbrueckii were evaluated for tolerance to ethanol, SO$_2$ and different temperatures, H$_2$S and biogenic amines production, maximum population size, foam generation, killer and flocculation phenotype.

Conclusions
Most of S. cerevisiae strains tolerated the selective pressures assayed, exhibiting growth at 16% ethanol, 250 ppm SO$_2$ or 37 °C. Lower tolerance limits were observed for non-Saccharomyces yeasts, with strains of C. zemplinina, I. orientalis and T. delbrueckii growing well at concentrations of 14% ethanol or 200 ppm SO$_2$. Most strains did not produce histamine, tyramine, putrescine, cadaverine phenylethylamine, tryptamine and isoamylamine, presented low foaming, no-flocculation or killer phenotype and were resistant to killer toxins. Substantial intra- and inter-species differences were observed in H$_2$S production. Present results highlight the phenotypic diversity among indigenous yeasts and reveal strains with prominent oenological traits that could be exploited in local wine production.

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DEVELOPMENT AND EVALUATION OF YOGHURT FLAVOURED WITH TAMARIND (TAMARIDUS INDICA)

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Background

Yoghurt is produced by bacterial fermentation of milk sugar (lactose), using Streptococcus thermophilus and Lactobacillus bulgaricus, producing lactic acid give yoghurt its texture and its characteristic tang. To modify certain properties of yoghurt, the fermented milk could be blended with ingredients fruits (for flavor and color) and sucrose or aspartame (sweetness). Exotic natural or artificial flavors to boost yoghurt like vanilla, orange, pineapple, strawberry, raspberry, cinnamon among others. However, tropical underutilized flavours like tamarind (Tamarindus indica) have not been exploited.

Objectives

To develop and evaluate yoghurt flavored with Tamarind chemically, microbiologically and organoleptically.

Methods

Tamarind fruit was pulped to juice and treated in three ways (sweetened with sugar, honey or without sweetener). Skimmed milk and water are homogenized, pasteurized for 15 mins at 85°C, and cooled to 42 - 44°C. The starter culture inoculated, mixed and incubated for 12 hour fermentation, flavoured with graded levels (0, 2, 4, 6, 8, 10, 20, 30, 40, 50 %.) of tamarind juice and stored for 0 to 21 days with weekly monitoring. The sample was then subjected to proximate, micro-nutrient, microbiological and sensory analysis.

Conclusions

Micro-nutrient content (Mg, Na, K, C and Vitamin C) increased with the addition of the fruit juice. Total solids increased with increase concentration of the sweetened fruit flavour in yoghurt. There was no mould or coliform growth but the total viable count ranged from 6.3 x 10² to 2.34x 10⁷ cfu/ml after 21 days. The sugar sweetened samples were most acceptable over the honey and fruit juice without sweetener.
Background

Biogenic amines are nitrogenous compounds with biological activity produced, primarily, by a variety of lactic acid bacteria. Biogenic amines, such as histamine and tyramine, are commonly found in fermented food products. In cheese, high concentrations can result in adverse health effects for the consumer.

Objectives

The aim of this study was to screen a range of artisanal cheeses (n=10) for the presence of microbial populations capable of producing biogenic amines.

Methods

Segments of the histidine (hdc) and tyrosine (tdc) decarboxylase genes were amplified using previously published PCR primer pairs. PCR amplicons were then TOPO-cloned and subjected to Sanger sequencing. In addition, the Ion Torrent PGM sequencer was used to provide a novel, complementary, in depth analysis of amine forming communities.

Conclusions

Analysis of both Sanger and Ion PGM sequence data revealed hdc and tdc positive bacterial populations both within cheeses and across different cheese varieties. *Lactobacillus curvatus, Lb. brevis, Enterococcus faecium* and *E. faecalis* were identified as the predominant species capable of producing tyramine while *Lb. buchneri, Lb. sakei* and *Lactococcus lactis* were among the species found to harbour histaminogenic potential. High-throughput Ion sequencing alone revealed the presence of sub-dominant genera, including, among others, *Staphylococcus saprophyticus* and *Streptococcus thermophilus*. Actual levels of histamine and tyramine in the respective cheeses were quantified by HPLC.
BACTERIOLOGICAL QUALITY OF COMMERCIALLY PREPARED FERMENTED OGI (AKAMU) SOLD IN SOME PARTS OF SOUTH EASTERN NIGERIA

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Background

Food poisoning and infection by bacteria are of public health significance to both developing and developed countries.

Objectives

This study compared the bacteriological qualities of commercially and laboratory based fermented ogi (akamu) prepared from maize.

Methods

Samples of ogi (akamu) prepared from white and yellow variety of maize sold in Uturu and Okigwe were analyzed together with the laboratory prepared ogi for microbial quality using the standard microbiological methods. The analyses showed that both white and yellow variety had total bacterial counts (cfu/g) of $4.0 \times 10^7$ and $3.9 \times 10^7$ for the laboratory prepared ogi while the commercial ogi had $5.2 \times 10^7$ and $4.9 \times 10^7$, $4.9 \times 10^7$ and $4.5 \times 10^7$, $5.4 \times 10^7$ and $5.0 \times 10^7$ for Eke-Okigwe, Up-gate and Nkwo-Achara market respectively. The Staphylococcal counts ranged from $2.0 \times 10^2$ to $5.0 \times 10^2$ and $1.0 \times 10^2$ to $4.0 \times 10^2$ for the white and yellow variety from the different markets while Staphylococcal growth was not recorded on the laboratory prepared ogi. The laboratory prepared ogi had no Coliform growth while the commercially prepared ogi had counts of $0.5 \times 10^3$ to $1.6 \times 10^3$ for white variety and $0.3 \times 10^3$ to $1.1 \times 10^3$ for yellow variety respectively. Lactic acid bacterial count of $3.5 \times 10^6$ and $3.0 \times 10^6$ was recorded for the laboratory ogi while the commercially prepared ogi ranged from $3.2 \times 10^6$ to $4.2 \times 10^6$ (white variety) and $3.0 \times 10^6$ to $3.9 \times 10^6$ (yellow).

Conclusions

There are chances of contracting food borne diseases from commercially prepared ogi. Therefore, there is the need for sanitary measures in the production of fermented cereals so as to minimize the rate of food borne pathogens during processing and storage.
Background
Cheese (wara) is a local dairy product produced and consumed in many African countries. It is produced locally by the Fulanis who use Calotropis procera leaves as coagulating agent for the milk.

Objectives
The isolation and identification of the fungi species present in wara and their ability to produce mycotoxins was carried out to determine the safety level associated with consumption of the cheese.

Methods
Mycotoxin production was determined with the use of the enzyme-linked immunosorbent serological assay (ELISA) technique

Conclusions
Some of the isolated mycoflora were found to be mycotoxigenic producing aflatoxins at various levels.
INVITRO PROTEIN DIGESTIBILITY OF FERMENTED BAMBARA NUT (VOANDZEIA SUBTERRANEAN L. THOUARS) USING DIFFERENT SPECIES OF RHIZOPUS
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Background
Bambara nut as legume contains sufficient quantities of carbohydrate and protein high in lysine and methionine. Processing methods, such as soaking, cooking or fermentation can improve the quality of legume protein.

Objectives
Therefore, this research considered the effect of fermentation on in vitro protein digestibility of bambara nut

Methods
The bambara nut was obtained from a local market in Ogbomoso, Nigeria. Different species of Rhizopus for the fermentation were obtained from Ladoke Akintola University of Technology, Ogbomoso, and the Institute of Agricultural Research and Training, Ibadan to produce fermented bambara nut flour. The In vitro protein digestibility of the flour sample was consequently evaluated.

Conclusions
Fermentation process using Rhizopus species alone, and with their combinations showed improvement in the nutritive value of the fermented bambara nut in relation to in-vitro protein digestibility, and the highest rate of protein digestibility was obtained using R. oligosporus alone
Background
Various Lactobacillus species are responsible for the production of fermented food products. Prior to the use of a certain isolate for industrial purpose, it is advisable to classify it at least on species level. Nevertheless, closely related species are not easy to distinguish but molecular methods might be able to reveal the differences even on subspecies level.

Objectives
The aim of our experiments was to verify the usability of several molecular methods in discrimination of Lactobacillus species and compare the efficiency of these tools.

Methods
DNA of lactobacilli were subjected to investigations by PCR (general bacterial and species-specific primers) and qPCR (including newly developed primers and High Resolution Melting - HRM), RFLP and sequencing in order to compare the efficiency of these methods in separation of species.

Conclusions
The HRM had proved its power as a one-step tool for preliminary classification of lactobacilli. With this method it might be much easier to separate closely related species, though, additional techniques, e.g. traditional PCR (species-specific primers), sequencing and RFLP pattern analysis might be necessary to use in order to confirm the results.
GENOMICS OF STREPTOCOCCUS MACE DONICUS: MOVING FROM PATHOGENICITY TO ADAPTATION TO THE DAIRY ENVIRONMENT

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Background

Lactic acid bacteria (LAB) constitute a significant group of microorganisms for food fermentations and for human health. The *Streptococcus bovis/Streptococcus equinus* complex (SBSEC) within LAB includes members that have been implicated in human disease, but are also found in foods.

Objectives

In this study we compared the three available genome sequences of *S. macedonicus* strains isolated from dairy products. Only one strain has its genome complete sequenced and previous analysis showed diminished pathogenic potential and adaptation to the milk environment. Here we present the *in silico* analysis of these strains, in order to better understand the *S. macedonicus* species.

Methods

Chromosomal maps were constructed using DNAPlotter and whole genome sequence alignments were performed by progressiveMAUVE and Webact in order to visualize conserved genomic regions or chromosomal rearrangements. Genomic islands were identified and visualized by IslandViewer, potential bacteriocins were predicted by BAGEL3 and CRISPRs were analyzed by the tools available in the CRISPRcompar web-service.

Conclusions

The analysis revealed that *S. macedonicus* strains have lost genes involved in the catabolism of complex plant carbohydrates, in the adhesion to the host’s cells and in haemolysis that are present in pathogenic SBSEC. In addition, *S. macedonicus* carries two lactose operons and a proteolytic system characteristic of dairy LAB. Our whole genome analysis of *S. macedonicus* shows adaptation traits to the nutrient-rich milk environment.
INACTIVATION OF BROCHOTHRIX THERMOSPACTA BY ATMOSPHERIC COLD PLASMA.

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Background

Brochothrix thermosphacta is considered one of the predominant food spoilage organisms in meat. Atmospheric cold plasma technology (ACP) is proposed as a potential technology for elimination of microbial contamination in food products.

Objectives

The aim of the study was to investigate the antimicrobial efficacy of dielectric barrier discharge atmospheric cold plasma (DBD ACP) for inactivation of B. thermosphacta.

Methods

A large gap DBD ACP system was used to investigate the inactivation of B. thermosphacta (10^7-8 CFU/ml) in PBS, liquid meat model medium (beef extract broth) and raw lamb chops. Samples were placed inside sealed rigid polypropylene containers and were treated with cold plasma using modified atmosphere as the inducer gas. The effect of ACP critical control parameters, such as treatment time, voltage level, media composition and post treatment temperature storage conditions against both planktonic bacteria and biofilms were evaluated. The surviving bacterial populations were estimated by colony count assay.

Conclusions

ACP showed substantial reductions of bacteria depending on the growth media. ACP treatment (80kV) for 30s completely inactivated the bacterial population in PBS, while 5 min treatment showed 2 Log unit bacterial reduction with the meat model medium. The antimicrobial efficacy of plasma against bacteria on lamb chops showed a reduction around 0.5 log units over a 10 day storage period. The results indicate ACP could be effective for inactivation of B. thermosphacta if treated with sufficient treatment periods providing a prolonged shelf life for meats and meat products. Further studies will investigate the effect of extended ACP treatment times for complete inactivation.
LARGE-SCALE GENE DELETION IN LACTOCOCCUS LACTIS USING THE CRE-LOXP RECOMBINATION SYSTEM

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Background
Minimum genome factories (MGFs) can be defined as recombinant strains whose metabolic pathways have been optimized for special applications. The plasticity of genomes gives us hints that it is probable to streamline physiological pathways through genome rearrangements as strains possess a number of non-essential genes. Lactic acid bacteria (LAB) are low G-C content Gram-positive bacteria and have relatively small genome. Lactococcus lactis could be served as the starting platform for value-added metabolites biosynthesis. Construction of a set of chassis cells provides the opportunity to obtain specific strains with desired performance.

Objectives
To delete the large-scale genes in Lactococcus lactis and construct the set of chassis cells for further synthetic biology research.

Methods

The Cre-loxP recombination system was used to construct a large scale region (19.0 kb) deficient strain N8ΔL1. Two loxP sites were integrated into the genome at target sites, and the large scale region (L1) was replaced by a cat-cassette. The recombination between two loxP sites was achieved when the Cre recombinase was expressed by constructed pNZTS-Cre plasmid, and then the L1-null strains were isolated. Despite the loss of 19.0-kb regions, the strain N8ΔL1 still exhibited normal growth and similar growth profile was also observed compared to the parental strain.

Conclusions
The Cre-loxP recombination system can be used for large-scale genome deletion and generate sequential chromosomal modification mutants of L. lactis efficiently. Based on the precision of its deletion, further genome streamlining and advance the regulatory network study about synthetic biology could be easily achieved.
EFFECT OF RESPIRATIVE METABOLISM OF LACTOBACILLUS CASEI ON MODEL SOURDOUGH FERMENTATION

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Background

Lactobacillus casei is a lactic acid bacterium (LAB) used in the production of many fermented food and feed products. Furthermore, this specie comprises strains commercially exploited as probiotic cultures. Thus, novel strategies to improve their adaptation to adverse conditions could enhance their safety, functional and technological properties. Recently, it has been demonstrated that respiratory metabolism results in this species in the expression of a phenotype with enhanced technological and stress response properties (increase in biomass, synthesis of antioxidant enzymes, robustness to stress conditions).

Objectives

The aim of the study was evaluating the impact of respirative/anaerobic metabolism of L. casei N87 strain on model sourdough fermentation.

Methods

The strain L. casei N87, grown both in anaerobic and respirative conditions, was inoculated as starter in wheat flour in combination with a commercial baker’s yeast culture. After 0, 6 and 24 h of fermentation LAB count, pH and Titratable acidity values, free amino acid (FAA), volatile compounds (VOCs) (by SPME-GC-MS), antioxidant activity (by DPPH assay) and albumins/globulins, gliadins and glutenins protein fractions (by SDS-PAGE) were evaluated.

Conclusions

A major increase in biomass for the strain L. casei N87 grown in respirative condition was registered after 6h of fermentation. Results obtained by FAA and VOCs evaluation indicated that respirative metabolism of L. casei N87 influences the flavour of sourdough. Furthermore, SDS-PAGE highlighted a different wheat protein degradation in anaerobic/respirative cultures. In conclusion, respirative L. casei N87 performs well in sourdough definition and a better explanation of its role in sourdough production is considered opportune.
Background

Chenopodium quinoa and Amaranthus spp. are considered pseudocereals characterized by high nutritional and functional values which are associated with the quality and quantity of their proteins, fats and antioxidant compounds. In the recent years, these pseudocereals have attracted the interest of research for their exploitation in the production of gluten-free products and food products with improved nutritional and health benefits.

Objectives

To characterize quinoa and amaranth seeds (produced in Campania Region) for microbiological, technological and safety aspects with the aim to use them in the production of “functional foods”

Methods

Three samples of Amaranthus (accessions A1, A2 and A3) and three samples of Chenopodium quinoa seeds (2 Titicaca and 1 Puno varieties) were obtained by experimental fields of Vitulazio (Campania Region). Total mesophilic count, lactic acid bacteria (LAB), yeasts, moulds, Enterobacteriaceae, enterococci, total and faecal coliforms were assessed by standard pour-plate technique by using selective media. Total polyphenols content was determined using a method based on Folin-Ciocalteu reagent, while antioxidant activity was carried out spectrophotometrically using DPPH (2,2-diphenyl-1-picrylhydrazyl) radical scavenging.

Conclusions

Results evidenced a high LAB concentration in quinoa and amaranth seeds ranged between 2.5 and 7.5 log CFU/g and between 2.8 and 5.0 log CFU/g, respectively. Quinoa seeds showed higher level of polyphenol content compared with amaranth, whereas no significant differences were observed in antioxidant activity, registered in high concentration for both the seeds. In conclusion, the characteristics of these seeds are highly desirable for the production of both gluten-free products and food with high nutritional value.
Background

Meat products can host variable microbial communities according to seasonal changes and production processes. These may encompass pathogenic or spoilage bacteria which must be controlled to ensure safety and quality of the products.

Objectives

Our aim was to describe the microbial community of chicken legs packaged under modified atmosphere.

Methods

Whole bacterial populations were collected and stored frozen at -80°C. Their ability to regrow on meat was checked. Bacterial diversity was determined by cultural methods (23 samples) and by 16S rDNA pyrosequencing (10 samples).

Conclusions

Total viable counts varied with samples ($10^3 - 10^8$ CFU/g) and plating methods showed that lactic acid bacteria, *Brochothrix thermosphacta*, and *Pseudomonas* spp. were the main bacterial. The characterization of the bacterial diversity by 16S rDNA pyrosequencing confirmed the presence of *B. thermosphacta*, and revealed that *Pseudomonas* was mainly represented by *P. extremaustralis* and *P. cedrina*. As well the main LAB species were *Carnobacterium* and *Shewanella* species. The predominance of *Pseudomonas* was correlated to meat packaging under high oxygen concentration, except when *B. thermosphacta* was dominant, suggesting a competition between these species. No clear cut correlation could be observed between farming practices or meat processing and bacterial communities. However, our results showed similar microbial profiles of samples issued from the same slaughterhouse suggesting the main contamination may occur during this processing. The frozen communities will now be used to evaluate the abiotic and biotic (e.g. the *Brochothrix*-*Pseudomonas* competition) factors in reproducible challenge tests on meat to finally improve the storage life-time.
FEMS-1577
Food microbiology

ANTIFUNGAL ACTIVITY OF AQUEOUS POLYPHENOLIC EXTRACTS FROM CITRUS PEELS
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Background
Fungal growth is the main cause of fruit decay, and is usually control by the application of synthetic fungicides, however, currently their used have been restricted. Therefore, it is urgent to find alternative antifungal substances. Phenolic compounds are secondary metabolites of plants and naturally present in fruit which have been associated with antimicrobial effect. Citrus peel contains high amount of flavonoids and is the main byproduct of processing industry.

Objectives
Then, the aim was to investigate the inhibitory activity of phenolic extracts obtained from citrus peel against fungal fruit spoilage.

Methods
The antifungal activity of extracts from orange and lemon peels were tested in-vitro against 6 yeast strains (Cryptococcus spp., Torulaspora spp, Aerobasidium pullulans, Rhodoturula spp., Hanseniaspora uvarum, Meyerozyma caribbica) and 8 molds (Botrytis cinerea, Monilia laxa, Alternaria spp., Penicillium glabrum, Penicillium expansum, Penicillium corylophilum, Cladosporium uredinicola, Cladosporium cladosporioides), by following the ability to grow in a medium containing different concentrations of extracts.

Conclusions
Overall, the inhibitory effect of orange extracts (OE) against yeast strains was around 90% at concentrations higher than 0.75 g/L, whereas the effect of lemon extracts was more strain dependent and powerful, with values closed to 90% against four strains at 0.15 g/L. On the other hand, although inhibitory effect against molds was lower, OE presented a remarkable activity against B. cinerea, M. laxa and Alternaria spp. and moderated against Penicillium and Cladosporium strains. In conclusion, these results suggest the possibility of using citrus polyphenolic extracts as a safer alternative to synthetic fungicides to control postharvest decay of fruit.
FEMS-1827
Food microbiology

LISTERIA MONOCYTOGENES OF SEQUENCE TYPE 121 HARBOR SPECIFIC ADAPTATIONS SUPPORTING PERSISTENCE IN FOOD PRODUCTION PLANTS.
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Background

The foodborne pathogen Listeria monocytogenes is able to survive for months and even years in food production environments. Among a great strain diversity particularly strains belonging to sequence type (ST)121 are persistent in food production plants.

Objectives

To elucidate the molecular determinants responsible for persistence of L. monocytogenes.

Methods

We analysed the genomes of two L. monocytogenes ST121 strains, which persisted for up to eight years in food production plants in Ireland and Austria. Additionally we characterized two ST121 strain-specific genetic features: Tn6188 and lin0464/lin0465.

Conclusions

All ST121 genomes are highly similar and show a tremendously high degree of conservation among prophages and particularly among their plasmids, which are usually variable parts of genetic information in bacteria. This remarkably high level of conservation suggests a strong selective pressure.

In addition, all ST121 strains share adaptations related to persistence in food production environments such as the presence of Tn6188, a transposon responsible for increased tolerance against quaternary ammonium compounds, and the presence of homologues of the L. innocua genes lin0464 and lin0465, a transcriptional regulator and a putative pfpl protease. Deletion of lin0465 resulted in reduced survival under oxidative and alkaline conditions suggesting a role in stress response. Furthermore all ST121 strains reveal a yet undescribed insertion harboring recombination hotspot (RHS) repeat proteins, which are most likely involved in
competition against other bacteria. In conclusion we show that *L. monocytogenes* ST121 strains are highly similar to each other harboring conserved regions which provide fitness adaptations to survival in food production environments.
NOVEL VIRULENCE FEATURES OF LISTERIA MONOCYTOGENES ISOLATED FROM FOOD SAMPLES SOLD AT A ROMANIAN BLACK MARKET.

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Background

Listeria monocytogenes is a facultative intracellular foodborne pathogen responsible for listeriosis. Investigated neglected exogenous routes of transmission of foodborne pathogens into the EU, we have isolated 15 L. monocytogenes strains in food products illegally sold at a Romanian black market.

Objectives

The aim of this study was to characterize the subtype and the virulence features of these L. monocytogenes strains.

Methods

We determined invasion efficiency and intracellular growth in human intestinal epithelial Caco2 and macrophage-like THP1 cells; and analysed the sequence of three main virulence factors: PrfA, internalin A (InlA) and listeriolysin O (LLO).

Conclusions

Multilocus sequence typing revealed that these L. monocytogenes strains belong to six different sequence types (ST). In vitro virulence assays showed a high strain variability regarding the invasion efficiency in Caco2 cells and the intracellular growth rate in both cell types. In parallel we revealed a high diversity in the InlA and LLO amino acid sequences, however strains belonging to the same ST harbour identical sequences. We detected in total 30 different amino acid substitutions, resulting in seven different InlA variants, two of which have not yet been described. All ST121 and ST9 strains, harbouring a premature stop codon resulting in truncated InlA, were unable to invade Caco2 cells. In addition the number of LLO mutations correlated negatively with intracellular growth in both cell types; and all ST155 strains showed no proliferation inside macrophages. In conclusion, we show a high diversity in the amino acid sequence of main virulence factors in L. monocytogenes resulting in distinct virulence profiles.
Background

Interactions of bacteria living in the same microenvironment influence the behaviour of pathogens like *Listeria monocytogenes*. Therefore inter-strain competition between different *L. monocytogenes* strains could influence fitness and pathogenicity.

Objectives

This study investigated the impact of co-culture on growth and *in vitro* virulence of *L. monocytogenes* strains and the effect of cell-contact on the observed growth and virulence competition.

Methods

Growth of eight *L. monocytogenes* strains was determined in single and two-strain mixed cultures in tryptic soy broth at 10°C for 10 days. The effect of strain competition (24h, 10°C) on invasion efficiency and intracellular growth was investigated using human intestinal epithelial Caco2 cells. Two selected strains were inoculated singly, mixed or separated by 0.4μm PET-membrane to analyze whether the observed effects are cell-contact dependent.

Conclusions

Significant differences in growth between single and mixed cultures were observed for certain strains e.g. ScottA and 6179 showed reduced growth rates when co-cultivated with other strains. Cell-contact was essential for growth competition.

High virulent strain e.g. PL25 showed either increased or unchanged invasion efficiency when co-cultured with low virulent strains, whereas the invasiveness of low virulent strains like ScottA and 6179 was attenuated. These results suggest a competitive advantage for the strains displaying higher invasion efficiency. In parallel to growth the effect of strain competition on virulence is cell-contact dependent.

In conclusion competition between *L. monocytogenes* strains has a strain-dependent effect on fitness and virulence.
NEW INSIGHTS OF A NATURAL GUM: FOCUSING ON GUT ECOLOGY.
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Background

Gum odina obtained from bark of Odina wodier (Anacardiaceae) is evaluated as prebiotic in this study. The natural gum (polysaccharide) is chemically galactopyranose associated with arabinose side chain.[¹]

Objectives

The use of prebiotic to fortify gut flora is current aspect of research for the control and prevention of colon cancer as well as boosting of immune system.

Methods

In vivo prebiotic potential of gum odina was performed on Swiss albino mice by deliberate challenging of S.typhi 62 focusing on immunological parameters[²] and in vitro studies was investigated by Simulator for Human Intestinal Microbial Ecosystem (SHIME).[³]

Conclusions

The non-degradability of gum odina by simulated gastric fluid (SGF) and simulated intestinal Fluid (SIF) attributes to one of the major characteristics of prebiotics i.e. indigestible carbohydrate. Microbial analysis of SHIME studies shows increase in colonization of various probiotic organisms such as Lactobacillus sp. and Bifidobacterium sp. upon administration of odina. In vivo study revealed natural gum selectively stimulates probiotic strains and eliminates enteric pathogens and also boosts immune system by increasing slgA in gut. Moreover increase in levels of IFN γ signifies additional protection against various pathogen induced primary and secondary infections. This gum is also believed to possess colon carcinoma preventing properties as it liberates short chain fatty acids (SCFA) upon fermentation which we are planning to focus in our next studies.
USE OF BACTERIAPHAGE-BASED SOLUTION AND ELECTROLYZED OXIDIZING WATER AS ALTERNATIVE SANITIZING SOLUTIONS TO INACTIVATE LISTERIA MONOCYTOGENES ON FOOD CONTACT SURFACES FOR THE SEAFOOD PROCESSING FACILITIES

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Background
Listeria monocytogenes is an important foodborne pathogen that can cause serious public health-related issues worldwide. L. monocytogenes could be isolated from food contact surfaces (FCS) from the seafood processing facilities, e.g., slicers, utensils, knives, sieves, conveyors, towels, employer’s gloves, which could then link to the occurrence of cross-contamination of seafood products.

Objectives
Effective sanitizing agents are thus needed to reduce the incidence of Listeria contamination on FCS. In this study.

Methods
Phage-based solution LP-124 (LPS; broad host-range listeriaphage) and electrolyzed oxidizing water (EOW; pH 2.5 and ORP 1150 mV) were evaluated for the ability to inactivate L. monocytogenes cells that were artificially contaminated on four different FCSs (plastic cutting board, towel, stainless steel spoon, and sieve) at 4 logCFU/cm² on a given FCS.

Conclusions
Results showed that EOW and LPS led to significantly (p<0.05) decrease of L. monocytogenes counts, representing about 2 logCFU/cm² on a given FCS, [KV1] compared with the counts recovered from washing with water. L. monocytogenes counts were not detected in the previously used EOW solution stored at 4 °C during 7 days after used, while about 2.8 logCFU/ml was observed in used phage-based solution after 7 days of storage. Both EOW and LPS can effectively inactive L. monocytogenes on different food contact surfaces, and can further be used as alternative sanitizing solution for the seafood processing facilities.
PREVALENCE OF INTESTINAL PARASITES AMONG FOOD HANDLERS OF SARI, NORTHERN IRAN

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Background
Parasitic infection is highly prevalent throughout the developing countries of the world. Food handlers are potential source of infection of many intestinal parasites and other enteropathogenic infections as well

Objectives
The aim of this study was to determine the prevalence of intestinal parasites carriers among food handlers attending the public healthcenter laboratory in Sari, northern Iran for annual check-up

Methods
The study was performed from August 2011 through February 2012. Stool samples were collected from 1041 male and female food handlers of different Jobs aged between 18 to 63 years and were examined following standard procedures.

Sociodemographic, environmental and behavioral data of the food handlers were recorded in a separate questionnaire. Intestinal parasites were found in 161(15.5%) of the studied samples. Seven species of protozoan orhelminth infections were detected. Most of the participants were infected with Giardia lamblia (53.9%) followed by Blastocystis hominis (18%), Entamoeba coli (15.5%), Entamoeba histolytica/dispar (5.5%), Cryptosporidium sp. (3.1%), Iodamoeba butschlii (3.1%) and Hymenolepis nana (1.9%) as an only helminth infection.

Conclusions
The finding emphasized that food handlers with different pathogenic organisms may predispose significant risk on the consumers. Routine screening and treatment of food handlers is a proper tool in preventing the food-borne infections.
IDENTIFICATION AND EVALUATION OF ANTIBIOTIC RESISTANCE OF PROBIOTIC STRAINS OF BIFIDOBACTERIA IN BELARUS

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Background
Accurate identification and assessment of antibiotic resistance of bifidobacteria are essential to secure quality and safety of probiotics and food products, containing these microorganisms.

Objectives
The objective of the work was identification and evaluation of antibiotic resistance of 25 probiotic strains of bifidobacteria, isolated from feces of healthy adults, therapeutical preparations and dairy products, marketed in Belarus.

Methods
Sequencing of 16S rDNA and transaldolase gene, MALDI-TOF MS protein profiling, biochemical testing, analysis of fatty acid methyl esters (FAMEs) were used for bifidobacteria identification. PCR-analysis, disc-diffusion and broth micro-dilution methods were applied for determination of antibiotic resistance of bifidobacteria.

Conclusions
Results of molecular-genetic identification of bifidobacteria indicated that 7 strains belong to species Bifidobacterium animalis subsp. lactis, 7 strains – Bifidobacterium longum, 5 strains – Bifidobacterium bifidum, 5 strains – Bifidobacterium adolescentis, 1 strain – Bifidobacterium breve. MALDI-TOF MS analysis confirmed taxonomic affiliation of tested strains and proved to be a rapid and reproducible technique for bifidobacteria identification. Protein biomarkers suitable for species and strain discrimination were defined. Both biochemical testing, based on evaluation of enzymatic activity and carbohydrates fermentation, and FAMEs analysis did not provide accurate species identification of bifidobacteria, but were useful for strain differentiation and characterization. Tested strains of bifidobacteria were susceptible to chloramphenicol (100%), penicillin G (96%), amoxicillin (96%), ampicillin (88%), streptomycin (80%), vancomycin (80%), gentamycin (76%), resistant to erythromycin (40%), clindamycin (40%), tetracycline (52%), and harbor genes tet(W) (36%), tet(M) (16%), erm(X) (32%), erm(B) (8%). Gene tet(W) was detected in all B. animalis subsp. lactis strains, gene erm(X) – all B. longum strains.
INFLUENCE OF TEMPERATURE AND INOCULUM SIZE ON SPORES GERMINATION PERCENT OF CLOSTRIDIUM ALGIDICARNIS AND CLOSTRIDIUM ESTERTHETICUM

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Background
'Blown pack' spoilage causes high economic losses for Brazilian meat industry.

Objectives
This research aimed to determine the influence of temperature and inoculum size on spore germination percent of C.algidicarnis, isolated from spoiled vacuum meat and C.estertheticum DSM8809, both recognized for their package blowing ability.

Methods
A central composite design - 2 factors x five levels - was applied: storage temperature (-2,3,7,11, 15°C) and inoculum size(10¹,10²,10³,10⁴ and 10⁵ spores/mL, previously prepared). Experiments were conducted using RCM media, in anaerobic conditions. Counts were performed daily, during 30 days, using NMP method. Spores germination percent (SGP) for each day was calculated as: SGP (\%)=(count of germinated cells/initial spore inoculum)*100).

Conclusions
In all assays, for both microorganisms, germination was observed. C.estertheticum germinated faster than C.algidicarnis at lower temperatures: at 3°C/10² spores of inoculum, after 1 day all spores, initially inoculated, were germinated and for C.algidicarnis, 100% germination was reached after 4 days. In addition, at 11°C/10² sp/mL, germination of C.estertheticum was slower than C.algidicarnis, achieving 100% after 11 days. For inoculum of 10³ spores/mL, C.algidicarnis reached high germination percent quickly at 15°C (52.4% after 2 days) while C.estertheticum, at 7°C (39% at 2 days). Increasing initial inoculum size from 10² to 10⁴ spores/mL/3°C, increased the time for total germination from 4 to 10 days for C.algidicarnis and from 1 to 15 days for C.estertheticum. These results clearly shows that tested temperatures were no enough to inhibit spores germination of both microorganisms, even if initial population is 10⁴ spores/ml, emphasizing the need to avoid the vacuum packed red meat clostridial spore contamination before packaging.
Background

The probiotic microorganisms must be able to survive during passing through the hostile conditions of the gastrointestinal tract and have an ability to adhere to the intestinal epithelial cells. Nowadays there are many physical and chemical factors affecting these abilities of probiotics.

Objectives

In this work was studied the effects of electromagnetic irradiation at the frequencies 51.8 and 53 GHz (1h) and antibiotic ceftazidime (20 µM) on survival of *Lactobacillus acidophilus* VKMB-1660 *in vitro* in model of gastrointestinal tract.

Methods

Bacteria developed in artificial gastric juice (treatment time 20 min) with pepsin (0.3%) and different pHs (2.0, 3.0, 4.0, 5.0) and artificial intestine juice (treatment time 1 h) with bile salts (0.45%) and pancreatin (0.1%) at pH 8.0. Moreover it was studied the effects of antibiotics and different pHs (5.5, 6.5 and 8.0) on adhesiveness of bacteria.

Conclusions

The results obtained shown that the colony forming unit number significantly decreased, in artificial gastric juice than in intestine juice in comparison with untreated control samples. The irradiation at both frequencies and ceftazidime significantly decreased the viability of bacterial samples in both gastric and intestine juices. Moreover, ceftazidime suppressed the adhesion ability of bacteria at all pHs used in comparison with untreated samples and adhesiveness was much weaker at pHs 5.5 and 8.0 than at pH 6.5.

Thus, lactic acid bacteria *L. acidophilus* can survive in viable state in the gastrointestinal tract even after irradiation and treatment with antibiotics, but antibiotics can significantly decrease an adhesion of lactic acid bacteria to intestine mucosa.
HYGIENE BARRIERS IN FOOD PROCESSING FACILITIES MAY SERVE AS “TROJAN HORSES” FOR L. MONOCYTOGENES

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Background
Generally, hygiene sluices are efficient barriers against the transmission of spoilage and foodborne pathogen bacteria.

Objectives
The aim of the study was to determine the efficiency of hygienesluices on the reduction of hygiene indicator bacteria and zoonotic agents in food processing companies.

Methods
We included five food processing facilities located in Upper- and Lower Austria. Samples were taken before and after sanitation comprising personal fingerprints, swab samples from shoes and the hygiene lock, and water residues and investigated according to microbiological standard methods. L. monocytogenes were subtyped by Serogroup PCR and pulsed-field gelelectrophoresis (PFGE).

Conclusions
One shoe sample each was found positive for E. coli (rfbE positive) and Salmonella spp. after sanitation in slaughterhouse B and RTE-food producer C, respectively.

Coliform bacteria and L. monocytogenes on shoes could not be reduced after passing the hygiene locks. In detail, 29% of the shoe samples were detected L. monocytogenes positive before and after the foot bath. Most of L. monocytogenes isolates were found on shoes of two slaughterhouses and one RTE-food company working staff. Subtyping revealed that the slaughterhouse A harbored L. monocytogenes serogroup 1/2a, 3a and 4b, 4d, 4e (n=21) resulting in five PFGE pulsotypes. In slaughterhouse B L. monocytogenes serogroup 1/2a, 3a, 1/2c, 3c and 4b, 4d, 4e (n=11) was present, representing five PFGE pulsotypes. In one RTE-food producing company genetic lineage II (1/2a, 3a; 1/2c, 3c; n=10) were over represented, including persistent strains. Hygiene locks, if not efficiently working, could be a reservoir for human pathogen bacteria and widely distributed L. monocytogenes clones.
DIVERSITY AND ANTI-LISTERIAL POTENTIAL OF BACTERIAL FLORA ON HARD-CHEESE RINDS

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Background

Vorarlberger Mountain cheese Protected Designation of Origin (PDO) owes its special characteristics from the raw milk of extensively farmed cows grazing wild herbs on alpine pastures during the summer months.

Objectives

The aim of the study was to determine the diversity of bacterial flora on hard-cheese rinds isolated from artisan Vorarlberger Mountain cheese. A further goal was to test their antilisterial capability.

Methods

The bacterial rind flora of seven cheese samples, including the variety of three ripening cellars (A, B, C) and two different ripening stadiums, were characterized. Furthermore, five cheeses were investigated during a six month period, to determine the variations in bacterial groups on cheese surfaces due to failures or instabilities in cheese-curing. Bacterial cheese flora isolates were enumerated and confirmed applying standard agar isolation methods and 16S rRNA sequencing. The antilisterial potential of 56 cheese rind bacteria was tested in an agar-overlay model against Listeria spp. Additionally, cheese rind bacteria with antilisterial potential and a brine-adapted L. monocytogenes isolate were introduced in a challenge experiment on cheese during ripening.

Conclusions

The majority of isolates comprised following genera and species: Brevibacterium spp. (30.9%), Staphylococcus equorum (20.9%), Corynebacterium casei/variabilis (11.4%) and Brachybacterium alimentarium (10.9%). Antilisterial potential in the agar model was observed from 30.4% of test strains. In the challenge experiment higher L. monocytogenes spike levels (10^6 cfu) were positive after enumeration and enrichment until the end of smearing at day 14. Applying a brine with higher salt concentration (>20%) resulted in higher growth rates during ripening (average 3.98x10^3 cfu/g).
MICROBIAL QUALITY OF EDIBLE INSECTS

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Background
Current animal protein sources will not be sufficient for the growing population. Therefore, alternative solutions need to be found. Edible insects are considered as a sustainable and nutritionally equivalent alternative to meat. Nowadays, there is a lack of profound insight into the microbiological quality of edible insects.

Objectives
The purpose of this research was to investigate the microflora of edible insects in a quantitative and a qualitative way, in particular of the yellow mealworm (*Tenebrio molitor*) and the grasshopper (*Locusta migratoria migratorioides*).

Methods
Living insects for human consumption were purchased from an eco-shop (Belgium). Both insect species were subjected to culture-dependent analyses (classical microbial counts) to determine the microbial load (total aerobic count (TAC), *Enterobacteriaceae*, lactic acid bacteria (LAB), yeasts and moulds (Y&M) and aerobic bacterial spores). In addition, culture-independent analyses (454 pyrosequencing) were performed to gain insight into the bacterial community composition.

Conclusions
Average microbial counts (3 batches, log cfu/g) in mealworms were 8.0 TAC, 7.3 *Enterobacteriaceae*, 7.4 LAB and 5.4 Y&M. Lower counts were observed for sporeforming bacteria ranging between <1,0-3,5 log cfu/g. Grasshoppers showed average counts (2 batches, log cfu/g) of 7.2 TAC, 5.6 *Enterobacteriaceae*, 7.0 LAB, 4.2 Y&M and 4.2 bacterial spores. The culture-independent analyses demonstrated a high bacterial diversity in mealworms and grasshoppers. Most bacteria belonged to the *Enterobacteriaceae* and LAB, however, the dominant bacterial species differed between both insect species. Hence, raw edible insects contain a high microbial load and diversity, including spoilage organisms and potential pathogens, a decontamination step before consumption is required.
Background

Microorganisms are of great importance in post-harvest spoilage of marine resources. Both quality-reducing and potential pathogenic bacteria may reduce the first-hand value, the export potential, and the reputation of the seafood industry.

Objectives
To examine microbiological conditions of the commercially most important pelagic fish species harvested in Norway, with respect to quality, hygiene and food safety.

Methods
Microbial fish quality was evaluated by heterotrophic plate counts (HPC) and counts of H$_2$S-producing bacteria, whereas hygienic- and safety conditions were assessed by analysing for faecal indicator organisms, e.g. presumptive *E. coli* and enterococci, and the pathogenes *Listeria monocytogenes, Salmonella* and staphylococci. Examined contact points in the production environment at vessels and factories, included surface samples of equipment and water samples associated with fish catch, -storage, and -production. Contact points were examined for HPC, Enterobacteriaceae, presumptive *E.coli*, enterococci, *Salmonella*, and *L. monocytogenes*. For evaluation, an assessment scheme with recommended microbiological limits suitable for fresh pelagic fish products are proposed based on contemporary EU- and Norwegian guidelines.

Conclusions
Quality-, hygienic- and food safety conditions were according to the recommended assessment scheme in all samples from 23 samplings. However, 18 samplings had samples that were not optimal and contamination of fish, the pump nozzle, sift box, sorting chambers, storage tanks, landing tanks and conveyor belts were most often recurring. Two samplings revealed that contamination early in the production chain followed the fish throughout the process, thus an increased focus on clean equipment during capture and storage is highly suggested.
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YEAST BIOFILM DEVELOPMENT AND RHEOLOGICAL PROPERTIES
DEVELOPMENT ON TURBULENT FLOW REGIMES.
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Background

In food processing lines or in complex equipment, microorganisms are exposed to varying hydrodynamic conditions caused by the flow of liquid food, and biofilms grown under a wide distribution of hydrodynamic strengths.

Objectives

The aim of the present work was to investigate the in situ thickness, architecture, and rheological properties of yeast biofilms growing on stainless steel under turbulent flow.

Methods

The yeast species used (Rhodototula mucilaginosa, Candida krusei, Candida kefyr and Candida tropicalis) were isolated from an apple juice industry. Biofilm formation in turbulent flow were performed in a Rotating Disk System (RDS) already described (Brugnoni et al., 2011). Viscoelastic properties of biofilms were determined by small deformation dynamic oscillatory measurements in a Paar Physica rheometer MCR301 (Anton Paar GmbH, Graz, Austria), using parallel plates.

Conclusions

Results show yeasts biofilms formed on stainless steel at Reynolds (Re) numbers ranging from 294,000 to 1.2 × 10⁶. These growth phases transform adherent blastospores to well-defined cellular communities. Biofilm formation increases with Re and time. Flow conditions impacted biofilm composition, with a predominance of C. krusei. Under turbulent flow in biofilm thickness increased >100 μm and cell morphology was governed by hyphal structures and rounded cells. Biofilms resulted viscoelastic materials with a solid-like behavior. Rheological values were not significantly affected by flow conditions or growth time. At large deformations their weak structure collapsed beyond a critical strain.

ROLE OF THE COLD SHOCK DOMAIN FAMILY PROTEINS (CSPS) IN NISIN AND BENZALKONIUM CHLORIDE STRESS TOLERANCE OF LISTERIA MONOCYTOGENES
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Background

Listeria monocytogenes is an important foodborne pathogen that causes rare but serious illness (listeriosis) and high mortality in those with diminished immunity. Proteins of the cold shock domain family (CspS) are global gene expression regulators that promote various stress adaptation responses in bacteria. Nisin and Benzalkonium chloride (BC) are widely used antimicrobials for food preservation and disinfection of food processing environments, respectively.

Objectives

To investigate the functional role of CspS in the innate resistance of L. monocytogenes to nisin and BC stresses.

Methods

L. monocytogenes EGDe wild type and csp deletion mutant strains were phenotypically compared under nisin and BC stress in growth assays. Impact of nisin and BC stress on gene expression was determined using quantitative reverse transcription PCR (RT-qPCR).

Conclusions

In absence of Csp functions the sensitivity of L. monocytogenes EGDe to nisin and BC stress exposure was increased. Both growth and survival capacities of the L. monocytogenes EGDe strain lacking CspS were significantly diminished compared to the wild type strain when exposed to nisin and BC stress in BHI cultures. In addition the Csp-lacking EGDe strain also showed increased susceptibility to cell envelope-targeting antibiotics. Induction of csp genes upon exposure to nisin but not BC was also detected using RT-qPCR. A comparative gene expression analysis revealed an altered expression in genes encoding peptidoglycan-binding proteins in absence of Csp functions. Our results suggest that Csp-dependent gene expression regulation
contributes to optimal intrinsic nisin and BC resistance mechanisms in the foodborne pathogen *L. monocytogenes*. 
Background

*Listeria monocytogenes* is a food-borne pathogen of public health and food safety concern responsible for highly invasive disease (listeriosis) and high mortality in those with diminished immunity.

Objectives

The objective of this study was to characterize *L. monocytogenes* strains recovered from different food matrices in Switzerland between 2011 and 2014 with respect to their genotypic and phenotypic properties.

Methods

Genotypes were determined through DNA sequencing (MLST) and PCR (stress survival islet (SSI-1), *qacH* and *brcABC*) methods. BC resistance (*BC*) was determined based on minimum inhibitory concentrations on MH agar and biofilm formation by the crystal violet staining method.

Conclusions

One hundred and forty two strains isolated from various food products between 2011 and 2014 in Switzerland were examined. The strains comprised serotypes 1/2a (64%), 1/2b (7%), 1/2c (12%), 3c (2%) and 4b (15%). There were 61 MLST sequence types (ST) determined including 24 new ST that had not been previously described. Fifty percent of the strains were SSI-1 PCR positive whereas 31% were negative. Remaining strains either harbored smaller than expected PCR amplicon (15%) or the SSI-1 PCR primers failed (4%) to amplify. BC was detected in 25 (18%) strains including 20 (80%) and 3 (12%) that possessed *qacH* and *brcABC* BC genetic determinants, respectively. Ninety one percent of the strains were (129/142) were classified as weak, 8% (11/142) as moderate and only 1% (2/142) as strong biofilm formers. Our results indicate a high genetic and phenotypic diversity among *L.
monocytogenes strains that contaminate different types of food products in Switzerland.
Background

Freshwater fishes are commonly used for fish preparations in Latvia and the hygienic status of a lake from where the freshwater fishes were originated may alter microbiological quality of fresh caught fish.

Objectives

The aim of the present study was to detect the microbiological quality of freshly caught freshwater fish in Latvia.

Methods

Altogether, 36 fishes were collected, among them 25, four and seven were European eel (Anguilla anguilla), silver bream (Blicca bjoerkna) and European perch (Perca fluvialitis), respectively. Samples were collected between September and November, 2014 from fishermen from lakes in Latvia. Surface swabs of a 5x5 cm² of freshly caught fish skin were examined according to ISO methods for total bacterial count (TBC), Enterobacteriaceae, fecal coliforms, psychrotrophic microorganisms, Salmonella spp., Listeria spp. and Yersinia spp.

Conclusions

Counts of TBC, Enterobacteriaceae, fecal coliforms and psychrotrophic microorganisms were found to be significantly lower on eels than on perch and silver bream (p>0.05). Eels in Latvia are introduced to lakes in elder or glass eel stage in the frame of eel breeding program and this could influence the contamination rates of eel microflora as they are mostly not a native part of freshwater environment. Observed significant differences (p>0.05) between the counts of TBC,
Enterobacteriaceae, fecal coliforms and psychrotrophic microorganisms may be explained with the hygienic status of lake of origin of freshwater fish. Absence of Salmonella spp., Listeria spp. and Yersinia spp. in tested samples indicates that they do not share pathogens of public health significance.