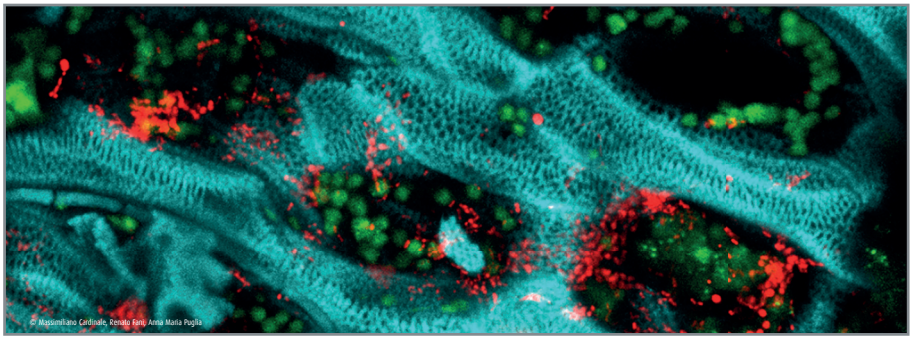
15th Symposium on

BAGECO 15

Bacterial Genetics and Ecology



Ecosystem drivers in a changing planet

26–30 May 2019
Lisbon, Portugal

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Abstracts

Invited Lectures

Horizontal gene transfer, mobile genetic elements and antibiotic resistance

IL 1

The transferable resistome of produce – the missing link?

K. Smalla

Julius Kühn Institut Federal Research Centre for Cultivated Plants, Braunschweig, Germany

IL 2
Contaminant antibiotic resistance in water environments
C. M. Manaia
Centre for Biotechnology and Fine Chemistry, Porto, Portugal

IL 3
Life in the dark – patterns and functions of groundwater microbiomes
K. Küsel
Friedrich-Schiller-Universität Jena, Institut für Biodiversität, Jena, Germany

IL 4

Probiotic bacteria as disease control agents in marine fish larval rearing

L. Gram

Technical University of Denmark, DTU Bioengineering, Department of Biotechnology and Biomedicine, Lyngby, Denmark

IL 5

Microbiome manipulation of marine hosts and its potential to foster ecosystem resilience

R. S. Peixoto

Rio de Janeiro Federal University, Brazil, and University of Carlifornia at Davis (UC Davis), USA

Host-microbe interactions – ecology and evolution

IL 6
Sponge holobionts – from microbes to ecosystems
U. Hentschel
Helmholtz Centre for Ocean Research, Kiel, Germany

IL 7
Modulation of fish microbe-interactions in recirculating aquaculture systems (RAS)
N. C. Gomes
University of Aveiro, Department of Biology & CESAM, Aveiro, Portugal

IL 8
Central role of microbial biofilms in the biodegradation of hydrocarbons
F. Coulon
University of Cranfield, UK

IL 9
P. V. Morais
Lisbon, PT

IL 10
Horizonal transfer of antibiotic resistance genes in urban water systems
P. V. Morais

IL 11

Role of chemical mediators in aquatic interactions across the prokaryote-eukaryote boundary – the sea lettuce Ulva only gets into shape with the right bacteria

T. Wichard[1], M. Kwantes[1], G. Califano[1], A. Weiss[1]

[1]*Friedrich Schiller University Jena, Institute for Inorganic and Analytical Chemistry, Jena, Germany*

There is a growing interest in the occurrence and diversity of metabolites used as chemical mediators in cross-kingdom interactions within aquatic systems. Bacteria produce metabolites to protect and influence the growth and life cycle of their eukaryotic hosts. In turn, the host provides a nutrient-enriched environment for the bacteria.

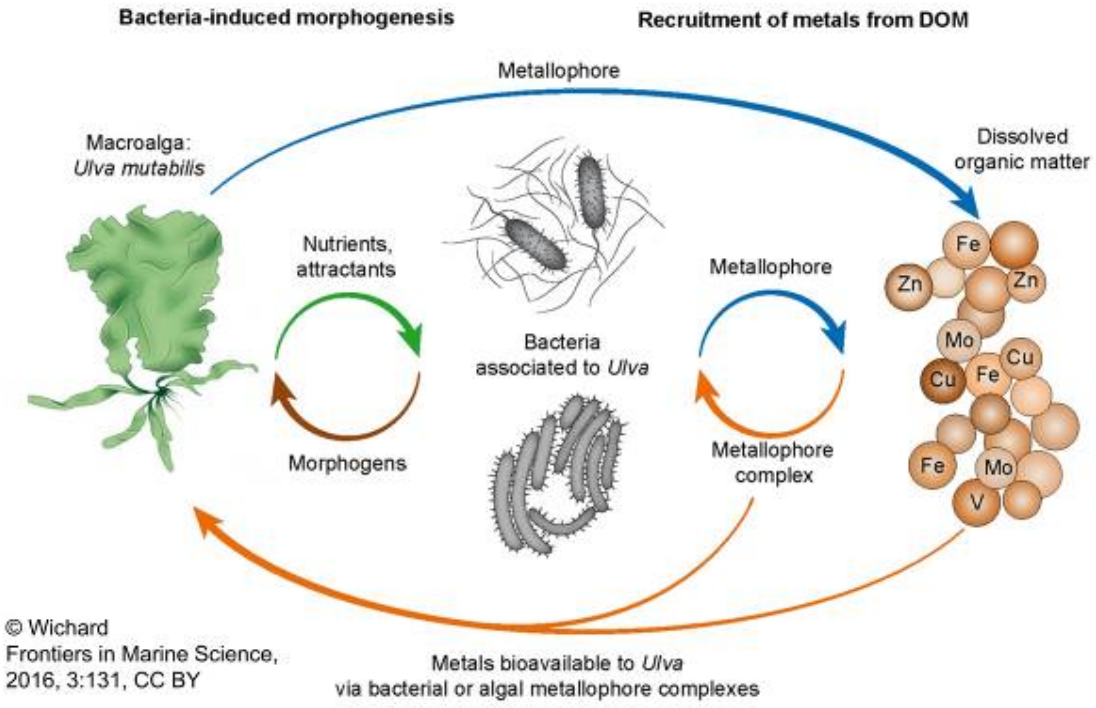
Here, I discuss the role of waterborne chemical mediators that are responsible for interactions in the green seaweed *Ulva* (Chlorophyta) and its associated bacteria. Growth and morphogenesis of *Ulva* depend on associated bacteria which release morphogenetic compounds that induce cell division and differentiation. Under axenic conditions, the model organism *Ulva mutabilis* develops into a callus-like-phenotype with cell wall protrusions. The combination of two bacteria, *Roseovarius* sp. MS2 and *Maribacter* sp. MS6 recovers the morphogenesis completely.

Results: Stable community and chemosphere evolution requires several essential steps that integrate the biofilm interface summarized in the following working model for *Ulva*: **(1) Finding a partner**: Macroalgae release dimethylsulfoniopropionate (DMSP), chemotactically attracting *Roseovarius* sp. MS2 (and other bacteria). **(2) Providing a carbon source**: *Ulva* delivers a glycerol boundary layer as a carbon source for *Roseovarius* sp., supporting biofilm formation. Bacteria successively assemble, depositing a self-produced mucilage layer. **(3) Inducing algal growth**: Upon establishing initial interactions, morphogenetic compounds stimulate *Ulva* cell divisions (*Roseovarius*-factor) and rhizoid formation (*Maribacter*-factor), promoting biomass production and connecting the alga directly with the bacterial biofilm through rhizoid formation induction. Higher algal biomass implies higher glycerol production, promoting bacterial growth. **(4) Exploiting a common chemosphere**: Close vicinity allows mutual benefit via the production of, for example, antibiotics or organic ligands for recruiting essential trace metals (Figure 1).

Conclusion: We conclude that many bacteria can use DMSP as a reliable signal indicating a food source and promote the subsequent development and morphogenesis in *Ulva* via morphogens.

References: Wichard and Beemelmanns (2018) Journal of Chemical Ecology 44:1008–1021 (review); Wichard et al. (2015) Frontiers in Plant Science 6:86 (review); Kessler et al. (2018) Molecular Ecology 27: 1808-1819; DeClerck et al. (2018) Current Biology 28: 2921-2933.

Fig. 1



Sattellite meeting:
COST Action HUPLANTcontrol – Control of human pathogenic microorganisms in plant production systems

IL 12
Plasmid mediated adaptation of plant-associated bacteria
K. Smalla
Julius Kühn Institut Federal Research Centre for Cultivated Plants, Braunschweig, Germany

IL 13
The natural resistome of microbial communities in plants and lichens
T. Cernava

IL 14
Microbial interactions as potential drivers for small scale spatial organisation of the plant root microbiome
S. Sørensen
University of Copenhagen, Denmark

IL 15
Evolution of microbiome traits and antibiotic resistances
J. D. van Elsas
University of Groningen, RUG Department of Microbial Ecology, Groningen, Denmark

Sattellite meeting:
COST Action HUPLANTcontrol – Control of human pathogenic microorganisms in plant production systems

IL 16
The plant microbiome within the one health concept
G. Berg
Technical University of Graz, Austria

IL 17

Built environments – new insights into the microbiome and resistome

G. Berg
Technical University of Graz, Austria

IL 18

What are the mechanisms behind alternative community states and how do we distinguish between them?

K. Faust
University of Leuven, Belgium

Oral Presentations

O 1

Mobile DNA in arctic and antarctic microbiocenoses – diversity, horizontal transfer and role of plasmids in adaptation of psychrotolerant bacteria to extreme environments

K. Romaniuk[1], A. Ciok[1], P. Decewicz[1], A. Gorecki[1], M. Dziurzynski[1], M. Styczynski[1], P. Golec[1], L. Dziewit[1]
[1]*University of Warsaw, Department of Bacterial Genetics, Warsaw, Poland*

Introduction: Plasmids, are self-replicating, extrachromosomal mobile genetic elements, and thus play a key role in horizontal gene transfer between bacteria. However, the knowledge about plasmids of cold-active bacteria originating from Arctic and Antarctica is scarce.
Objectives: The aim of the study was to reveal the diversity and role of plasmids in adaptation of psychrotolerant bacteria to extreme environments, and to analyze the range and directions of horizontal gene transfer in polar microbiocenoses.
Materials and methods: Standard molecular biology methods were used. Growth kinetics, biofilm formation and minimum inhibitory concentrations of toxicants were analyzed according standard procedures.
Results: Over 150 plasmids originating from cultivable psychrotolerant bacteria, as well as various plasmid contigs retrieved from metagenomic data were characterized. Identified replicons are mostly small, cryptic plasmids, including miniature replicons (<1 kb) found in glacier-inhabitants - *Variovorax* spp. Detailed analysis of larger (>10 kb) plasmids revealed the presence of numerous genes, that seem to affect bacterial adaptation to extreme environmental conditions. Amongst identified genes of adaptive value, we found and characterized determinants that: (i) protect bacteria against reactive oxygen species and UV radiation, (ii) confer resistance to heavy metals, (iii) enable assembly of iron-sulfur [Fe-S] clusters, (iv) enable utilization of hardly-degradable carbon sources and (v) increase biofilm formation abilities.
Conclusions: Many identified plasmids revealed to be beneficial for their bacterial hosts. Interestingly, as shown in the similarity network analysis, closely related genes were found within plasmids of bacteria belonging to various taxonomic groups, which exemplify the possible directions of horizontal gene transfer in polar environments. The direct examples of horizontal transfer of plasmids amongst co-residing bacteria were also found.

O 2

IncN plasmids are vectors disseminating colistin resistance in wastewater microbiota

Z. Yu[1], R. Pinilla-Redondo[1], J. Nesme[1], J. Madsen[1], N. Andersen[1], A. K. Olesen[1], H. Ananbeh[2,3], A. Dechesne[4], B. Smets[4], S. J. Sørensen[1]
[1]*University of Copenhagen, Department of Biology, Section of Microbiology, Copenhagen, Denmark*
[2]*Mendel University in Brno, Department of Chemistry and Biochemistry, Brno, Czech Republic*
[3]*Brno University of Technology, Central European Institute of Technology, Brno, Czech Republic*
[4]*Technical University of Denmark, Department of Environmental Engineering, Kongens Lyngby, Denmark*

Wastewater treatment plants (WWTPs) collect residual pharmaceutical compounds and massive amounts of human-gut associated bacteria, some of which are closely related to human pathogens. As high cell densities and recurrent antibiotic selective pressure favour plasmid-mediated dissemination occur in WWTPs, those are regarded as particularly active hot spots for the spread of plasmid-borne antibiotic resistance genes (ARGs). Plasmids belonging to the IncN incompatibility group are often associated with the dissemination of clinically relevant ARGs. Their presence in WWTPs and effluent waters together with their broad host range, high transfer rate and ability to replicate in Enterobacteriaceae, a group comprising several critical pathogens make them a critical concern to public health. However, little is known about their transfer dynamics in urban water systems.
In this work, we describe the capture and characterization of conjugative plasmids harbouring clusters of resistance determinants from a municipal WWTP activated sludge sample (Odense, DK). Plasmids were recovered by exogenous isolation using an *Escherichia coli* recipient strain. Nanopore sequencing revealed the presence of a 73Kbp IncN plasmid carrying a complete set of functional conjugative genes and multiple ARGs. Antibiotic susceptibility testing showed that this plasmid can transfer resistance of colistin, a last-resort antibiotic, to a sensitive *Escherichia coli* recipient. Direct isolation from the WWTP community confirmed the presence of colistin-resistant strains in that environment. Most were identified as *Pseudochrobactrum* sp. and *Ochrobactrum* sp. using full-length 16S rRNA gene sequencing. Interestingly, a *Pseudochrobactrum* isolate hosted a similar-sized IncN plasmid also encoding complete conjugative transfer systems and multiple ARGs. Further filter mating experiments with a colistin-sensitive recipient strain confirmed the mobility of the IncN plasmid and its associated colistin resistance. However, the responsible determinant could not be readily identified by sequence homology as one of the already characterized mobilized colistin resistance (*mcr*) gene variants. The identification of a potentially novel determinant of colistin resistance transferable to *Escherichia coli* is of significant importance for surveillance purpose. Together, these results uncover the dynamic nature of self-transmissible IncN plasmids and their role in the dissemination of colistin resistance in the WWTP environment.

O 3

Genetic Globetrotter – a marine plasmid hitch-hiking vast phylogenetic and geographic distances

J. Petersen[1], V. Ringel[1], A. K. Kaster[2], J. Vollmers[2]
[1]*Leibniz-Institute DSMZ, Microbial Ecology and Diversity Research, Braunschweig, Germany*
[2]*Karlsruhe Institute of Technology, Institute for Biological Interfaces 5, Karlsruhe, Germany*

Introduction: Horizontal gene transfer (HGT) plays an important role in bacterial evolution and triggers the origins of bacterial diversity and versatility. HGT events often involve mobile genetic elements like plasmids, which can promote their own dissemination by associating with adaptive traits in the gene pool of the so called mobilome. Novel traits that evolve through HGT can therefore lead to the exploitation of new ecological niches, prompting an adaptive radiation of bacterial species.
Results and Conclusion: In this study, we present phylogenetic, biogeographic and functional analyses of a novel, previously unrecognized RepL-type plasmid (pLA6_12) found in diverse members of the marine Roseobacter group all across the globe. Our study includes the first reported instance of identical plasmids observed in phylogenetically and geographically distant marine bacteria, revealing a previously overlooked, but environmentally highly relevant vector for HGT. The genomic and functional characterization of pLA6_12 showed a completely conserved backbone dedicated to replication, stability and mobilization as well as an interchangeable gene cassette with highly diverse, but recurring, motifs. The majority of the latter appear to be involved in mechanisms coping with toxins and/or pollutants in the marine environment. Experimental transfer of pLA6_12 into the model organism *Phaeobacter inhibens* DSM 17395 documented that its chromate resistance cassette confers a tenfold increased tolerance against extremely toxic hexavalent chromium. Furthermore, we provide experimental evidence that the plasmid has the potential to be transmitted across bacterial orders, thereby increasing our understanding of evolution and microbial niche adaptation in the marine environment.

Petersen J, Frank O, Göker M, Pradella S (2013) Extrachromosomal, extraordinary and essential--the plasmids of the Roseobacter clade. *Appl Microbiol Biotechnol*. 97: 2805-2815.
Michael V, Frank O, Bartling P, Scheuner C, Göker M, Brinkmann H, **Petersen J** (2016) Biofilm plasmids with a rhamnose operon are widely distributed determinants of the 'swim-or-stick' lifestyle in roseobacters. *ISME J*. 10: 2498-2513.
Brinkmann H, Göker M, Koblížek M, Wagner-Döbler I, **Petersen J** (2018) Horizontal operon transfer, plasmids, and the evolution of photosynthesis in *Rhodobacteraceae*. *ISME J*. 12: 1994-2010.

O 4

Phylogenomics illuminates the evolution of acquired and intrinsic resistance in environmental multidrug-resistant (MDR) *Stenotrophomona*

P. Vinuesa[1], L. E. Ochoa-Sánchez[1], J. Rivera-Campos[1], B. Contreras-Moreira[2]
[1]*Center for Genome Sciences - UNAM, Cuernavaca, Mexico*
[2]*EMBL-EBI: European Bioinformatics Institute, Hinxton, United Kingdom*

The genus *Stenotrophomonas* (*Gammaproteobacteria*) has a broad environmental distribution. Its best known species is *S. maltophilia*, an emerging MDR opportunistic pathogen, causing diverse life-threatening infections. Taxonomic problems in this species and genus have precluded the establishment of clear species-phenotype associations. Using phylogenetic and population genetic analyses on multilocus data of a large collection of Mexican environmental isolates, we have previously shown that the so-called *S. maltophilia* complex (Smc) contains at least 5 distinct lineages. Only the one we defined as *S. maltophilia sensu lato* (Smsl) is MDR and expresses metallo-beta-lactamases (MBLs) (1). This was confirmed by a phylogenomic study based on core- and pan-genome phylogenies, performed on a large genome dataset (*n*=119), including 10 complete Mexican Smc genomes, using our new software package GET_PHYLOMARKERS (GP) (2). We assembled and annotated 50 additional complete genomes from our collection and estimated a robust species tree for the genus with GP, encompassing the genus diversity.
To understand the evolution and genomic basis of resistance in *Stenotrophomonas*, presence-absence data for antibotic-resitance genes and efflux pump loci (EPs) were mapped on the species tree, revaling a lineage-specific accumulation of particular EPs along the phylogeny (Fig. 1), explaining the high intrinsic resistance experimentally determined for our Smsl isolates. These bacteria lack plasmids, but phylogenetic and comparative analyses revealed horizontally-acquired resistance genes (ARGs) not previously reported for *Stenotrophomonas*, particularly at sewage-polluted sites, which are associated with chromosomally-encoded mobile genetic elements, like a novel Tn*402*-like class-1 integron (Fig. 2), and diverse genomic islands. A selection of these loci were functionally characterized by heterologous expression en *E. coli* and by structural modelling of encoded proteins. Genome-wide surveys for genes under positive selection (PS) revealed that several components of specific EPs and MBLs have experienced bursts of episodic PS in the branch subtending the Smsl clade.
We conclude that the evolution of resistance in *Stenotrophomonas* is largely governed by lineage-specifc gene acquisition of EPs, HGT of ARGs and diversification by PS.
FUNDING: PAPIIT-UNAM IN206318
1. Ochoa-Sánchez L. and Vinuesa P. 2017. Front Microbiol. 8, 1548
2. Vinuesa P. et al. (2018). Front. Microbiol. 9:771

Fig. 1

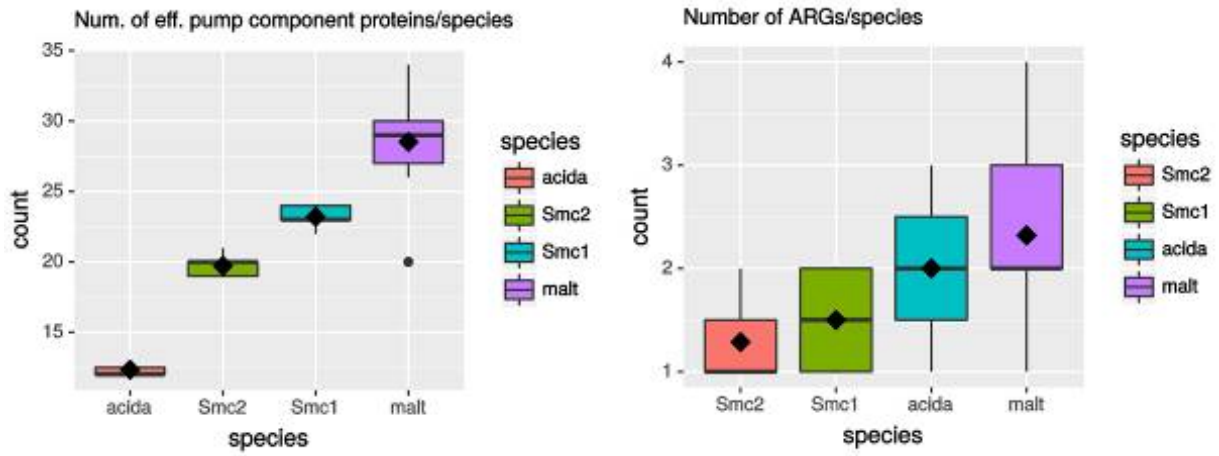
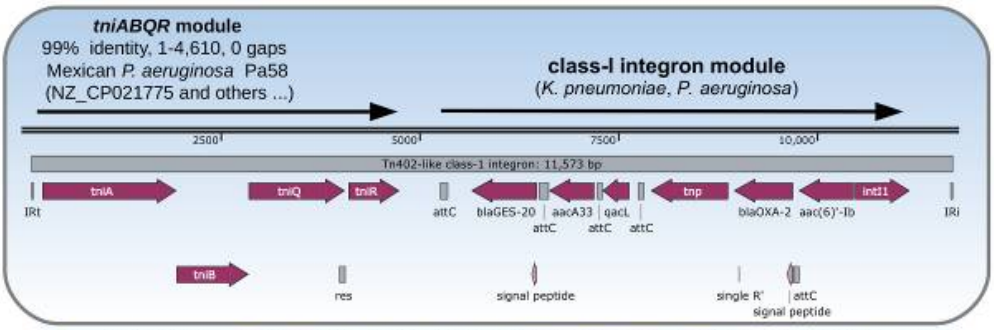


Fig. 2



Gene cassette array (5' → 3') in the *S. acidaminiphila* TEXM2D_MKIMI4_3 Tn*402*-like class-1 integron

- **AAC(6)'-Ib**, aminoglycoside N(6')-acetyl transferase, type I
- **BlaOXA-2/OXA-544 – like** (99.86% ID; 1 mismatch), oxacillin-hydrolyzing *class D* β-lactamase
- **D,D(35)E transposase**
- **QacL / EmrE,** quaternary ammonium compound efflux, SMR transporter
- **AAC(6')-33**, aminoglycoside 6'-N-acetyltransferase
- **BlaGES-20 -like** (with 54 nt-long in-frame deletion), *class A* ESBL coferring carbapenem resistance

O 5

***Bacillus subtilis*: bacterial sex is more popular between less related organisms**

P. Stefanic[1], K. Belcijan[1], B. Kraigher[1], R. Kostanjšek[2], I. Mandić Mulec[1]

[1]*University of Ljubljana, Department of food science and technology, Chair of Microbiology, Ljubljana, Slovenia*

[2]*University of Ljubljana, Department of Biology, Ljubljana, Slovenia*

Questions: *B. subtilis* is a soil dwelling bacterium with a diverse social life that includes interactions resulting in bacterial equivalent of sex, which subsequently have a profound influence on bacterial evolution. Recently we discovered kin discrimination among highly related strains of *B. subtilis*, where less related strains showed antagonistic behaviour towards each other in the form of killing, however, the role of kin discrimination in bacterial sexuality remains unknown. Our aim was to identify how kin discrimination affects horizontal gene transfer and to find mechanisms responsible for gene exchange.

Methods: Swarming assays were performed and samples were taken from the meeting points of kin and non-kin swarms. DNA concentration, DNA exchange and activation of competence genes was determined by QuantiFluor® system, selective plating and fluorescent microscopy, respectively. Cell integrity was investigated by electron microscopy.

Results: We show that during swarming, strains of *B. subtilis* preferably take up DNA from less related non-kin strains. We also demonstrate that this mechanism stems from a higher induction of competence at the meeting point of two non-kin strains due to cell-envelope stress response and not from increased DNA concentration resulting from killing at the meeting point of non-kin strains.

Conclusions: Our results demonstrate an important evolutionary mechanism of "promiscuous but safe sex": a type of bacterial cell-contact dependent DNA exchange that could promote innovation and adaptation inside the species but still bring low risk of integrating toxic DNA. Our discoveries also imply that primary function of this mechanism is diversification instead of genome repair. Our research might help understand the vast genomic diversity within this species despite existing mechanisms limiting less-related DNA integration during transformation.

O 6

Microbes in the city – a metagenomic trait-based analysis of microbial metacommunity structure along urbanization gradients

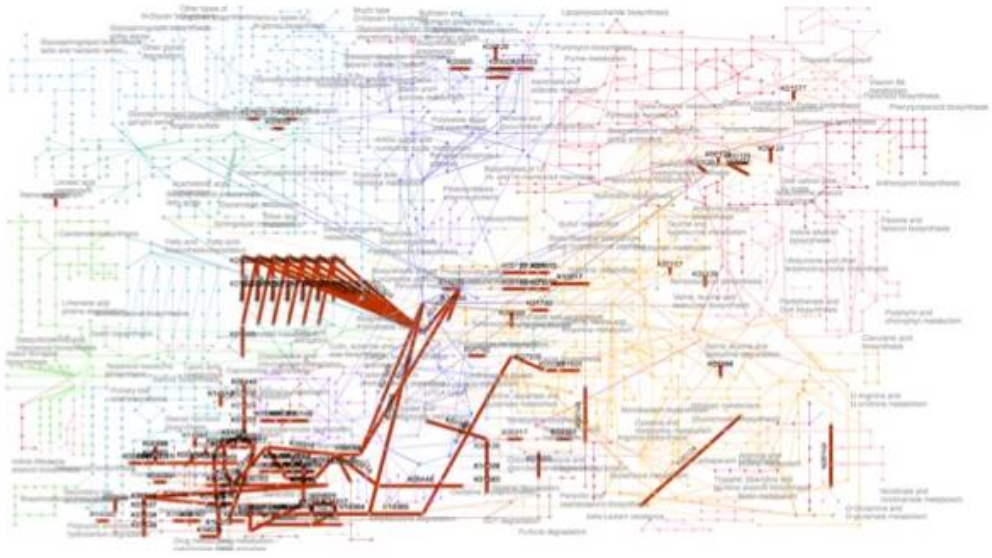
S. Mukherjee[1], Y. Darzi[2,3], A. Kumar Anand [4,5], C. Souffreau[1], T. Yamada[2], J. Raes[3], L. De Meester[1]
[1]KU Leuven, Biology, Leuven, Belgium
[2]Tokyo Institute of Technology, Department of Biological Information, Tokyo, Japan
[3]KU Leuven, Department of Microbiology and Immunology, Rega Institute, Leuven, Belgium
[4]University of Cambridge, School of Clinical Medicine, Cambridge, United Kingdom
[5]University of Cambridge, Department of Paediatrics, Wellcome - MRC Cambridge Stem Cell Institute, Cambridge, United Kingdom

Introduction: Urban ecosystems are one of the most crucial examples of the overwhelming impact that humans impose on the natural environment, leading to a deep biodiversity crisis. Given the facts that microbes play a pivotal role of in driving biogeochemical cycles and overall ecosystem functioning, and are often the first responders to environmental perturbation; it is of utmost importance to gain insight into the mechanisms underlying microbial adapation to anthropogenic stress.

Materials and methods: In this study, we used freshwater ponds located across well-characterized gradients of urbanization in Belgium as model systems, in order to determine the impact of urbanization on microbial metacommunities. A GIS based selection was used to identify 27 plots in three categories of urbanization (determined by % built-up area); cut-off points for the % built-up area were set at 0-3% for "low" urbanization, 5-10% for "moderate" urbanization, and >15% for "high" urbanization. Within each of the 27 larger plots, 3 subplots again representing "high", "low" and "moderate" urbanization were identified such that they harbour a shallow pond. Shotgun metagenomic sequencing of DNA extracted from bacterioplankton samples collected from 63 ponds was performed on an Illumina HiSeq 2500 platform with a coverage of ~5Gb per sample. Quality trimmed and assembled scaffolds were used for gene prediction and were further summarized into a gene-catalog of ~10 million genes. This gene-catalog was annotated against multiple databases including KEGG, Pfam, TIGRFAM and eggNOG to gain multi-view insights into functional responses of microbes to urbanization. Molecular trait matrices of relative frequencies of KEGG pathways and modules were created and their responses to urbanization were determined.

Results and conclusions: KEGG pathways associated with degradation of Atrazine, Caprolactam, Styrene, Flurobenzoate, Chloroalkane and Chloroalkene were significantly enriched in urban ponds. We further zoomed into responses of 318 individual kegg orthologs associated with 21 xenobiotic degradation KEGG pathways. This analysis revealed a significant increase in relative abundances of genes involved in degradation pathways of BPA, PET, DDT, cyanuric acid, Hexachlorocyclohexane and Mandelic acid in ponds located along areas with high % built-up. These results provide a glimpse of urbanization-associated molecular signatures of bacterial communities in aquatic habitats.

Fig. 1



Pathway enrichment(globaltest algorithm) : xenobiotic degradation genes enriched in urban ponds are highlighted

O 7

Impact of environmental perturbations on multiple ecological processes of adaptation in ammonia oxidisers

C. Gubry-Rangin[1], A. Aigle[1], C. Thion[2], J. Pett-Ridge[3], G. W. Nicol[2], M. Firestone[4], J. Prosser[1]
[1]*University of Aberdeen, Aberdeen, United Kingdom*
[2]*Ecole Centrale de Lyon, Lyon, France*
[3]*LLNL, Berkeley, CA, United States*
[4]*University California, Berkeley, CA, United States*

Niche specialisation and differentiation are crucial concepts for understanding environmental adaptation of microbial communities. However, they are often difficult to demonstrate in natural environments because of the complexity of microbial communities, lack of information on physiological characteristics of environmental significance and functional redundancy. Alternative approach is to use knowledge gained from ecophysiological studies on cultures in controlled microcosms.
Information is available on the ecophysiology of archaeal and bacterial ammonia oxidisers (AOA and AOB), which perform the same ecosystem function and control rates of soil nitrification, the oxidation of ammonia to nitrate via nitrite; ammonia oxidisers therefore present a valuable model for studying terrestrial adaptation. This study adopted a hypothesis-based approach to investigate niche specialisation of AOA and AOB and their differential responses to perturbations in two potentially important environmental characteristics, soil pH and temperature.
Hypothesis predictions of responses of AOA and AOB abundance, activities and community structure to perturbations in soil pH and temperature were tested experimentally in soil microcosms in which both groups were present and potentially active by measuring temporal changes in gene and transcript abundance and levels of stable isotope assimilation. These measures of growth and activity were complemented by analysis of the community composition at a very fine phylogenetic scale using a newly developed Illumina MiSeq sequencing approach, employing a bioinformatics pipeline adaptable to any functional gene. Fine-scale diversity of active microbes was also matched to a comprehensive database of pH preferences of soil AOA.
The findings provide evidence for niche specialisation of AOA and AOB following environmental perturbations and demonstrate the importance of considering prokaryote richness and phylogenetic resolution for understanding ecosystem function and assessment of functional redundancy. Environmental perturbation also led to preferential selection and growth of generalist or specialist AOA, depending on the intensity of pH perturbation. The study therefore demonstrates the need for well-defined hypotheses for understanding microbial adaptation in complex ecosystems and provides novel experimental and conceptual approaches for studying richness-function relationships, functional redundancy and niche specialisation concepts.

O 8

Understanding the reversion of soil CH4 flux across the forest-to-pasture conversion in the Amazon rainforest

D. Obregón[1], M. Tuzzin de Moraes[1], L. Fonseca[1], D. Navroski[1], L. W. Mendes[1], J. L. M. Rodrigues[2], B. J. M. Bohanann[3], P. Camargo Barbosa[1]
S. Saleska[4], C. Wagner- Riddle[5], K. Dunfield[5], S. M. Tsai[1]
[1]*University of São Paulo, Center for Nuclear Energy in Agriculture, Cell and Molecular Biology Laboratory, Piracicaba, Brazil*
[2]*University of California, Department of Land, Air and Water Resources, Davis, CA, United States*
[3]*University of Oregon, Institute of Ecology and Evolution, Corvallis, OR, United States*
[4]*University of Arizona, Ecology and Evolutionary Biology, Tucson, AZ, United States*
[5]*University of Guelph, School of Environmental Sciences, Guelph, ON, Canada*

Our previous data indicated that Amazonian forest soils are important sinks of atmospheric CH4, but the forest-to-pasture conversions turn them into net emission sources. Presumably, this switch occurs due to transformations of methane-cycling microbial communities, which are likely associated to alterations in physicochemical properties of the soil. In this work, we describe a multidisciplinary study analyzing this multidimensional phenomenon, including CH4 fluxes measurement, isotope analysis for identification of isotopic signature of CH4 (δ13C and δ2H), characterization of soil physical and chemical properties. We coupled these measurements to molecular quantifications of methanogenic and methanotrophic microorganisms and the taxonomical characterization of the microbial communities, based on high-throughput amplicon sequencing of 16S rRNA gene from Bacteria and Archaea. The dataset was generated from samples (soil and gases) collected in areas of forest and pastures at the Tapajos National Forest, at the Eastern Amazonia (Brazilian State of Pará). The gases and soil were sampled during the rainy and dry seasons of 2017, including in sites of forests growing in two types of soil textures (clay and sandy) for each land use. Samplings soil profiles were at 0-10, 10-20, 20-30 and 30-40 cm, respectively. Changes *in situ* methane flux were strongly associated with land use changes, as native forest soils maintained methane consumption throughout the seasons at deeper depth (0-40cm) when compared to the pasture soils (0-20 cm). Alterations in the taxonomic profiles of methanotrophic bacterial communities were observed, predominantly the genus Methylocystis and AlphaI cluster in forest and pasture soils, respectively. However, the main alterations were found within the methane emissions by Archaea, most significantly in sandy pasture soils. The isotopic signature of CH4 suggested that increases in CH4 emission in pasture soils, especially in sandy soils, were associated to the hydrogenotrophic pathway of methanogenesis. These results are responding to a scientific question raised 20 years ago, and confirm the importance of the integration of biogeochemical sciences to respond to the challenge of lowering methane emissions in the planet, by stressing the need for avoidance of practices such as the conversion of forest to pasture within the Amazonian Basin.

O 9
No publication allowed.

O 10
Biogeographical and depth patterns of global marine prokaryotic community structure and function
C. Astudillo-Garcia[1], G. Lear[1]
[1]University of Auckland, School of Biological Sciences, Auckland, New Zealand

Environmental factors are an important determinant of microbial community assembly processes. While the influence of these factors on microbial community structure has been investigated across small spatial scales, a much broader perspective is needed to decipher ecological patterns driving microbial community structure and functionality at a global scale. The TARA Oceans expedition systematically collected more than 35,000 samples for morphological, genetic, and environmental analyses across multiple depths at the global scale. The dataset"s breadth provides an excellent opportunity to interrogate the specific role of environmental variation as a determinant of global latitudinal and depth gradients in species richness, functional potential, genome size and microbial co-occurrence patterns. We selected 134 prokaryote-enriched microbial metagenomes collected from around the world from surface, deep-chlorophyll maximum and mesopelagic seawater. Pre-assembled metagenomes were re-annotated and average genome size was calculated as a proxy for microbial metabolic versatility to investigate putative gradients of increasing microbial genetic complexity. We showed that, while latitude did not significantly affect microbial species richness, it influenced functional diversity, which increased significantly towards the equator. Depth was the main driver of change in microbial community richness, functional diversity and average genome size, with microbial assemblages from the mesophilic zone presenting highest values for every factor. Comparative analyses were conducted to identify specific functions and/or genes enriched along the depth profile. Surface communities were significantly enriched in genes involved in intracellular trafficking, secretion and vesicular transport as well as extracellular structures; deep-chlorophyll maximum in genes related with the cytoskeleton; and the mesophilic zone with energy production and conversion, amino acid transport and metabolism, secondary metabolites biosynthesis, transport and catabolism. Moreover, microbial co-occurrence patterns explored using correlation networks were also affected by depth. Larger and more compartmentalized networks where found when increasing depth, presenting different keystone species. Overall, our results show that microbial species richness, functional diversity, average genome size and inter-taxa correlations are differently affected by gradients in environmental factors and geographic distance

O 11

Facilitation and strain variability maintain persistence of generalist microbial communities in the European seabass gut

F. Kokou[1], G. Sasson[1], J. Friedman[2], A. Cnaani[3], I. Mizrahi[1]
[1]Ben-Gurion University of the Negev, Department of Life Sciences, Be'er Scheva, Israel
[2]Hebrew University, Rehovot, Israel
[3]Agricultural Research Organization, Rishon LeZion, Israel

The term core microbiome has been coined for microbes consistently present in a particular habitat. If conditions in that habitat are highly variable, then core microbes may also be considered ecologically generalists. However, little is known about whether metabolic competition and microbial interactions influence the ability of some microbes to persist in the core microbiome while others cannot. As a model, we investigated microbial communities in three gut sites of the European seabass under four dietary conditions. These were compared with previous results from other fish species. We identified generalist core microbial populations in each gut site that are shared across fish, present under multiple diets, and persistent over time. We find that core microbes tend to show synergistic growth in co-culture, and low levels of predicted metabolic competition. Within core microbial species, we find higher intraspecific variability than in other microbes. Thus, both intraspecific variability and interspecific facilitation may contribute to the ecological stability of the animal core microbiome.

O 12

Biological Denitrification Inhibition (BDI) in the field – strategy to improve plant nutrition and growth

W. Galland[1,2], F. Piola[2], A. Burlet[3], C. Mathieu[3], M. Nardy[1], S. Poussineau[2], L. Blazère[2], J. Gervaix[1], S. Puijalon[2], L. Simon[2], F. E. Haichar[1]
[1]UMR 5557 CNRS (Lyon 1 university), Ecologie Microbienne Lyon, Villeurbanne, France
[2]Université de Lyon, UMR5023 LEHNA, Université Lyon 1, CNRS, ENTPE, LEHNA, Villeurbanne, France
[3]Station d'Expérimentation et Information , SERAIL, Brindas, France

To meet the needs of human populations, agriculture is more intensive, using excessively a large number of nitrogen fertilizers to increase yields. The challenges of tomorrow's agriculture are to cope with an ever-expanding population, while limiting the impact on our environment. These fertilizers are used because nitrogen is one of the limiting factors of plant growth (1). However, in soils nitrate is also used by denitrifying bacteria that reduce it to N_2O (greenhouse gases) and N_2. Therefore, plants are in direct competition with these bacteria for the assimilation of nitrate (2). Recently, the group has highlighted a strategy developed by some plants consisting of secondary metabolite production: procyanidins, that inhibit the denitrification of microbial communities in soils *in situ* (3). This strategy could make nitrate more available in the soil, that could be used by plants for their growth. However, the extent to which procyanidins can affect plant growth and nutrition *via* BDI under field conditions has not yet been investigated. In this study, we tested the effect of exogenously applied procyanidins in the field on *Lactuca sativa* crops nutrition and growth.Our results show that the addition of procyanidins in fields at different concentrations results in an inhibition of microbial denitrification activity in soils (N_2O emission measured by gas chromatography), an increase in available nitrate (ion chromatography), counter-selects denitrification communities in the roots adhering soil of lettuce (qPCR of *nirK* and *nirS* genes) and a significant gain in plant productivity on treated crops. This study allowed us to propose in the short term the development of a more environmentally friendly method of sustainable agriculture, by limiting fertilizer inputs, nitrogen losses in the soil, and greenhouse gas emissions, while increasing agricultural productivity.

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O 13

Reduced potential for the degradation of phenolic compounds in the rhizosphere of apples grown in replant soils

V. Radl[1], J. Winkler[1], S. Kublik[1], L. Yang[1], G. Vestergaard[1], T. Winklenmann[2], P. Schröder[1], M. Schloter[1]
[1]Helmholtz Zentrum München Deutsches Forschungszentrum für Gesundheit und Umwelt, Oberschleißheim, Germany
[2]Leibniz Universitaet Hannover, Woody Plant and Propagation Physiology Section, Hannover, Germany

Apple replant disease (ARD) is a phenomenon that occurs in areas where apple plants or closely related species have been cultivated for many years. Even though ARD is a well-known phenomenon, which has been observed on different regions and soil types, the causes behind its emergence remain unclear. In the present work, we tested the hypothesis that ARD is caused by changes on assembly of the soil microbial community rather than just changes on pathogen loads. To test this hypothesis, we performed pot experiments where apple plants were grown in virgin (CO) or diseased (ARD) soils from an experimental nursery area, localized in Ellerhoop (Schleswig-Holstein, Germany). Microbial communities were assessed using 16S rRNA amplicon and shotgun metagenome. Utilization of plant derived carbon by soil microbes was investigated using 13CO2 labeling experiments performed in climate chambers. Ten weeks after apple plantlets were transferred to soils we could detected lower plant biomass in ARD compared to control pots. We observed larger differences in composition of bacterial communities of ARD and CO after 12 weeks. Shotgun metagenome showed a clear differentiation between the rhizosphere and bulk soil compartments. However, differences associated the treatment were only evident at the rhizosphere, for which we detected changes on the abundance of major bacterial genera. Even though reads assigned to pathogenic fungi were detected in the libraries, those were very low abundant and did not significantly differ between the treatments. Remarkably, we detected a significantly lower abundance of many genera of the phylum Actinobacteria. Moreover, we observed a lower potential for the degradation of aromatic compounds in replant soils, which is probably connected with higher concentration of phenolic compounds, such as benzoic acid, generally associated with ARD. In fact, incorporation of plant derived carbon by the soil microbes was higher in virgin soil. We did also observed clear differences in the metabolic profiles from ARD and virgin soils. Our data indicate that long-term release of plant derived carbon causes changes in the soil, thereby, affecting the activity and composition of the soil microbiome, which probably facilitates the establishing of pathogenic organisms in the roots of the young apple plants.

O 14

Plant protection from a distance

A. Ossowicki[1], S. Jafra[2], J. M. Raaijmakers[1], P. Garbeva[1]
[1]NIOO-KNAW, Microbial Ecology, Wageningen, Netherlands
[2]Intercollegiate Faculty of Biotechnology of University of Gdansk and Medical University of Gdansk, Gdańsk, Poland

Soil and plant microbiomes are essential to maintain plant growth, health and protection against biotic and abiotic stresses. The phenomenon of soil suppressiveness, where plants show significantly reduced disease symptoms even if the pathogen and favourable conditions are present, is strongly related to the microbial activity and secondary metabolites production. Nevertheless, not much is known about microbial mechanisms in suppressive soils especially when it comes to Volatile Organic Compounds (VOCs). Due to their physicochemical properties, VOCs easily diffuse through gas- and water-filled pores in soil and rhizosphere environments, allowing long-distance interactions. Many bacterial VOCs were found to possess strong antifungal activity. One of the examples is the volatile-mediated antimicrobial activity against fungal and oomycete plant pathogens expressed by rhizosphere isolate *Pseudomonas donghuensis* P482. We investigated the role of the GacS/GacA system on VOCs production in *P. donghuensis* P482. The results obtained demonstrated that VOCs emitted by *P. donghuensis* P482 have strong antifungal and antioomycete, but not antibacterial activity. The production of certain antifungal/antioomycete volatiles such as dimethyl sulfide, S-methyl thioacetate, methyl thiocyanate, dimethyl trisulfide, 1-undecan and HCN is depended on the GacS/GacA two-component regulatory system.
However, can we correlate soil fungistasis with soil suppressiveness? Our recent findings based on the screening of 28 soils from the Netherlands and Germany indicate that VOCs-mediated soil suppressiveness and soil fungistasis does not always correlate with each other. VOCs-mediated interactions only partially explained soil suppressiveness of *Fusarium culmorum*. Currently, we are identifying the microbial consortia and their secondary metabolites (both water-soluble and VOCs) involved in soil suppressiveness.

O 15

Bioinformatics tools and strategy to select microbial strains for fermented food products

H. Falentin[1], S. Deutsch[1], V. Gagnaire[1], A. Thierry[1], S. Dérozier[2], C. Nédellec[2]
[1]INRA, Microbiology, Rennes, France
[2]INRA, Jouy-en-Josas, France

Introduction: Fermented foods are complex biological ecosystems, harbouring diverse microbial communities which contribute to the quality of the products. In most industrial processes, these communities are advisedly chosen as starter culture for their adaptation to (i) the substrates to be fermented (milk, vegetable) (ii) the process (°C, O_2, NaCl) and for the desired overall quality of the food product in terms of texture, flavour, nutrition and health benefits. For this purpose, technological properties of microbial strains are mainly assessed by time-consuming *in vitro* screenings. We hypothesis that the huge amount of bibliographic and microbial genomic data available in public databases would enable an *in silico* preselection of the strains of interest before wet laboratory screening experiments.
Question: Our aim was to provide (i) adapted bioinformatic tools to mine the diversity of microbial technological properties and metabolisms (ii) a strategy to preselect strains based on their phenotypes and on their genetic potential. Finally, the tools developped will be applied for a selection of strains able to ferment diverse vegetable raw material and to produce specific aroma coumpounds. Tne identified combination will be tested in wet lab.
Methods: We used a bioanalysis approach to provide insight into the presence of enzyme-encoding genes responsible for targeted technological properties. KEGG, Metacyc, NCBI and CAZY databases were queried by a search on enzyme numbers, names and sequence alignments. We developed Florilege (http://migale.jouy.inra.fr/Florilege/), a database of microbial phenotypes based on text-mining to gather microbial growth conditions.
Results: Combining both approaches, we set up a bioinformatic preselection of lactic acid bacteria able to degrade sugar from milk, cereal or legume based juice to develop yogurt-like products. Database queries provided a list of microbial species with genes encoding key targeted enzymes. A set of preselected anaerobic, mesophilic and thermophilic strains has been successfully screened on different milk and juice in our wet laboratory providing potential new starter strains.
Conclusion: The developed tools and the strategy can be usefully applied to other domains like bioremediation and white biotechnology.

O 16

High-throughput screening for beneficial synthetic microbial communities

J. Friedman[1], J. Kehe[2], A. Kulesa[2], A. Ortiz[2], J. Gore[2], P. Blainey[2]
[1]Hebrew University, Plant Pathology and Microbiology, Rehovot, Israel
[2]MIT, Cambridge, MA, United States

Goal: Microbial communities have numerous potential applications in biotechnology and agriculture. However, current efforts to elucidate the properties of such communities, and to engineer beneficial communities are limited by the combinatorial complexity of interactions between species and their dependencies on environmental conditions. The goal of this work is to develop a complementary approach wherein synthetic communities are combinatorially constructed and assayed in high throughput, enabling direct screening for communities that perform desired functions, such as suppression of pathogens or facilitation of plant growth-promoting bacteria.
Methods: We have developed the kChip, a nanodroplet-based platform that performs rapid, massively parallel, bottom-up construction and screening of synthetic microbial communities. The kChip platform enables parallel construction and quantitative screening of ~100,000 synthetic microbial communities per day, and requires no robotic liquid handling. The platform screens communities composed of up to 19 inputs (e.g. strains or media) randomly selected from a larger library of potential inputs. Drawing on advances in micro-compartmentalization, the kChip platform generalizes a high-density microwell array approach that groups and merges sets of nanoliter droplets that each carry input components.
Results: As a first demonstration of the utility of high-throughput screening, we discovered specific compositions of bacteria isolated from soil that promote the growth of a model plant symbiont Herbaspirillum frisingense. To do so, we measured the yield of a GFP-expressing H. frisingense strain in co-culture with ~100,000 synthetic communities comprising up to 19 soil isolates. We discovered specific compositions that promote Hf growth in a manner that is robust to the available carbon sources and to the presence of additional strains.
Conclusions: kChip screening can identify multistrain consortia possessing any optically assayable function, including facilitation of biocontrol agents, suppression of pathogens, degradation of recalcitrant substrates, and robustness of these functions to perturbation. Moreover, large datasets collected through kChip screening can be leveraged to characterize how a given species responds across a large array of biotic and abiotic environments, elucidate how these factors drive microbial interactions, and uncover design principles for functional consortia.

O 17

Vibrio cholerae in food vacuoles expelled by protozoa are more infectious _in vivo_

G. Espinoza-Vergara[1], P. Noorian[1], D. McDougald[1]
[1]University of Technology Sydney, ithree Institute, Sydney, NSW, Australia

Vibrio cholerae is an aquatic bacterium that is the aetiologic agent of the acute diarrheal disease cholera, which is endemic in many countries. Outbreaks are linked to inadequate access to clean water and sanitation and it is estimated that there are 1.3 to 4.0 million cases and 21,000 to 143,000 deaths annually worldwide. In aquatic environments,V. cholerae interacts with a wide variety of organisms, including heterotrophic protists (protozoa). Several species of these bacterial predators have been reported to release live, undigested bacteria in expelled food vacuoles (EFVs) when feeding on certain pathogens. While the production of EFVs has been reported, their biological role as a vector for the transmission of pathogens remains unknown.
To produce EFVs, V. cholerae was co-incubated with Tetrahymena pyriformis. Briefly,T. pyriformis were enumerated by microscopy and adjusted to 10^3 cells ml^{-1} and added to co-cultures of V. cholerae adjusted to 10^8 cells ml-1. After overnight incubation EFVs were purified by filtration, incubated for 1 h with gentamicin 300 µg ml^{-1} to kill any extracellular bacteria. After gentamicin treatment, V. cholerae-EFVs pellets were collected by centrifugation, washed three times and suspended in 1 ml of 0.55 X NSS.
Using co-incubation assays, we report that T. pyriformis releases large numbers of EFVs when feeding on V.cholerae. The EFVs are stable, the bacterial cells within are protected from multiple stresses (low pH, antimicrobials and starvation) and vast numbers quickly escape when incubated at 37°C or in the presence of nutrients. We show that OmpU, a major outer membrane protein positively regulated by ToxR, plays a significant role in the production of EFVs. Importantly, cells released from EFVs have growth and colonisation advantages over planktonic cells both in vitro and in vivo and are highly infectious (as shown in the infant mouse model of infection). Our results suggest that EFVs facilitate V. cholerae survival in environment and in the gastric environment, enhancing infectious potential and may significantly contribute to the dissemination of epidemic V. cholerae strains. These results establish a new understanding of the mechanisms of persistence and the modes of transmission of V. choleraeand may further apply to other opportunistic pathogens that have been shown to be released by protists in EFVs. Results presented here will improve the identification and tracking of pathogens in the environment.

O 18

Heritability and domestication of the cereal seed microbiota

M. Cardinale[1], E. Flory[2], Y. Abdullaeva[2], B. Schneider[2], B. Ambika Manirajan[2], S. Schnell[2]
[1]University of Salento, Department of Biological and Environmental Sciences and Technologies (DiSTeBA), Lecce, Italy
[2]Justus-Liebig-University Giessen, Giessen, Germany

In the last years, the plant seed microbiome has received much attention from microbial ecologists and agricultural microbiologists. The reason is, that plant seeds (previously considered free of microorganisms inside) were discovered to stably harbor a highly diverse community of microbes[1], which can be transferred to the next plant generation[2]. This discovery expanded the current concept of plant holobiont to a more evolutionary context, since the possibility to vertically inherit microbes leads to the hypothesis that microbe selection by the plant, and their transmission using seeds as vectors, is a driving force shaping the plant microbiome assemblage and functionality. In this work, we analyzed the seed microbiota of 8 cereal species: 1 barley, 3 wheats, and their corresponding ancestors (all cultivated and maintained under identical conditions). We sequenced 16S rRNA gene libraries with IonTorrent NGS, using a new PNA blocker that increased the number of bacterial reads up to 70 times. Crop seeds showed a significantly higher proportion of *Cutibacterium*, while the wild ancestors had more *Pseudomonas*. This is coherent with the discovery, in grapevine plants, of *Propionibacterium zappae*, a bacterium whose speciation occurred at the beginning of grapevine domestication[3]. To assess the extent of the vertical transmission of seed-associated bacteria to the next plant generation, we analyzed the seed microbiota of the 1st and the 2nd barley plant generation, in greenhouse. Although the microbiome structure was notably different (probably due to the differences between greenhouse and field conditions), we could calculate that 30% to 70% of the bacteria in the 2nd generation seeds likely originated from the 1st generation seeds. Our results suggest that cereal crop domestication modified also the seed microbiota, by enriching typical human-associated species. Moreover, it appears that the extent of bacterial vertical transmission to the next plant generation is higher than previously thought.

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O 19

Insights into the diazotrophic microbiome of wetland rice and its N_2-fixation activity

H. Schmidt[1], D. Seki[1], D. Randi[1], L. Dufour[1], A. Schintlmeister[1], D. Woebken[1]
[1]University of Vienna, Department of Microbiology and Ecosystem Science, Vienna, Austria

The microbiomes of numerous plant species are currently being revealed; yet, studies focusing on specific functional guilds are still scarce. N_2-fixing microorganisms (diazotrophs) represent an important guild, as they are able to convert atmospheric N_2 into ammonia thus promoting plant growth. For wetland rice, diazotrophs can alleviate N-shortage, and their activities will be affected by the gradients of carbon sources (via root exudates) and oxygen in the soil-plant interface. However, major questions regarding the identity of active microbial players, their spatial distribution, and the transfer of N to rice plants remain unanswered.

We have performed mesocosm experiments to investigate the diversity, spatial distribution, and activity of diazotrophs in association with wetland rice. Amplicon sequencing of dinitrogenase reductase (*nifH*) genes and transcripts was performed to study the effects of soil type, rice variety, and biocheochemical factors on the diversity and potential activity of diazotrophs in soil-plant microenvironments (bulk soil, rhizosphere, root). N_2 fixation activity in these microenvironments was assessed via whole-plant incubations with $^{15}N_2$-containing atmospheres followed by single-cell analyses through high-resolution secondary ion mass spectrometry (NanoSIMS).

N_2 fixation activity was detected in microorganisms inhabiting bulk soil, as well as rhizosphere and rhizoplane of rice roots. *NifH* gene and transcript sequence data indicate that the diazotrophic communities were distinct across the different microenvironments, and that the diversity of diazotrophs decreased from bulk soil towards the roots. These data indicate that different biogeochemical properties present in these sites were driving the diazotrophic community differentiation. In addition, soil type and plant genotype influenced the recruited communities. In order to reveal the location of active diazotrophs in the rhizoplane of rice roots (a potential site for N transfer), we have recently developed a Gold-FISH – NanoSIMS approach. This approach allows us to link the *in situ* visualization of single bacteria on rice roots with their incorporation of ^{15}N in $^{15}N_2$ incubations.

Overall, we will provide novel insights into biological N_2 fixation of diazotrophs associated with wetland rice, ranging from the scale of microbial communities to the individual players - single diazotrophic cells.

O 20

Biologically regulated biomineralization promotes persistent infections and biofilm formation

A. Keren-Paz[1], I. Karunker[1], M. Cohen-Cymberknoh[1], T. Olender[1], E. Kerem[1], I. Kolodkin-Gal[1]
[1]Weizmann Institute of Science, Rehovot, Israel

In nature, bacteria form complex and differentiated multicellular communities, known as **biofilms**. Bacterial biofilms are associated with persistent bacterial infections, and thus pose a global threat of extreme clinical importance. To date, the ability of biofilm-forming bacteria to form complex architectures was attributed exclusively to their **organic** extracellular matrix (ECM). However, we have recently shown that microbial biofilms contain a **robust internal mineral layer,** composed of calcium carbonate. This mineral component strengthens the biofilm architecture and serves as a frame to support larger bacterial populations. In addition, this mineral structure protects bacteria from antibiotics (1,2). In this work, we exposed a differentiated subpopulation of metabolically active biofilm cells promoting biomineralization. Using Cryo-Scanning Transmitting Electron Tomography, we produced 3D reconstructions of dividing biofilm cells revealing localized intracellular calcium foci. Furthermore, analysis of th**e transcriptome architecture of calcifying biofilm cells** revealed novel enzymatic pathways promoting both biofilm formation and mineralization. After primary production, the organic ECM serves as a template for the calcite growth to form an active diffusion barrier.

Tight biological regulation of calcification can promote mineral formation in unfavorable environments, e.g: a human host. Therefore, we studied *Pseudomonas aeruginosa* dependent calcite formation in clinical settings. Our results indicate that *pseudomonas* biofilms can form calcite crystals during persistent lung infections, and that those crystals are tightly associated with bacterial cells. Our work demonstrated how studying the molecular mechanisms that promote biofilm mineralization may reveal numerous novel, previously overlooked targets for anti-biofilm antibiotic and thus is of enormous clinical significance, as it can yield novel drugs to combat biofilm infections.

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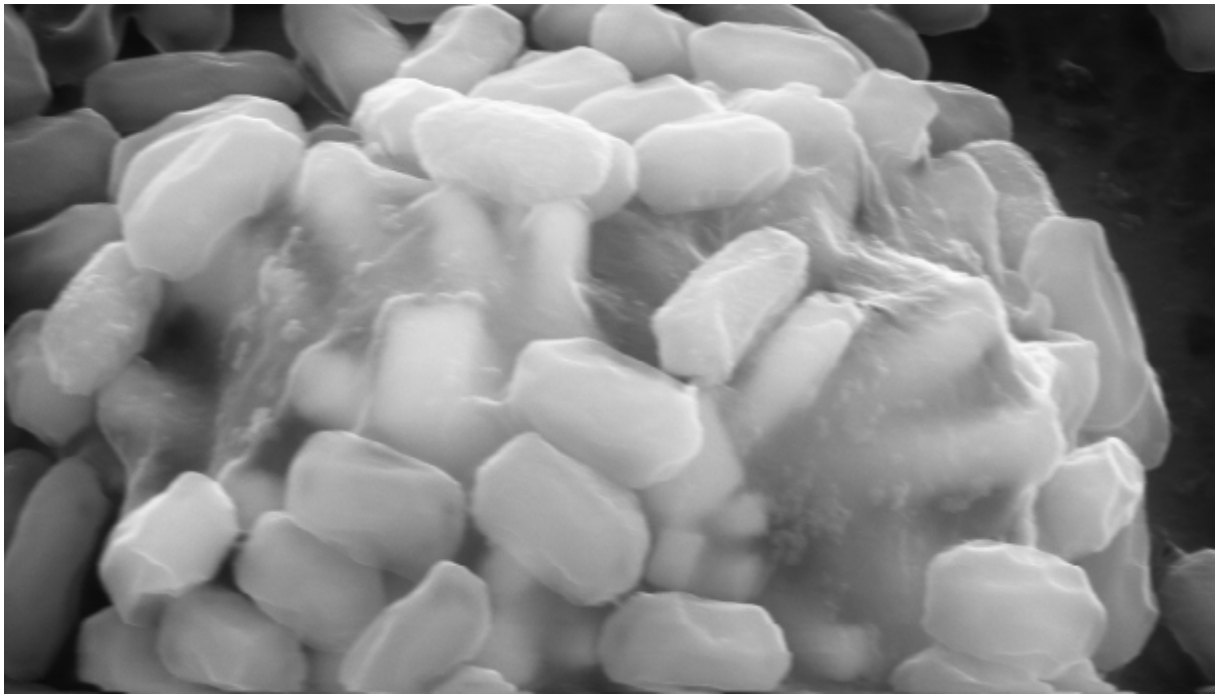
Fig. 2



O 21

DNA- and transcriptome-stable isotope probing together with the genome analysis of *Zoogloea oleivorans* reveal taxonomic and functional insights into microaerobic toluene degradation

A. Táncsics[1], L. Bradford[2], M. Farkas[1], A. R. Szalay[2], B. Kriszt[3], T. Lueders[2]
[1]*Szent István University, Regional University Center of Excellence in Environmental Industry, Gödöllő, Hungary*
[2]*Helmholtz Zentrum München, Institute of Groundwater Ecology, Neuherberg, Germany*
[3]*Szent István University, Department of Environmental Safety and Ecotoxicology, Gödöllő, Hungary*

Introduction: The availability of oxygen is often a limiting factor for the degradation of aromatic hydrocarbons in subsurface environments. However, while both aerobic and anaerobic degraders have been intensively studied, degradation betwixt, under micro- or hypoxic conditions has rarely been addressed. Long-term monitoring of a shallow BTEX-contaminated groundwater in Hungary led to the assumption that bacteria harbouring subfamily I.2.C-type catechol 2,3-dioxygenase (C23O) enzymes may have essential role in this process.
Objectives: Bacterial communities at the site were dominated by members of the *Rhodocyclales*, but the majority of the detected C23O genotypes could not be affiliated to any known bacterial degrader lineages. Therefore, the main objective of the study was to identify key microaerobic toluene degraders by stable isotope probing-based approaches.
Materials & metods: Site sediments were incubated with $^{13}C_7$-toluene under microoxic conditions. Following incubation both DNA and total RNA were isolated from the sediment samples and were subjected to isopycnic centrifugation and fractionation. Density gradient fractions were analysed by 16S rRNA gene amplicon pyrosequencing and RNA sequencing.
Results: Analysis of the gradient fractions revealed that uncultured members of the *Rhodocyclales* (related to *Quatrionicoccus* spp.) and *Zoogloea oleivorans* were most abundant both in ^{13}C-DNA and in ^{13}C-rRNA, while well-known aerobic degraders such as *Pseudomonas* spp. remained unlabeled. mRNA of phenol hydroxylase genes were highly labeled and abundant, while other transcripts of toluene-activation were not detected. Clear labeling of subfamily I.2.C-type C23O but not C12O transcripts supports the hypothesis that this group of extradiol dioxygenases is adapted to low oxygen concentrations. Genome sequence analysis of type strain of *Zoogloea oleivorans* revealed a cluster in the genome containing the genes of a multicomponent phenol hydroxylase together with genes coding for a complete *meta*-cleavage pathway. Another cluster flanked by transposases contained the subfamily I.2.C-type C23O gene but coded only a partial *meta*-cleavage pathway.
Conclusion: This study provided new evidence that the known diversity of hypoxic degraders of BTEX compounds is still incomplete. Moreover, alignment of ^{13}C-mRNA sequence reads on the genome of *Zoogloea oleivorans* will hopefully shed light on the biodegradation pathway of toluene under microaerobic conditions.

O 22

Effects of plant and soil inoculum on abundance of bacterial and archaeal N-cycling guilds after remediation of heavy metal contaminated soil

M. Suhadolc[1], S. Pintarič[1], A. Kaurin[1], D. Kastelec[1], D. Leštan[1], I. Maček[1]
[1]*University of Ljubljana, Biotechnical Faculty, Ljubljana, Slovenia*

Heavy metals (HM) are among the most important pollutants in Europe and worldwide, thus several remediation techniques have been developed over the last decades. Soil washing with ethylenediamine tetraacetate (EDTA) water solution has been demonstrated as a very successful method for the removal of Pb, Zn and Cd from soil (Leštan, 2017), however our question of interest was to evaluate its effects on soil functioning.
Soils used in the study were selected according to the total HM contents and soil properties, two soil samples were from long-term HM contaminated sites, and one was non-contaminated (control). Plant (*Lolium perenne*) and inoculum (rhizosphere soil) ammendment were included as treatments. In the outdoor pot experiment, HM availability ($CaCl_2$ extraction); and the abundance of the total bacterial, archaeal and fungal community, as well as abundance of nitrogen cycle microbial guilds (qPCR targeting *nifH, amoA, nirK, nirS*, *nos*ZI and *nos*ZII genes) were followed. EDTA remediation significantly reduced total Pb, Cd and Zn contents, however several soil properties also changed, and were affecting microbial communities after remediation. Microbial biomass was significantly lower in remediated soils in comparison to original even four months after remediation. Altered soil conditions after remediation have also reflected in lower abundance of N-cycling functional genes, especially in soils with the higher pH and higher organic matter content, thus changes in N pathways could be expected. The presence of plant increased microbial biomass and the abundance of all studied communities in remediated soils.
Our study clearly showed that plants play an important role in the revitalization of remediated soil, while the addition of inoculum, which had negative effect on the abundance of all studied communities, needs to be further investigated.

O 23

Development and optimization of an efficient treatment strategy for bioremediation of chlorinated hydrocarbon contaminated areas: from laboratory to field

Z. Nagymáté[1], C. Romsics[1], L. Jurecska[1], F. Tóth[1], V. Bódai[2], P. Sátorhelyi[2], î Mészáros[1,3], B. Erdélyi[2], K. Márialigeti[1]
[1]*Eötvös Loránd University, Deparment of Microbiology, Budapest, Hungary*
[2]*Fermentia Ltd., Budapest, Hungary*
[3]*Institute of Agricultural Sciences, Lindau, Switzerland*

Short-chain chlorinated aliphatic hydrocarbons (CAHs) (e.g.: tetrachloroethene - PCE, trichloroethene - TCE) are one of the most serious pollutants. Bioaugmentation, applying cultures containing *Dehalococcoides* sp. capable of complete reductive dechlorination, provides a promising technique to remediate CAHs contamination.
Our aim was to develop an efficient treatment strategy including the development of a new bioaugmentation culture capable of complete dechlorination. Our goal was to perform pilot tests to verify the efficiency of our bioaugmentation agent.
Dechlorinating consortia were isolated from contaminated sites and were enriched in microcosm experiments at bench-scale and at industrial-scale (in 100 L fermenter). To monitor the effectiveness of the dechlorination in laboratory and field experiments polyphasic approach was applied using gas chromatography, water chemistry measurement, terminal restriction fragment length polymorphism, next generation sequencing and fluorescence *in situ* hybridization techniques.
Dechlorinating microbial communities were successfully enriched at laboratory- and industrial-scale. Thereafter, pilot-scale test was conducted with dosage of the consortia five times along with continuous application of biostimulation agent during a 29 months" period. Complete dechlorination of TCE to ethene was observed both in laboratory and field experiments which could be linked to three ribotypes of *Dehalococcoides* sp. and to genera *Sulfurospirillum.* Due to the applied bioremediation technique the total CAHs contamination was decreased by up to 90% while cell counts increased. Microbial community altered in a way that anaerobic fermenting Bacteria and methanogenic Archaea were dominant originated from bioaugmentation and biostimulation agents.
We concluded that dehalorespiring microorganisms originated from the bioaugmentation agent and was enhanced by biostimulation indicating the effectiveness of our bioremediation approach.

O 24

Metagenome assembled genomes from petroleum reservoirs and oil-degrading methanogenic enrichments unveiling syntrophic associations for crude oil biodegradation

I. N. Sierra-García [1,2], D. Belgini[2], G. Borrel[3], A. Torres-Ballesteros[4], E. Santos-Neto[5], N. Gray[6], V. M. de Oliveira[2]
[1]*Unidades Tecnologicas de Santander , Ciencias Básicas , Bucaramanga, Colombia*
[2]*Microbial Resource Division, Research Center for Chemistry, Biology and Agriculture (CPQBA), University of Campinas - UNICAMP, Campinas, Brazil*
[3]*Department of Microbiology, Unit Evolutionary Biology of the Microbial Cell, Institut Pasteur, Paris, France*
[4]*Rothamsted Research, Harpenden, United Kingdom*
[5]*PETROBRAS/R&D Center, Rio de Janeiro, Brazil*
[6]*School of Civil Engineering and Geosciences, Newcastle University, Newcastle Upon Tyne, United Kingdom*

Molecular biology techniques (mainly 16S rRNA gene-based approaches) have been applied in many oil reservoir environments for characterization of microbial communities. Although these studies have greatly enriched our knowledge on microbial diversity in these environments, they are limited in addressing the function of major players. The metabolic capacities of the microorganisms inhabiting petroleum reservoirs are related with the hydrocarbon transformations with significant impacts on energy sources. Geochemical evidence suggests that anaerobic methanogenic biodegradation is the main mechanism driving biodegradation of crude oil in subsurface petroleum reservoirs. In a previous study, we obtained methanogenic active oil-degrading enrichments from petroleum samples. In this work, we applied a metagenomics-based approach combining assembly and binning aiming to define the dominant microbial members and decipher their functional capabilities in the original petroleum sample and in the derived methanogenic enrichments. Shotgun metagenomic data from crude oil and active methanogenic petroleum enrichments, were analysed using Metabat2. Although whole shotgun metagenomics and 16S rRNA gene clone libraries were applied previously to investigate the petroleum samples, the metagenomic-binning approach enabled a higher taxonomic resolution and allowed the identification of several bacterial and archaeal candidate phyla (Microgenomates, Atribacteria, among others). Hydrocarbon activation is being mediated by fumarate addition and putative carboxylation mechanisms carried out by *Syntrophus*, *Syntrophomonas* and by Candidatus Atribacteria. Further metabolism is then associated to a lower diversity of fermentative groups and finally, intermediary metabolites are converted to methane by both hydrogenotrophic and acetoclastic pathways, which seem to coexist in both the oil samples and the methanogenic oil-degrading enrichments. These results expand our knowledge of the taxonomic and metabolic diversity in the bacterial and archaeal members associated with petroleum environments and our comprehension of the role of the indigenous microbiota in oil fields linked with oil biodegradation.

O 25

Microbial community analysis of a Dutch drinking water treatment plant, with a special focus on microorganisms involved in N, C and Fe cycling

L. Poghosyan[1], J. Frank[1,2], M. van Kessel[1,2], T. van Alen[1,2], H. Op den Camp[1,2], M. Jetten[1,2], H. Koch[1,2], S. Lücker[1,2]
[1]Radboud University, Microbiology, Nijmegen, Netherlands
[2]Radboud University, Nijmegen, Netherlands

In the Netherlands, both surface (35%) and groundwater (65%) are used for drinking water production. Anaerobic groundwater entering the drinking water treatment plant (DWTP) in Breehei, the Netherlands, contains high concentrations of ammonium (NH4+), iron (Fe), manganese (Mn) and methane (CH4). Elevated concentrations of these chemical compounds in groundwater can cause severe problems during drinking water production and distribution. Microbial communities mediate the biodegradation of organic and inorganic matters, most prominently in drinking water filtration systems.
In this study we used high-throughput sequencing in combination with differential coverage and sequence composition-based binning to recover high-quality metagenome assembled genomes (MAGs) and to characterize the microbial communities involved in N, C and Fe cycling. Samples were collected during June 2016 and September 2018, from the primary and secondary sand-bed (PSB, SSB) filters, in addition to wall biofilm (WB). Besides, WB samples were also collected in May 2017. We recovered 50 MAGs with an estimated completion above 70% and with less than 10% redundancy. Metagenomic analyses indicated that microbial communities colonizing the WB and the granular material of the PSB were dominated by bacteria that can utilize one-carbon compounds and were affiliated with the *Methylococcaceae* and *Methylophilaceae.* The abundances of these bacteria drastically decreased in SSB samples. Iron oxidizers of the genus *Gallionella* were highly abundant mainly in the WB, where colloidal iron deposition was observed. The different groups of nitrifying microorganisms were also among the most abundant microorganisms. Both canonical nitrite- and the newly discovered complete ammonia-oxidizing (comammox) *Nitrospira* were recovered from all samples. Notably, these novel comammox *Nitrospira* displayed the highest abundance of all nitrifying microorganisms, particularly in the WB. The DWTP samples also harbored novel microorganisms, the metabolic role of which remain to be determined.
In conclusion, we demonstrated that the DWTP Breehei represents an interesting model system to study microbial community composition involved in drinking water production. The knowledge obtained here will help to advance our understanding of the role of microorganisms in the removal of contaminating compounds from groundwater and thus to safeguard human health.

O 26

Evolution and metabolic diversity of the Thaumarchaeota

P. Sheridan[1], Y. Meng[1], W. Williams[1], J. Bhambra[1], S. Raguideau[2], C. Quince[2], J. Prosser[1], T. Williams[3], C. Gubry-Rangin[1]
[1]University of Aberdeen, Aberdeen, United Kingdom
[2]University of Warwick, Warwick, United Kingdom
[3]University of Bristol, Bristol, United Kingdom

Introduction: Ammonia oxidising archaea (AOA), the Thaumarchaeota, are prevalent in many soil and marine ecosystems, playing an important ecological role in global nitrogen cycling. However, the metabolic diversity of this phylum, including the diverse lineages of non-AOA Thaumarchaeota, remains understudied. Recent advances in genomics have provided a wealth of thaumarchaeotal genomes, and an opportunity to improve our ecological and evolutionary understanding of this important phylum.
Objectives: This study aimed to provide a robust whole-genome phylogeny for this phylum and understand the gene origin, duplication, loss and transfer events that have driven Thaumarchaeotal evolution and niche specialisation.
Materials and methods: More than 150 Thaumarchaeota genomes (including novel genomes affiliating to the two most abundant, but previously undescribed, soil lineages) were analysed using cutting-edge phylogenomic, gene content and metabolic pathway reconstruction approaches.
Results: Some of the newly acquired genomes suggest potential for a mixotrophic lifestyle, which contrasts to known autotrophic AOA. The transition from non-AOA to AOA has been accompanied by a large number of *de novo* gene acquisitions, including genes encoding oligotrophic traits such as inorganic sulfur and carbon assimilation and synthesis of several amino acids and vitamins. In contrast, deeply-rooted non-AOA genomes have extensive metabolic variation, with some organisms employing alternative chemolithotrophic strategies, such as sulfur oxidation, and this physiological diversity mirrors their environmental diversity.
Conclusion: This study links ecology and evolution for an ecologically important microbial lineage using modern methodological tools. It presents the first representative genomes of abundant lineages acquired using ultra-deep metagenomics and pinpoints gene acquisitions and other evolutionary changes that facilitated the expansion of Thaumarchaeota into diverse ecosystems.

O 27

Populating the tree – expanding our understanding of the metabolic versatility of environmental *Nitrospira*

H. Koch[1], L. Kop[1], S. Haaijer[1], E. Spieck[2], M. Jetten[1], S. Lücker[1]
[1]*Radboud University, Nijmegen, Netherlands*
[2]*Universität Hamburg, Hamburg, Germany*

Autotrophic nitrification, the stepwise oxidation of ammonia to nitrate via nitrite, is performed either in a mutualistic interaction of ammonia-oxidizing microorganisms and nitrite-oxidizing bacteria (NOB), or by complete nitrifiers that catalyze both nitrification steps on their own. All known complete nitrifiers belong to the genus *Nitrospira*, which previously was assumed to comprise only canonical NOB. This genus can be phylogenetically divided into at least six lineages and represents the most environmentally widespread NOB clade. In biotechnological processes, such as drinking water and wastewater treatment, *Nitrospira* are key nitrifiers driving the removal of nitrogen (N) compounds from these waters. Although distribution studies identified *Nitrospira*-like bacteria also in a wide range of natural ecosystems, most genomes analyzed so far were obtained from isolates or metagenomes from engineered systems and represent just two of the six known phylogenetic *Nitrospira* lineages.
To fill the knowledge gap that exists due to the biased phylogenetic distribution of genome-sequenced representatives of this important N-cycling clade, we sequenced and analyzed genomes of *Nitrospira* species isolated from natural systems and affiliated with *Nitrospira* lineages not containing any genome-sequenced representatives. While the core genome of *Nitrospira* includes key pathways like the reductive tricarboxylic acid cycle for CO2 fixation, all five respiratory chain complexes and the nitrite oxidoreductase for nitrite oxidation, the accessory genome enables the use of alternative substrates such as ammonia, hydrogen and formate as well as alternative nitrogen sources for assimilation, like nitrite, cyanate and urea. This metabolic versatility opens the possibility to occupy different niches and to respond to fluctuating nutrient availability, which might be one reason for the global distribution and the high diversity of *Nitrospira*-like bacteria.

O 28

Unveiling methanogenic and methanotrophic populations through metagenome assembled genomes of enriched Amazonian soils

R. Gouvêa Taketani[1,2], D. dos Santos[1], K. Alves[1], F. Andreote[1]
[1]*University of São Paulo, Departament of Soil Sciences, Piracicaba, Brazil*
[2]*Centre of Mineral Technology, Rio de Janeiro, Brazil*

Question: The expansion of agriculture into areas of forest is considered the second most important greenhouse gas (GHG) source after the burn of fossil fuels. Therefore, the Amazonian agriculture frontier is quite important in this scenario. Among the GHGs, the methane is naturally produced and oxidized by soil microorganisms. However, the isolation and growth of these organism is difficult and slow or, hitherto, impossible.
Methods: Thus, in the current study, we have we have recovered metagenome assembled genomes MAGs from methanogenic and methanotrophic organisms obtained from metagenomes of soil enrichments. Soils were enriched with methane, acetate, methanol, and H2:CO2. The DNA extracted from the enrichments were sequenced by Illumina HiSeq technology assembled by IDBA-UD, binned with Metabat2, and the overall quality evaluated with CheckM.
Results: The metanotrophic enrichments yielded 91 MAGs, 12 of this could be assigned to metanotrophic bacteria. These bacteria belonged to the Gammaproteobacteria and Alphaproteobacteria. This MAGs contained genes related to the oxidation of methane (*pmo*). The methanogenic enrichments yielded only 4 low quality MAGs of methanogenic Archaea, however this MAGs contained *mcr*Agenes. The taxonomic affiliation of the MAGs was consistent with the substrates used for the enrichment of these soils.
Conclusion: In conclusion, the use of soil enrichments coupled with the assembly and binning of metagenomes can unveil the metabolic potential of certain uncultured microorganism and shed light on its role in the environment.

O 29

Marine bacteria and fungi as promising source for new antibiotics

J. Wiese[1], J. F. Imhoff[1]
[1]GEOMAR Helmholtz Center for Ocean Research Kiel, Marine Microbiology, Kiel, Germany

Natural products and derivatives thereof are of considerable importance in the discovery of new pharmaceuticals e.g. for the treatment of cancer, diabetes, inflammation diseases, and infection diseases caused by bacteria, fungi, viruses, or parasites. The great biodiversity of marine microorganisms is reflected in their huge chemical diversity, which provides a rich source of biologically active compounds. We are well advised to use this high potential for the exploration of promising compounds to be developed to new drugs for the treatment of infection diseases. With the aim to bear this challenge and to find new compounds exhibiting antibiotic activities, we investigated bacteria and fungi affiliating to phylogenetic diverse taxa from unique marine habitats such as deep-sea sediments, algae, sponges, bryozoa, and corals with a particularly large portion of antibiotic producing microorganisms. Examples are (i) the new polyketide mayamycin, which was produced by *Streptomyces* sp. HB202 isolated from the marine breadcrumb sponge *Halichondria panicea* collected at the Baltic Sea (ii) bacicyclin, a cyclic hexapeptide obtained from *Bacillus* sp. BC028, originated from the mussel *Mytilis edulis*, an inhabitant of the Baltic Sea; (iii) the polyketides engyodontochones A - F produced by *Engyodontium album* LF069 from the sponge *Cacospinga scalaris* (Mediterranean Sea) and (iv) lindgomycin, a polyketide isolated from strain KF970, which was derived from Arctic seawater and affiliated to the family *Lindgomycetaceae*. In addition, the bottlenecks in the development of drugs using the large marine natural product pipeline will be discussed.

O 30

***Actinobacteria* from the South Pacific – chemical diversity of specialized metabolites**

A. Cumsille[1], A. Undabarrena[1], L. Zamora[1], N. Serna[1], N. Zamorano[1], V. González[1], F. Claverías[1], B. Cámara[1]
[1]Universidad Tecnica Federico Santa Maria, Chemistry and Centre of Biotechnology, Valparaíso, Chile

As pathogenic bacterial resistance continues arising worldwide, there is an urgent need to focus efforts on the discovery of new antimicrobial compounds. Natural products are still the main source for bioprospection in drug discovery, targeting *Actinobacteria* from underexplored habitats. Chilean marine ecosystems provide dynamic pressures that shape the microbial communities to develop specialized metabolites, potentially crucial for ecological adaptation. In this context, our group aims to explore the actinobacterial culturable biodiversity obtained from Chilean coasts and assess their metabolic potential to produce antimicrobial compounds. Our collection comprises 32 genera belonging to 20 families, considering sediments and invertebrate"s marine samples. Several sampling points alongside the Chilean coast were explored: Chañaral Island, Easter Island, Valparaíso bay, Chiloé Island, Comau fjord and Penas gulf. Identification of isolates was accomplished through 16S rRNA gene sequencing and subsequent phylogenetic analyses. Exploration of antimicrobial potential was performed in several actinobacterial strains, testing different culture conditions and model bacteria. Crude extracts with positive bioactivities were dereplicated using liquid chromatography-high resolution mass spectrometry. A hierarchical cluster analysis was performed in order to select those strains with new chemical entities where further purification efforts will be achieved. Comparison to databases evidenced that 37% of the identified metabolites do not present matches with fragmentation patterns of known metabolites, suggesting promising candidates for novel compounds. Among our collection, three strains showed interesting compounds. These results suggest that Chilean marine environments represent an interesting niche for biodiscovery, validated by the chemical diversity obtained from our strain collection.

O 31

Mutualism is not supported by Fe processing but by growth-related factors in Fe-cycling co-cultures

R. Cooper[1], C. E. Wegner[1], S. Kügler[1,2], N. Ueberschaar[2], R. Poulin[2], G. Pohnert[2], K. Küsel[1,3]
[1]*Friedrich Schiller University Jena, Institute for Biodiversity, Jena, Germany*
[2]*Friedrich Schiller University Jena, Institute of Inorganic and Analytical Chemistry, Jena, Germany*
[3]*The German Centre for Integrative Biodiversity Research (iDiv) Halle-Jena-Leipzig, Leipzig, Germany*

Mutualism is defined as an interaction between individuals of different species that results in positive effects on reproduction and/or survival of interacting populations. The coexistence of Fe-oxidizing and Fe-reducing microbes in habitats with fluctuating redox conditions is a prime example of mutualism in nature, where both organisms profit from the Fe produced by the partner organism that is needed in its oxidized or reduced state as either electron acceptor or donor. We hypothesized that mainly core genes related to Fe metabolism would be upregulated under co-culture conditions. Thus, we set up a series of cell-free supernatant exchange and liquid co-culture batch experiments to determine the benefit provided to each organism in the presence of the partner"s metabolome (without physical contact) or with the partner. We found that the partner"s metabolome stimulated the activity of the other organism, thus enhancing the rates of *Sideroxydans* CL21 Fe oxidation 1.3-fold or *Shewanella oneidensis* Fe reduction 1.5-fold when amended with the partner"s cell-free supernatant. Next, liquid co-culture batch incubations were conducted to gauge the impact of physical contact of each partner organism. qPCR showed that both *Sid.* CL21 and *S. oneidensis* 16S rRNA gene copy numbers increased between 1-2 orders of magnitude over 6 days in mono- and co-culture incubations. RNAseq analyses of these incubations revealed 14-20% of total genes were differentially expressed (DEGs) in *Sid.* CL21 and *S. oneidensis,* respectively. The overall transcriptome profiles indicated that genes involved in Fe-cycling were not upregulated when grown in co-culture. Instead, the most upregulated DEGs in *Sid.* CL21 in co-culture were involved in biopolymer transport and general growth factors, while hydrogenases were the most downregulated. Genes involved in biofilm formation, putrescine synthesis and degradation, and tungstate and zinc transport were some of the most upregulated DEGs in *S. oneidensis.* Our RNAseq results were used as groundwork for targeted metabolomic profiling of the coexisting microbes enabling us to correlate changes in transcription expression patterns and metabolite abundance, for example, the presence of putrescine degradation products and zinc compounds. Together, our results show how coexistence depends on contributions from each partner"s metabolism during Fe cycling in nature and how these coexisting microbes potentially rewire their response to the partner organism.

O 32

Forest soil bacteria – an underexplored niche of novel biocatalysts for biomass conversion

C. Algora[1], S. Hollá[1], P. Baldrian[1], R. López-Mondéjar[1]
[1]*Czech Academy of Sciences, Institute of Microbiology, Prague, Czech Republic*

Lignocellulose is one of the most abundant biomass on Earth, which additionally is a renewable alternative for the sustainable production of chemicals without the use of fossil fuels. Lignocellulose is highly abundant in forest ecosystems, and soil microorganisms have evolved to decompose the recalcitrant lignocellulosic polymers. High diversity of bacteria equipped with a myriad of genes for the degradation of various compounds is found in forest soils. Among those compounds, structural carbohydrates (i.e., cellulose, hemicellulose) and the carbohydrate-active enzymes as biocatalyts are of major interest. Here, we investigated the microbial community of a temperate deciduous forest in Czech Republic decomposing complex carbohydrates (cellulose, xylan, glucomannan, and beta-glucans). For that, mesh bags containing the various complex carbohydrates were *in situ* incubated for three weeks in the forest topsoil. Identification of the microbial communities colonizing the various complex carbohydrates in the mesh bags with Illumina high-throughput sequencing and cultivation methods indicated a role of *Gammaproteobacteria* decomposing most complex carbohydrates. Cultivation experiments using selective media with the various complex carbohydrates as carbon source yielded the isolation of many bacteria within the *Firmicutes* and *Proteobacteria* (with members of the classes *Alpha*-, *Beta*-, and *Gammaproteobacteria*). The screening of more than 1,000 isolates cultivated under the various complex carbohydrates revealed that members of the order *Enterobacterales,* class *Gammaproteobacteria*, were abundantly present in most complex carbohydrates. Interestingly, among all complex carbohydrates tested, glucomannan yielded the highest diversity of isolates. These novel isolates with hydrolytic properties for the degradation of structural complex carbohydrates may be of further interest for biotechnological processes in the biofuel and biomass industry.

O 33

Exploring a soil metagenome for novel bacterial phyla and biosynthetic gene clusters

M. Liles[1,2], A. Pereira[1], M. Sandoval-Powers[1], Y. Zhang[3], A. Calderón[3], D. Mead[2]
[1]*Auburn University, Biological Sciences, Auburn, AL, United States*
[2]*Varigen Biosciences Corporation, Madison, WI, United States*
[3]*Auburn University, Drug Discovery and Development, Auburn, AL, United States*

Introduction: Most soil microbes are recalcitrant to cultivation under laboratory conditions. In this study a large-insert soil metagenomic library was mined for biosynthetic gene clusters (BGCs) and phylogenetically informative genome sequences.
Objective: To explore the diversity of microbial genomes present within an agricultural soil including the identification and heterologous expression of BGCs to produce bioactive metabolites.
Materials and Methods: We identified BGCs using PCR, targeting Polyketide Synthases (PKS), or using a NGS strategy in which clone DNAs were pooled, sequenced with Illumina, and contigs were screened for BGCs, rRNA operon and other sequences. Clones that contained BGCs were conjugally transferred into *Streptomyces coelicolor* M1154 and screened for antimicrobial activity, followed by LC/MS characterization of bioactive metabolites.
Results: The soil metagenomic library was comprised of sequences derived from diverse microbial genomes, including sequences obtained from putative Candidate Phyla Radiation (CPR) taxa. We identified 474 contigs that contained a PKS and/or NRPS pathway among 1,656 BGCs identified. Cloned BGCs were divergent from known pathways, with the nearest BLAST hit of keto-synthase domains ranging from 22 to 80% amino acid identity. BGCs identified via PCR (n=84) were a subset of the clones identified via NGS, which were both numerically more abundant and included highly divergent clades (Fig. 1). BAC clones expressed in *S. coelicolor* M1154 were identified that produced bioactive metabolites that inhibited the growth of multidrug-resistant bacterial or fungal pathogens.
Conclusion: These results indicate that novel bacterial phyla and BGCs can be identified from complex metagenomes using a direct cloning and NGS approach, thereby avoiding PCR biases. **This is the first report of CPR-affiliated phyla from any soil sample.** BGCs from a soil metagenome can be heterologously expressed to produce bioactive secondary metabolites.

Fig. 1

O 34

No publication allowed.

O 35

Drinking water microbiota – astounding biodiversity, almost unexplored

A. Bruno [1], A. Sandionigi[1], D. Magnani[1], A. Panio[1], F. Orizio[1], M. Bernasconi[2], M. Labra[1], M. Casiraghi[1]
[1]*University of Milano-Bicocca , Biotechnologies and Biosciences Department , Milan, Italy*
[2]*Metropolitana Milanese S.p.A., Milan, Italy*

While safe and of high quality, drinking water can host an astounding biodiversity of microorganisms, dismantling the belief of its "biological simplicity". We focused on what happens to the microbial communities from the source to the tap. We collected water samples from groundwater throughout the main steps of the potabilization process, and from the drinking water distribution system, in an urbanized area in Northern Italy. We integrated microbiological analyses and molecular techniques in order to explore drinking water microbiota.
We were able to isolate and identify more than one hundred bacteria, peculiar of drinking water ecosystem, and no one of these was pathogenic. In this way, we created a collection of drinking water specific bacteria, useful for further characterizations. Our results revealed that the composition of bacterial communities varies across the drinking water treatment plant and distribution system. We showed that carbon filters harbour a microbial community seeding and shaping water microbiota downstream, introducing a significant variation on incoming (groundwater) microbial community. Chlorination seems to not affect the altered microbiota. We were also able to correctly predict (through machine learning analysis) samples belonging to groundwater, but the assignation was not reliable with carbon filter samples, which were incorrectly predicted as chlorination samples. The presence and abundance of specific microorganisms allowed us to hypothesize their role as indicators. We reported the presence of the so called "*microbial dark matter*" (those microorganisms accounting for a large proportion of life and biodiversity but whose basic metabolic and ecological properties are not known, Solden et al., 2016), across the entire drinking water supply. New questions arise about the role of microbial dark matter in drinking water. Indeed, our results suggested that these bacteria might play a central role in the microbial dynamics of drinking water.
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Solden, L., Lloyd, K. and Wrighton, K., 2016. The bright side of microbial dark matter: lessons learned from the uncultivated majority. *Current opinion in microbiology*, *31*, pp.217-226.

O 36

Automated diagnostic system for pathogen and resistome profiling in clinical and environmental settings

R. P. Abreu Pereira[1], L. Villiers[2], C. Baussmerth[3], M. Hendling[4], V. D'Onofrio[5], I. Gyssens[5], I. Barisic[4], M. Lorenz[3], B. Corgier[6], S. Jones[7], C. Marquette[2]
E. Wellington[1]
[1]*Universiy of Warwick, School of Life Sciences, Coventry, United Kingdom*
[2]*Université Claude Bernard Lyon 1, Lyon, France*
[3]*Molzym GmbH, Bremen, Germany*
[4]*Austrian Institute of Technology, Vienna, Austria*
[5]*Hasselt University, Hasselt, Belgium*
[6]*AXO Science, Lyon, France*
[7]*Bee Robotics, Gwynedd, United Kingdom*

The widespread occurrence of multidrug-resistant bacteria is a major public health problem and has been frequently linked to the extensive use of antibiotics in human and veterinary medicine to treat pathogenic bacteria. Besides, there is an increasing concern about the human exposure to antibiotic resistant bacteria in freshwater systems and the spread and persistence of these bacteria in these ecosystems. Thus, faster and accurate methods are currently required to improve the time for diagnosis of infectious diseases, the subsequent patient"s antibiotic treatment as well as the monitoring of multidrug-resistant pathogens in the environment. In our study we aimed to develop, optimise and validate a benchtop automated system to allow a rapid detection of bacterial pathogens and characterisation of their genotypic antimicrobial resistance in clinical and environmental samples. An automated diagnostic system that integrates differential lysis, multiplex target enrichment and microarray-based detection was developed. *In silico* predictive analysis and *in vitro* validation of the assays were performed. Analytical validation was accomplished by spiking human blood and water samples with a diverse range of multidrug resistant bacteria frequently detected in clinical specimens and freshwater systems. The approach was applied to a set of clinical and environmental samples. We confirmed that the developed diagnostic system has a fast turnaround time, is reproducible and provides a highly efficient automated DNA extraction. The integrated approach showed high *in situ* sensitivity and specificity, supporting the use of the multiplex and microarray technology targeting 52 bacterial targets and 45 antibiotic resistance genes. The similar performance and good agreement observed between the system results and the data obtained with the methods routinely used in diagnostics, i.e. culture-based methods and qPCR, strengthen the methodology validation.
The novel system, contingent upon successful clinical trial results, has the potential to substantially improve and shorten the length of evidence-based decision-making in sepsis diagnosis. Plus, it can become a valuable tool in environmental diagnostics and research for the detection of antibiotic-resistant waterborne and water-based pathogens.

O 37

High-throughput omic approaches to characterize the microbiome-cervix frontier during HPV infections and cervical dysplasia

F. Godoy-Vitorino[1], E. Lemuel-Tosado[1], N. Chorna[2], J. Romaguera[3]
[1]*University of Puerto Rico, School of Medicine, Microbiology and Medical Zoology, San Juan, Puerto Rico*
[2]*University of Puerto Rico, School of Medicine, Biochemistry, San Juan, Puerto Rico*
[3]*University of Puerto Rico, School of Medicine, Obstetrics and Gynecology, San Juan, Puerto Rico*

Introduction: The human cervix is regarded as a protective barrier, having a microbiome community that acts as a biochemical barrier against infections. Human papillomavirus (HPV) is the etiologic agent for cervical dysplasia and carcinoma, and our group has given the first step towards understanding the microbiome-host interactions in the cervix of Hispanics living in Puerto Rico.
Objectives: We characterized the cervical microbiome and mycobiome of Hispanic patients with HPV infections and dysplasia, and determined early-detection biomarkers for high risk infections using microbiome and mycobiome approaches, as well as Gas chromatography-mass spectrometry (GC-MS).
Patients and methods: Patients that were recruited provided urine, cervical swabs and cervical lavages during the visit to the gynecologist. 16S rRNA V4 region and ITS-2 fungal regions were amplified and sequenced using Illumina technology. GC-MS was employed on the urine-derived products and on the cervical lavages, for metabolomic analyses.
Results: Cervical bacteria were more diverse in patients with CIN3 pre-cancerous lesions but not significantly associated to high-risk HPV. Nonetheless fungal diversity was significantly higher in cervical samples with high-risk HPV. Fungal biomarker signatures for cervix include Sporidiobolaceae and *Malassezia* for high-risk HPV infections. Urine metabolomic analyses revealed three biomarkers associated to high-risk HPV infections, 5-Oxoprolinate, Erythronic acid and N-Acetylaspartic acid. We have improved recruitment and are currently applying GC-MS to cervical lavages to characterize the metabolites at the cervical frontier.
Conclusion: Our combined omics datasets were able to uncover possible microbes and metabolites associated to high-risk HPV infections. The disruption of the balance of the normal cervical microbiota (dysbiosis), leads to the appearance of anaerobic taxa related to infections of the genital tract, as well as new yeast that may be related to carcinogenesis. Together these methods offer new opportunities for early detection of HPV infections to prevent cervical cancer.

O 38

Bacterial survival in microscopic wetness

N. Kashtan[1], M. Grinberg[1], T. Orevi[1], S. Steinberg[1]
[1]*The Hebrew University of Jerusalem, Plant Pathology and Microbiology, Rehovot, Israel*

Microscopic wetness occurs in many microbial habitats including the phyllosphere – the above ground parts of plants – which is a huge microbial habitat of global importance. There is increasing evidence that during daytime, microscopic wetness in the form of thin films and droplets, invisible to the naked eye, persist on leaf surfaces. This wetness is suggested to occur on most plants worldwide and results from deliquescence of hygroscopic aerosols that are ubiquitous on leaves. Little is known on the impact of such microscopic wetness on the ecology of bacteria and on cells' survival in particular. Here we study how such microscopic wetness affects central features of microbial life, in particular cells' survival. We developed an experimental system that create microscopic wetness on artificial surfaces. Using advanced microscopy and image processing we analyzed microscopic wetness, bacterial self-organization and survival as well as the interplay between the three. We revealed that stable microscopic droplets are formed around bacterial aggregates on a drying surface under moderate relative humidity, at least partially due to deliquescence. Notably, droplet size correlated with aggregate size, and cell survival of both solitary and aggregated cells was higher within larger droplets. Similar results were observed for >10 different strains, two of them – Pseudomonas fluorescens and P. putida – studied in depth here. The formation and retention of microscopic droplets around bacterial aggregates during daytime are likely central features of the leaf surface as a microbial habitat and key for bacterial survival on plant leaf surfaces.

Fig. 1

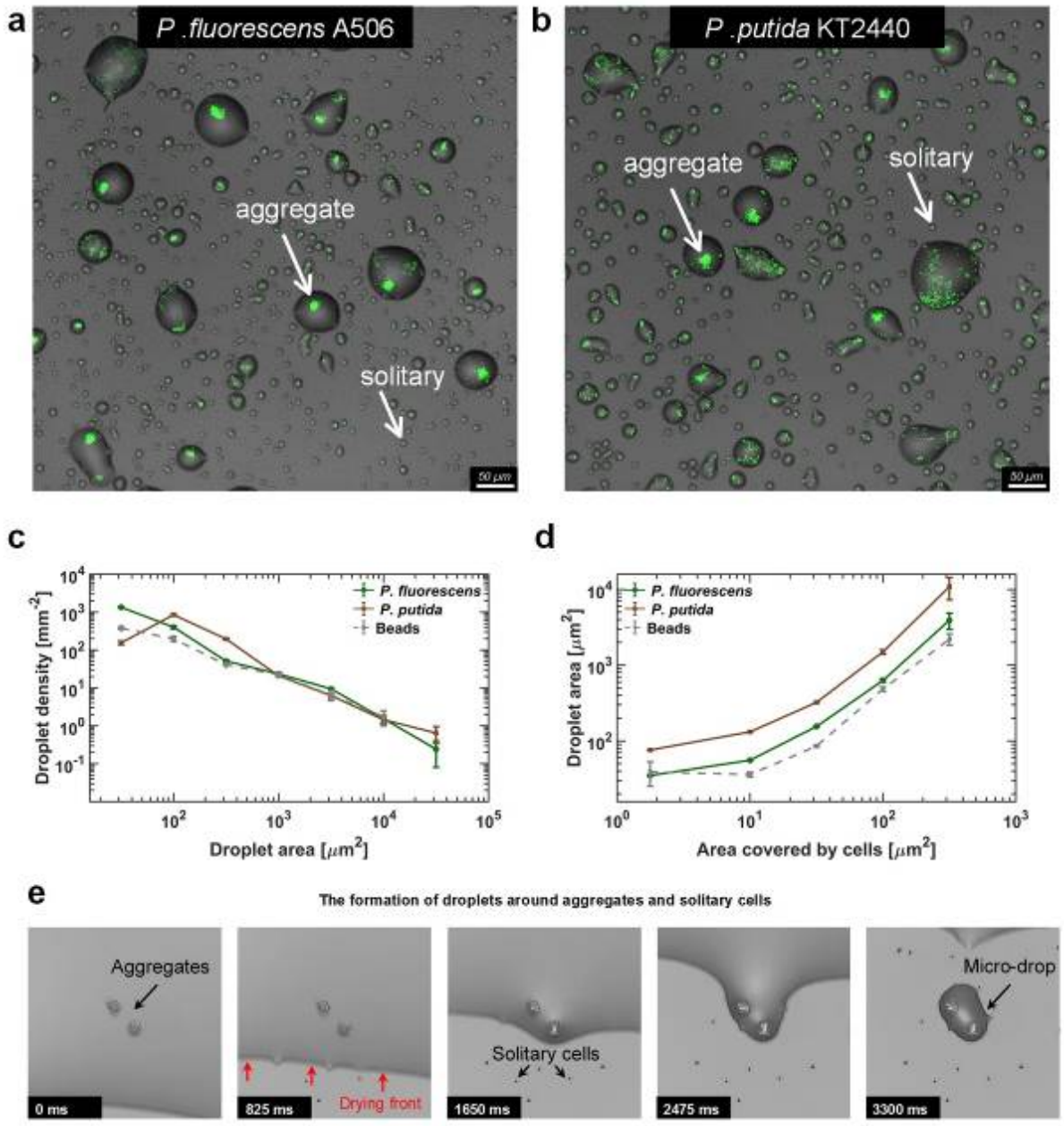
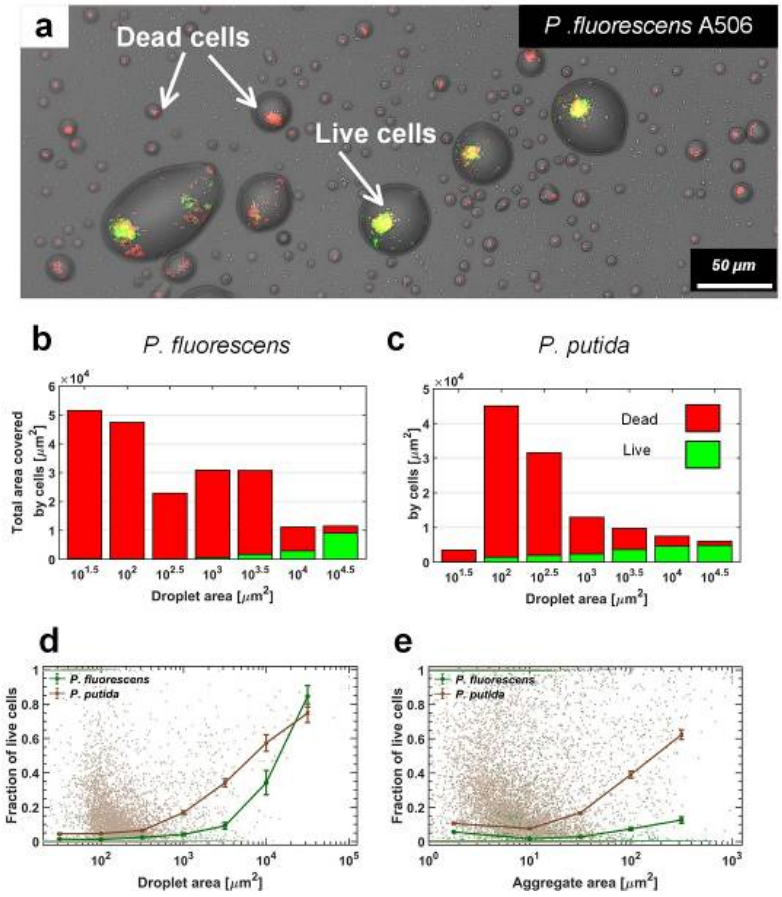

a
P .fluorescens A506
aggregate
solitary
50 μm
b
P .putida KT2440
aggregate
solitary
50 μm
c
Droplet density [mm⁻²]
P. fluorescens
P. putida
Beads
Droplet area [μm²]
d
Droplet area [μm²]
P. fluorescens
P. putida
Beads
Area covered by cells [μm²]
e
The formation of droplets around aggregates and solitary cells
Aggregates
0 ms
825 ms
Drying front
Solitary cells
1650 ms
2475 ms
Micro-drop
3300 ms

Fig. 2


a
Dead cells
Live cells
P .fluorescens A506
50 µm
b
P. fluorescens
Total area covered by cells [µm²]
Droplet area [µm²]
c
P. putida
Dead
Live
by cells [µm²]
Droplet area [µm²]
d
P. fluorescens
P. putida
Fraction of live cells
Droplet area [µm²]
e
P. fluorescens
P. putida
Fraction of live cells
Aggregate area [µm²]

O 39

Unravelling the ecological processes shaping the microbial rare biosphere

X. Jia[1], F. Dini-Andreote[2], J. Falcão Salles[1]
[1]University of Groningen, Groningen Institute for Evolutionary Life Science, Groningen, Netherlands
[2]Netherlands Institute of Ecology, Department of Microbial Ecology, Wageningen, Netherlands

Soil teems with microorganisms, with relatively few dominant species co-existing alongside a high number of rare species, i.e. microbial rare biosphere. However, most of our current knowledge of microbial community is based on dominant species rather than rare species. To fill this gap, we investigated the ecological mechanisms mediating the structure of the microbial rare biosphere across space and time on a salt mash chronosequence (Schiermonnikoog, the Netherlands). Using high-throughput 16S cDNA sequencing, we observed that the species composition of rare biosphere was different from that of dominant biosphere. In rare biosphere, most species were permanently rare and conditionally rare, whereas only a small fraction was transiently rare. By disentangling the ecological processes via combining phylogenetic community structure with ecological null model analysis, we found that homogenizing selection was the predominant ecological process contributing to the variation of rare biosphere structure, suggesting that most rare species persist in low abundance due to their special life-history strategies or ecological trade-offs. Together, these results elucidated how the highly diverse soil microbial rare biosphere is assembled, which will promote a better understanding of the main processes underpinning the huge diversity found in soil microbial communities.

O 40

Microbial life strategies in the detritusphere – DNA-SIP provides quantitative data on carbon utilization by copiotrophs and oligotrophs

M. Uksa[1], H. Pagel[2], K. Müller[1], S. Gschwendtner[3], C. Poll[1], S. Kublik[3], H. Haslwimmer[1], M. Schloter[3], T. Streck[2], E. Kandeler[1]
[1]University of Hohenheim, Institute of Soil Science and Land Evaluation, Soil Biology, Stuttgart, Germany
[2]University of Hohenheim, Institute of Soil Science and Land Evaluation, Biogeophysics, Stuttgart, Germany
[3]Helmholtz Zentrum München, Research Unit for Comparative Microbiome Analysis, Neuherberg, Germany

Microbial functional traits and life strategies control carbon (C) transformation processes at the soil-litter interface. This study aims at identifying key functional groups in the detritusphere and quantifying their contribution to C turnover.
Therefore we studied C flow from maize litter of different age into an arable soil after applying ^{13}C-labeled and unlabeled maize litter using a microcosm system. A reciprocal transplantation of the litter allowed us to track the C flow into bacteria and fungi at the early (0-4d), intermediate (4-12d), and late phase (28-36d) of decomposition. ^{13}C-atom fraction excess in the microbial DNA was determined by quantitative DNA-SIP and barcoding of bacterial 16S rRNA gene and fungal ITS2 region using high throughput sequencing techniques. OTUs were categorized into functional groups with respect to life strategies based on utilization of litter-derived C for growth. Bacteria were furthermore subdivided into slow- or fast-growers using the number of 16S rRNA copies per genome as a proxy for their potential maximum growth rate.
Fast-growing bacteria (e.g., *Bacillus*), *Mortierella*, and basidiomycetous yeasts were the dominant utilizers of litter-derived C in the early phase. On average, fungi exhibited a higher ^{13}C-enrichment than bacteria (39 vs. 11 atom-%). In contrast, C utilization in the late phase was lower and distributed across a more diverse microbial community, especially slow-growing bacteria (e.g., Acidobacteria, Gemmatimonadetes, and Chloroflexi) and Ascomycota. These results provide quantitative evidence of the successive utilization of litter-derived C in soil by fast-growing copiotrophs and slow-growing oligotrophs supporting current microbial ecological theory. Quantitative data on C flow into functional groups will be used to inform a new C turnover model that explicitly considers microbial life-history traits.

O 41

Bacterial warfare – the arsenal goes viral

J. Gama[1], I. L. Domingues[2], A. F. Carvalho[2], F. Dionisio[2]
[1]*Microbial Pharmacology and Population Biology Research Group, Department of Pharmacy, UiT The Arctic University of Norway, Tromsø, Norway*
[2]*Centre for Ecology, Evolution and Environmental Changes, Department of Plant Biology, Faculty of Sciences of the University of Lisbon, Lisbon, Portugal*

It has been argued that organisms can use their parasites as weapons in the same way they use toxins. For example, humans have taken advantage of pathogens in warfare for centuries.
We conducted a series of experiments with *Escherichia coli* and phage λ, respectively as models for host and virus. Lysogens (cells carrying the virus integrated in their genome) are immune to the pathogen, but when induced can produce and release viral particles. The virus can then kill susceptible non-lysogenic competitor cells. However, if there are too many viruses it is more likely that susceptible cells become lysogenic instead of dying.
We performed competition experiments between lysogenic and susceptible *E. coli* and show that the outcome depended on habitat structure. In structured habitats (e.g., on a Petri dish), resources not used by susceptible cells that died could be only consumed by immune cells near the killing zone, increasing the frequency of lysogens locally, and overall in the whole population. This effect ceased after several days of competition due to the conversion of susceptible into lysogens, which occurred after substantial amplification of the virus. In unstructured habitats (liquid culture), resources were equally distributed throughout the whole population and the advantage of lysogens was rarely observed.
We then generalized this hypothesis to viruses that are unable to integrate into the host"s chromosome (like most pathogens) and do not confer immunity. This was demonstrated experimentally with *E. coli* and a lytic derivative of phage λ unable to integrate chromosomally. Computer simulations with randomly generated parameters supported this demonstration by showing that the frequency of virus-carrying cells increased over time in more than half of the parameter sets.

O 42

Mechanisms of interaction between members of a biofilm community

S. Rice[1], S. Subramoni[1], S. Booth[1], R. Williams[1], S. Kjelleberg[1]
[1]*Nanyang Technological University, The Singapore Centre for Environmental Life Sciences Engineering, Singapore, Singapore*

To begin to understand how microbial biofilm communities function, we have investigated a three species biofilm community, Pseudomonas areuginosa, Pseduomonas protegensand Klebsiella pneumonia, and shown that this mixed community displays emergent properties, such as increased biomass and stress tolerance, compared to comparable single species biofilms. We propose that these emergent properties are the combined effect of changes in gene and protein expression as well as the impact of sharing of metabolites and biofilm matrix components. By changing media composition, we can demonstrate not only changes in species composition, but also clear evidence of metabolic cooperation. We have therefore undertaken a combination of "omics" based studies as well as investigation of the effects of media composition on biofilm development and function. There were few changes in gene expression in single species biofilms across time for any of the species tested. However, when challenged with SDS, genes associated with stress resistance were induced for P. aeruginosaand K. pneumonia, but not for P. protegens, which is consistent with the impact of SDS on biofilms of these bacteria. The results show that a surprisingly small cohort of genes are specifically affected by growth in a mixed species biofilm and this similarly reflected in changes in protein expression for the mixed and single species biofilms. Comparison of metabolite profiles also supports the observed changes in gene and protein expression, showing that there are distinct changes in central metabolism and strongly suggests that metabolic cooperation or competition play important roles in mixed species biofilm development. Finally, by generating targeted gene deletion strains, we can begin to define the molecular mechanisms that underpin mixed species community interactions. For example, here we show that the quorum sensing system of P. aeruginosais important in regulating the production of effectors that mediate interactions with other community members. In conclusion, we have been able to develop a highly reproducible community biofilm model for experimentation. The results show that this community displays emergent properties of enhanced biomass and shared defence mechanisms. Further, the system enables a detailed molecular understanding of the mechanisms of community assembly and function.

Poster Presentations

Session 1

Horizontal gene transfer, mobile genetic elements and antibiotic resistance

P 1

Phytochemical composition and antibacterial activity of extracts of Cajanus cajan (L.) Millsp., Vernonia amygdalina Delile and Psidium guayava L. used in the treatment of diarrheal infections in Benin

A. Afoussatou[1,2]

[1]*United of research in applied microbiology and pharmacology of naturals substances, Biology, Cotonou, Benin*

[2]*High School Faucon , Biology, Abomey Calavi, Benin*

Introduction: As a result of therapeutic failures and the rising costs of treating enteric pathogen infections, scientists are trying to find alternative care. Hence, the resort to plants of the traditional pharmacopoeia for their many pharmacological activities of *K. pneumoniae, K. rhinocleromatis, K. oxytoca, P. oryzihabitans, P. aeruginosa, E. coli, C. freundii, S. cholereasius and S. flexner.*

Objective: The present study was initiated in the general objective, to evaluate the chemical composition of the aqueous and ethanolic extracts of some plants of Southern Benin namely *Cajanus cajan* (leaves), *Vernonia amygdalina* (leaves) *and Psidium guayava* (leaves and roots) and their activities on multi-resistant bacterial strains.

Methods: The content of the total polyphenol and flavonoid extracts was measured by the method of Basli and *al.* and Kim *and al..* The toxicity of the extracts was assessed by the larval toxicity test using *Artemia salina* larvae. The agar and liquid diffusion methods were used for the sensitivity test and the determination of the minimal inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) of plant extracts on enteropathogenic strains.

Results: Selected plants all had high levels of flavonoids and polyphenols. All these plants are nontoxic at the concentration of 100 mg / ml. The MICs and MBCs of the active extracts varied according to the extracts and bacterial strains studied. The ethanolic extract of *Psidium guajava* leaves showed bactericidal activity on the strains of *Pseudomonas aeruginosa, Salmonella cholereasius* and *Shigella flexneri.* The ethanolic extracts of the leaves of *Cajanus cajan* and *Vernonia amygdalina* showed bactericidal activity on the strain of *Shigella flexneri*.

Conclusion: The various extracts of selected plants showed bactericidal activities against the various enteropathogenic strains tested and can therefore constitute substituents for antibiotics following transformations into improved traditional medicines.

Key words: Enteropathogenic strains, antibacterial activity, *Cajanus Cajan, Vernonia amygdalina, Psidium guayava.*

P 2

The impact of sediment concentrations on the survival of *bla*CTX-M-15-producing *E. coli* in wastewater released to seawater and freshwater

Y. Bashawri[1]

[1]*Bangor University, Environmental science, Bangor, United Kingdom*

Antibiotic resistant bacteria and their genes have become a public health issue. Particularly, *bla*CTX-M-15 produced by *E. coli* has rapidly become the most common type of Extended-Spectrum β-Lactamase enzymes (ESBLs) in many countries during the last decade. Wastewater treatment plants release large amounts of ESBL-producing bacteria into the environment [1]. Thereafter, their survival is dependent on many factors, including shielding by sediments, which could protect them from inactivation by UV radiation. The aim of this study was to assess the impact of sediment concentrations on the inactivation rate of *bla*CTX-M-15 -producing *E. coli* released from human wastewater into seawater and freshwater.

Microcosms containing either sea or freshwater with low, medium and high turbidity were inoculated with *bla*CTX-M-15 -producing *E. coli* and then exposed to the average duration of UV radiation in winter (14 min) and in summer (4.5 h) of north Wales. Counts of *bla*CTX-M-15 -producing *E. coli* were performed on Brilliance™ ESBL Agar plates, then the identified bacterial isolates and its gene (*bla*CTX-M-15) confirmed by biochemical and PCR tests. Furthermore, the physiochemical properties of the waters (electrical conductivity, pH, phosphate, nitrate, total organic carbon and total organic nitrogen) were determined.

The results showed that sediment concentration had a significant impact on the survival of *bla*CTX-M-15-producing *E. coli*, with greater survival under higher turbidity in both seawater and freshwater. In the first thirty minutes, the survival of *bla*CTX-M-15-producing *E. coli* was reduced slightly (20-28%) at all turbidity levels in both waters, with the exception of freshwater of lower turbidity (45% reduction). However, after 4.5 h UV exposure, the survival was significantly reduced (85-100%) at all turbidity levels in sea and freshwater. Overall, the number of *bla*CTX-M-15-producing *E. coli* recovered from the microcosms declined over 8 h, particularly so in seawater of lower turbidity.

In conclusion, this study found that there was a positive relationship between sediment concentrations and survival of *bla*CTX-M-15-producing *E. coli* in water. Survival in freshwater was greater than in seawater and under UV exposure rates, more typical of winter conditions.

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Fig. 1

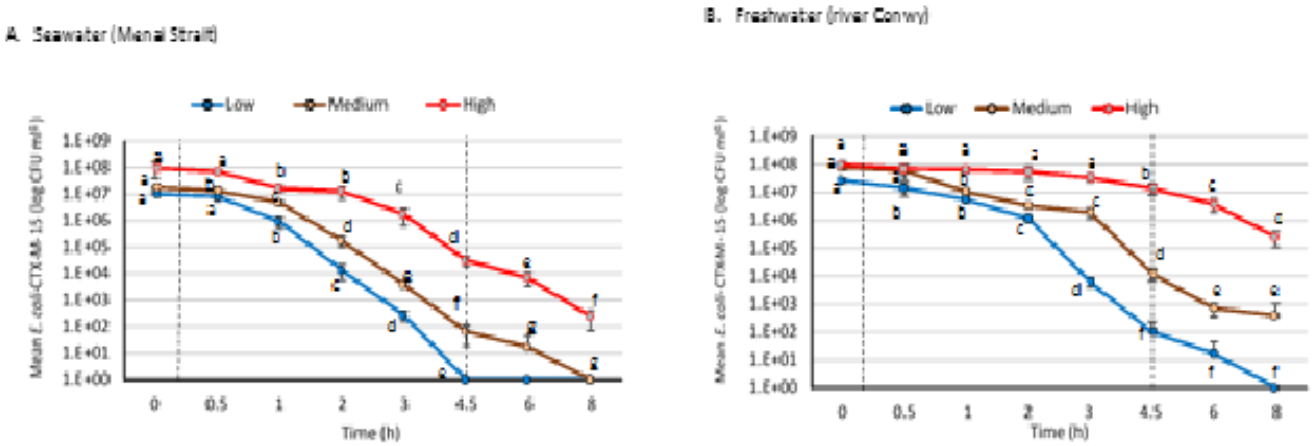


Figure 1 Mean (CFU ml^{-1}) (± SD; n=3) of $bla_{CTX-M-15}$-producing *E. coli* in (A) seawater and (B) freshwater recovered from the different levels of turbidity following different durations of exposure to UV radiation. The dashed lines represent the average of long time exposure in winter (14 min) and summer (4.5 h). Letters denote the significant differences (Mann-Whitney *U* test, P<0.05)

P 3

***Phaeobacter inhibens*DSM 17395 – plasmid conjugation as a smart strategy for niche adaptation**

L. Birmes[1], H. Brinkmann[1], J. Petersen[1]

[1]*Leibniz-Institute DSMZ - German Collection of Microorganisms and Cell Cultures, Microbial Ecology and Diversity Research, Braunschweig, Germany*

Question: Uptake of plasmids by conjugation appears to be the most fundamental strategy of *Rhodobacteraceae* to adapt rapidly to various environmental conditions. The model organism *P. inhibens* DSM 17395 was used as a recipient for plasmid conjugation of genes from the denitrification pathway such as the nitrate reductase (*nar*). The objective was the directed transfer of two missing plasmid-encoded denitrification genes from distally related donor strains. Established transconjugants were expected to have acquired the metabolic capacity to reduce nitrate to nitrite and to use this ability to grow anaerobically on nitrate.

Methods: Undirected transposon mutagenesis was applied to label the plasmids of interest with antibiotic resistant genes. The exchange of plasmids between recipients and donor strains was launched with a mating procedure during the mid-exponential phase of growth. Successful transfers were identified by subsequent growth on plates with the respective antibiotics. The distribution of denitrification genes in more than 300 genome-sequenced *Rhodobacteraceae* was investigated via multi-gene BLAST analyses.

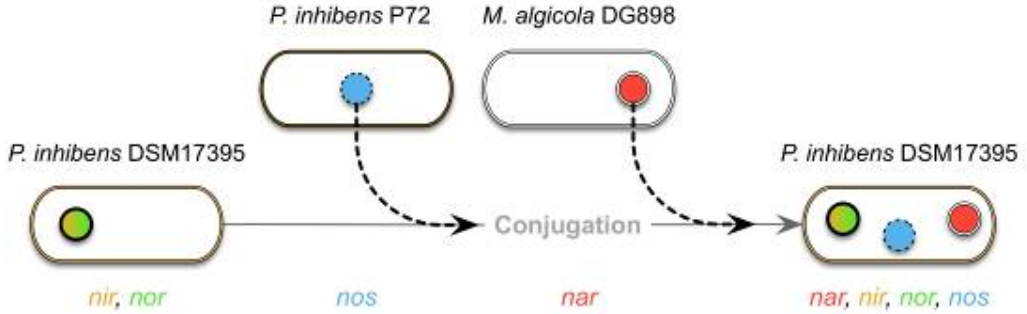
Results: Phylogenetic analyses revealed a strongly scattered distribution of the denitrification genes within the *Rhodobacteraceae.* Another striking observation was the persistent localization of these genes on extrachromosomal replicons (ECRs). Two plasmid-located denitrification genes (*nar*, *nos*) could be successfully transferred by conjugations from *Marinovum algicola* DG898 and *P. inhibens* P72 into *P. inhibens* DSM 17395. The recipient now harbors the complete set of genes required for denitrification (*nar, nir, nor, nos*). In contrast to the *Phaeobacter* wild-type the newly generated transconjugant obtained the ability to reduce nitrate, which correlates with the capacity to grow in the absence of oxygen.

Conclusion: This study showed the transmission of plasmids beyond the species border which exemplifies the impressive natural potential of marine bacteria for evolutionary adaptation to new habitats. The horizontal acquisition of a functional nitrate reductase paved the way for *P. inhibens* DSM17395 to grow anaerobically in the sediment.

Figure 1: Experimental conjugation scheme with overview of gene distribution

Petersen J, Frank O, Göker M, Pradella S (2013) Extrachromosomal, extraordinary and essential--the plasmids of the Roseobacter clade. *Appl Microbiol Biotechnol*. 97: 2805-2815.

Fig. 1



P 4

Profiling of naturally occurring antibiotic resistances in plant microbiomes

T. Cernava[1], G. Berg[1]
[1]*Graz University of Technology, Institute of Environmental Biotechnology, Graz, Austria*

Eruca vesicaria var. *sativa* (Mill.) Thell., commonly known as arugula, is a popular raw-eaten ingredient in salads due to its peppery, pungent taste. Similar to other leafy greens, *E. sativa* is colonized by a vast diversity of microbes, which complement the holobiont"s functioning. The indigenous microbiota only rarely allows foodborne pathogens to settle, indicating high competitiveness. Nevertheless, the prevalence of resistances in such microbial communities is only poorly understood. We analyzed the structure, abundance and functioning of the plant-associated microbiota in the arugula phyllosphere, rhizosphere and the corresponding bulk soil in an integrative approach. When compared to the rhizosphere, higher proportions of *Gammaproteobacteria*, including *Enterobacteriaceae* were observed in aerial plant parts. Their occurrence was verified by fluorescence *in situ* hybridization coupled with confocal laser scanning microcopy in different plant compartments. Complementary metagenomic profiling of the bacterial population indicated a higher prevalence of antibiotic resistances in plant-derived samples. We found general resistance mechanisms including various efflux pumps in the datasets, but also specific resistance mechanisms against fluoroquinolone, chloramphenicol and other antibiotics. Due to the high occurrence of *Enterobacteriaceae* in arugula samples, we screened a representative culture collection for resistances against eight common antibiotics. It was shown that more than 90% of the isolates were resistant against Ampicillin, Erythromycin, and Penicillin. Our findings suggest that antibiotic resistance is common in distinct raw-eaten plants; however, the implications for human health remain unclear.

P 5

Identifying drivers of antibiotic resistance in UK river systems

J. Delaney[1], C. Borsetto[1], E. Wellington[1], S. Raguideau[2], A. Singer[3], C. Quince[2]
[1]*University of Warwick, School of Life Sciences, Coventry, United Kingdom*
[2]*University of Warwick, Medical School, Coventry, United Kingdom*
[3]*Centre for Ecology and Hydrology, Wallingford, United Kingdom*

The development of resistance to antibiotics by bacterial pathogens is one of the significant threats to public health globally. A major challenge in understanding the environmental aspect of antimicrobial resistance (AMR) is to differentiate the impacts of correlation and causation associated with sewage treatment and manure processing. Antibiotics are detected in all our waste streams as they are often excreted partially metabolised or in some cases not metabolised at all. Resistance genes (ARG) are frequently mobilised on integrons and plasmids or other mobile genetic elements (MGEs) so may be disseminated into environmental bacteria by horizontal gene transfer (HGT) at random but selected by environmental pollutants. The ARG may also persist in human or animal associated bacteria contributing to ARG reservoirs in the environment. Both processes pose a risk to human and animal health and our research is attempting to elucidate mechanisms driving ARG dissemination.

The objectives of this study were to exploit a combination of newly available metagenome and qPCR data to analyse the presence of ARG in community DNA isolated from river sediments and relate this to a wide range of physico-chemical features of the riverine environment. Multivariate statistics were used to correlate ARG prevalence to factors such as concentrations of antibiotics, metals, biocides, in addition to land use and seasons.

Central to the analysis was the Thames Metagenome Database (TMD), which consists of over a terabase of microbial DNA sequence data derived from 69 locations in the River Thames Catchment. Current analyses showed that certain "hotspot" sites on the catchment had elevated levels of the sulphonamide resistance gene *sul1*. Also, there was a significant association between the presence of heavy metals such as Cadmium, Zinc and Titanium and the presence of antimicrobial genes such as *tetM, qacE and ermF.* The location of these *sul1* genes is the topic of focused research to establish if they are within environmental bacteria and or human associated bacteria derived from WWTP effluent. To date we have established most of the *sul1* genes were recovered on integrons associated with plasmids but the host background for these plasmids is still being investigated using Hi-C analysis.

In further studies, we also aim to apply Emulsion paired isolation and concatenation (EPIC) PCR, to link functional genes and phylogenetic markers of antibiotic resistance.

P 6

Improved bacterial hospitality to plasmids

J. Kloos[1], J. Gama[1], J. Hegstad[1], î Samuelsen[1,2], P. J. Johnsen[1]
[1]*Microbial Pharmacology and Population Biology Research Group, Department of Pharmacy, UiT The Arctic University of Norway, Tromsø, Norway*
[2]*Norwegian National Advisory Unit on Detection of Antimicrobial Resistance, Department of Microbiology and Infection Control, University Hospital of North Norway, Tromsø, Norway*

Plasmids are drivers of bacterial adaptation against antibiotics, but in the absence of selection, they often incur fitness costs in new hosts. Here we studied a non-mobilizable plasmid which encodes the *bla*$_{VIM}$ gene that confers resistance against carbapenems. This plasmid, originally isolated from *Klebsiella pneumoniae*, imposed a low fitness cost when acquired by an uropathogenic *Escherichia coli* isolate. We evolved this plasmid-carrying strain for >300 generations and showed that the fitness cost was mitigated during host-plasmid co-evolution. Neither the evolved nor the ancestral plasmid reduced the fitness of the evolved host. The evolved plasmid, however, still imposed a fitness cost on the ancestral *E. coli* host. Altogether, this finding suggests that compensatory evolution had resulted from chromosomal adaptation. The specific mechanisms and genetic events responsible for increased plasmid permissiveness are currently under investigation. We further analyzed whether this adaptation was plasmid specific. We replaced the *bla*$_{VIM}$-encoding plasmid with an unrelated conjugative plasmid also determining carbapenem-resistance (*bla*$_{NDM-1}$). This plasmid decreased the fitness of the ancestral host, but had a weaker effect on the host that co-evolved with the *bla*$_{VIM}$-encoding plasmid. Thus, host adaptation does not seem to be plasmid-specific. We report here a clinically relevant phenotypic pattern of host adaptation towards a non-conjugative plasmid that simultaneously ameliorates the fitness cost of an unrelated conjugative plasmid. This pre-adaptation may promote the acquisition of other plasmids and facilitate the emergence of multi-drug resistant bacteria.

P 7

Plasmid-associated antibiotic resistance genes in soil metagenomes from Fildes Peninsula (King George Island, Antarctica)

M. Giménez[1,2], G. Azziz[3], S. Batista[1], G. Iraola[2]
[1]*Instituto de Investigaciones Biológicas Clemente Estable, Unidad de Microbiología Molecular, Montevideo, Uruguay*
[2]*Institut Pasteur Montevideo, Microbial Genomics Laboratory, Montevideo, Uruguay*
[3]*Facultad de Agronomía, Laboratorio de Microbiología, Montevideo, Uruguay*

Horizontal gene transfer (HGT) in prokaryotes is one of the most important mechanisms involved in their adaptation to environmental changes. This evolutionary mechanism has undoubtedly contributed to the dispersion of antibiotic resistance genes (ARGs). Plasmids are one of the most important components of the mobilome as they are directly involved in the genetic exchange between bacteria. They are composed of a backbone organized in modules that play different roles such as replication, transference and maintenance. Metagenomics has proved to be a powerful approach to study the biology of plasmids and its influence in ARGs occurrence in different environments. However, there is no consensus about the tools and strategies to be used for the study of plasmids from metagenomics data as most of them have important drawbacks. In this work we assessed ARGs presence in three soil metagenomes from Fildes Peninsula (King George Island, Antarctica) in a gradient of human and animal impact. To accomplish this we designed a tool that allows the identification of plasmidic contigs by two different strategies. One of them is based on the detection of relaxases and replication initiator proteins (RIPs). We propose that domain" architecture can be an informative feature to differentiate plasmidic RIPs from similar chromosomal proteins. The following step was developed to classify plasmidic contigs. A replicon and MOB typing approach, based on HMMs, was developed. The other strategy is based on comparison of contigs, through blastn, against a curated plasmidic database. The advantage of this strategy is that it does not depend on the presence of RIPs or relaxases to classify a contig as plasmid-derived. All plasmidic contigs were used as input to search for ARGs presence. The same strategy was used with each complete metagenome assembly. Using this approach we could detect the presence of plasmid derived contigs in the analyzed samples. The metagenome with more anthropogenic impact had more plasmid-derived sequences compared to the other two metagenomes. This sample also had more ARG´s diversity, and was the only one whose plasmidic contigs could be associated with ARGs presence. A MexK efflux pump was found encoded in an IncP-like replicon in this sample. Resistance to aminoglycosides and beta-lactams was found to be conserved in the three metagenomes. Further studies are being done to assess the conjugative capacity of antimicrobial resistance traits in these samples.

Horizontal gene transfer, mobile genetic elements and antibiotic resistance

P 8

The abundance of plasmids in *E. coli* increases level of antibiotic resistance in urban waterways

K. Hristova[1], A. Kappell[1], R. Beattie[1], T. Skwor[2], I. Bessarab[3], K. Arumugam[4], R. Williams[3]
[1]*Marquette University, Biological Sciences, Milwaukee, WI, United States*
[2]*University of Wisconsin Milwaukee, Milwaukee, WI, United States*
[3]*National University of Singapore, SCELSE, Singapore, Singapore*
[4]*Nanyang Technology University, SCELSE, Singapore, Singapore*

The city of Milwaukee urban waterways represent a natural reservoir of antibiotic resistance, which may provide a source of transferable genetic elements to human commensal bacteria and pathogens. We hypothesize there is a greater abundance of multidrug-resistant (MDR) bacteria and transferable genetic elements in Milwaukee"s urban waterways compared to the human microbiome (influent wastewater or clinical isolates). We postulate hospital wastewater serves as a stronger reservoir of carbapenem-resistant and ESBL-producing *Escherichia coli* than municipal wastewater. The phenotypic antibiotic resistance of 259 *E. coli* isolated from urban waterways, sewage and clinical isolates was determined based on antibiotics covering 10 different families. Additionally, 126 *E. coli* from hospital wastewater and wastewater treatment facility influent, pre-chlorinated and post-chlorinated effluents were isolated on mTEC media with or without 2 µg/ml cefotaxime and screened for phenotypic resistance. Amongst hospital wastewater isolates, although all were susceptible to carbapenems, 7 % were ESBLs and 8% exhibited resistance to ceftazidime/clavulanic acid. Our data indicates high prevalence of MDR *E. coli* isolates in hospital wastewater and suggests resilience of these isolates to chlorination. The 219 strains identified with plasmids showed six highly represented agarose-gel banding patterns of which two were correlated with MDR. Statistical comparisons indicate that plasmid presence in the environmental isolates correlate with resistance to a greater number of antibiotics or location. The plasmid genome of eight *E. coli* strains were sequenced with both Illumina MiSeq sequencing and Nanopore MinION long read sequencing. Ongoing data analysis will reveal detailed information about this mobile resistome that will be presented. The implications of this study are significant to understanding the presence of resistance and the role of plasmids in urban freshwater environments.

P 9

Detection of population dynamics and low abundant Staphylococcus species in patients undergoing prosthetic joint replacement surgery using targeted *tuf* and 16S rRNA sequencing

S. Iversen[1], T. B. Johannesen[1], S. M. Edslev[1], S. Tevell[2,3], E. Månsson[3,4], î Nilsdotter[5], B. Söderquist[3,6], M. Stegger[1], P. S. Andersen[1]
[1]*Statens Serum Institut, Department of Bacteria, Parasites & Fungi, Copenhagen, Denmark*
[2]*Department of Infectious Diseases, Karlstad, and Centre for Clinical Research, Värmland, Sweden*
[3]*Örebro University, School of Medical Sciences, Faculty of Medicine and Health, Örebro, Sweden*
[4]*Region Västmanland – Uppsala University, Centre for Clinical Research, Hospital of Västmanland, Västerås, Sweden*
[5]*Linköping University, Department of Infectious Diseases, and Department of Clinical and Experimental Medicine, Norrköping, Sweden*
[6]*Örebro University, Department of Laboratory Medicine, Clinical Microbiology, Faculty of Medicine and Health, Örebro, Sweden*

Bacteria of the *Staphylococcus* genus are common inhabitants of a healthy human microbiota, present at various skin sites and on mucosal membranes. Carriage of *Staphylococcus* species with invasive potential constitutes a risk for infection, especially in patients undergoing surgical procedures like prosthetic joint replacement, and the threat is currently substantiated by the spread of multidrug resistant lineages in health-care facilities. Culture-based methods and 16S rRNA gene sequencing are standard methods used to detect bacteria in clinical samples, but the sensitivity of these methods is limited with regard to low abundant bacterial samples, which is a characteristic feature of skin samples.

Here, we evaluate a new set of genus-specific primers that specifically target the *tuf* gene of the *Staphylococcus* genus and assess sensitivity and reproducibility measures on staphylococcal mock communities. We apply the targeted *tuf* gene approach in combination with 16S rRNA gene sequencing of the V3-V4 region on samples collected from patients undergoing prosthetic joint replacements and analyze the effects of chlorhexidine showers on groin and nasal microbiotas. We report here the bacterial changes that occur at genus level as well as the dynamics of the *Staphylococcus* species associated with chlorhexidine showers.

This *tuf* gene sequencing approach enables consistent and reproducible community structure profiling of mock communities with a capacity to distinguish all currently known staphylococcal species. By targeting the *tuf* gene, this approach permits detection of low abundant staphylococcal species in groin and nasal samples and provides increased sensitivity and discriminatory power for the *Staphylococcus* genus in comparison with the more widely used 16S rRNA gene. Importantly, *tuf* gene sequencing offers precise staphylococcal composition estimates from low abundant samples, which facilitates studies of species dynamics and competition within the staphylococcal genus across diverse habitats.

P 10

Antibiotic resistance in agricultural soil associated to the application of cow manure-derived amendments from ecological and conventional livestock farms

L. Jauregi[1], L. Epelde[1], I. Alkorta[2], C. Garbisu[1]
[1]*NEIKER-Tecnalia, Conservation of Natural Resources, Derio, Spain*
[2]*Instituto BIOFISIKA (CSIC, UPV/EHU), Biochemistry and Molecular Biology, Bilbao, Spain*

The application of organic amendments of animal origin into agricultural soil can enhance its physical, chemical and biological characteristics, while providing essential nutrients to crop plants. Regrettably, this practice can also lead to the emergence and dissemination of antibiotic resistance (antibiotic resistance genes-ARGs, antibiotic resistant bacteria-ARB, mobile genetic elements-MGEs) in agricultural soil. Here, we studied this threat for environmental and human health in agricultural soil after the application of cow manure-derived amendments (i.e., composted manure, fresh manure, slurry) from ecological and conventional livestock farms. For this purpose, wheat plants were grown in amended soils. After harvest, the relative abundance of 95 ARGs and MGE genes from amended soil and wheat grains were determined using the Fluidigm Biomark HD System – qPCR. The impact of amendment application on the structure and composition of soil microbial communities was determined by next generation amplicon (16S, 18S) sequencing. The quantification of total bacteria in soil and wheat grain was measured via qPCR. Minimum inhibitory concentrations (MICs) against five antibiotics (meropenem, streptomycin, sulfamethoxazole, tetracycline and vancomycin) were calculated in soil and wheat grain samples. The abundance of ARGs and MGEs significantly differed between treatments. These abundances were significantly higher in soil samples than in wheat grain samples. Similarly, the composition of microbial communities differed among amended soils. Values of total bacteria were higher in amended soils. Finally, MIC values were higher in soil, compared to wheat grains. *This work was funded by the Basque Government through URAGAN-CRN 18-20 (18-00044).*

P 11

Fecal pollution can explain antibiotic resistance gene abundances in anthropogenically impacted environments

A. Karkman[1], K. Pärnänen[1], D. G. J. Larsson[2,3]
[1]*University of Helsinki, Department of Microbiology, Helsinki, Finland*
[2]*University of Gothenburg, Department of Infectious Diseases, Gothenburg, Sweden*
[3]*University of Gothenburg, Centre for Antibiotic Resistance Research (CARe), Gothenburg, Sweden*

Introduction: Discharge of treated sewage leads to release of antibiotic resistant bacteria, resistance genes and antibiotic residues to the environment. However, it is unclear whether increased abundance of antibiotic resistance genes in sewage and sewage-impacted environments is due to on-site selection pressure by residual antibiotics, or is simply a result of fecal contamination with resistant bacteria.
Objectives: Our goal was to develop an approach that can help in disentangling environmental on-site selection and/or horizontal dissemination of antibiotic resistance genes from passive dissemination/enrichment by fecal pollution.
Materials and Methods: Using crAssphage as a marker of human fecal contamination, we analyzed the extent of fecal pollution in human impacted environments using publicly available metagenomic data and correlated it with relative antibiotic resistance gene abundance.
Results: We find that the presence of resistance genes can largely be explained by fecal pollution with the exception of environments polluted by very high levels of antibiotics from drug manufacturing, where selection for antibiotic resistance is evident. We were able to detect true hotspots for antibiotic resistance gene selection in sediments receiving exceptionally high levels of antibiotics from industry. In addition, we show that in all other studied environments receiving anthropogenic waste, there was no clear evidence of wide scale selection.
Conclusions: Our results provide a framework to help disentangling dissemination of resistant human fecal bacteria from the possible selection and horizontal gene transfer of resistance genes in the environment. Our results demonstrate the necessity to consider fecal pollution levels to avoid making erroneous assumptions regarding environmental selection of antibiotic resistance. In addition, these results bring insight to the dynamics of antibiotic resistance genes in sewage receiving environments and highlight the importance of measuring fecal pollution when assessing the selection and dissemination patterns of antibiotic resistance genes in the environment.

P 12

Horizontal spread of transposons – transposition during natural transformation

J. Kloos[1], P. J. Johnsen[1], K. Harms[1]
[1]*UiT-The Arctic University of Norway, Tromsø, Norway*

Transposons are genetic elements that can translocate themselves within the host genome through transposase activity. They are confined to the host genome, unless they are located on mobile genetic elements or are themselves conjugative. Although conjugation is thought to be the main horizontal dissemination pathway for plasmid-borne transposons, recent *in vitro* studies have demonstrated that transposons can also effectively be transmitted through natural transformation. The pathway includes active uptake of free DNA by a competent recipient cell, expression of transposase from DNA transiently present in the cytoplasm, and recombination of the transposon with the host genome by transposase activity.
We investigated the requirements for transposition during natural transformation in the naturally competent *Acinetobacter baylyi* model organism. We inserted the non-conjugative transposon Tn*1* into a pSC101-derivative plasmid vector unable to stably replicate in *A. baylyi*, and we used the resulting plasmid as donor DNA to naturally transform *A. baylyi*.
Transformants by transposition ("transposants") formed at low frequencies (about 10^4-fold lower than homologous recombination and tenfold lower than plasmid recircularization). Transposant formation was directly proportional to donor DNA concentration (pseudo-one-hit kinetic) and required host (RecA, XerCD) and transposon (resolvase) functions. We identified the double-stranded DNA exonuclease RecBCD as a main inhibitor for transposition during transformation. Altogether, these results suggest the cytoplasmic formation of a transient DNA double-strand intermediate (e.g., circular or protected by Chi sequences) that allows temporary expression of the transposase gene.
About 80% of transposition events occurred in a small sector of the host chromosome around the presumed terminus of replication. The mechanistic aspects of this distribution are under investigation.

P 13

Hospital microbiome and resistome

K. Koskinen[1,2], S. Duller[1], P. Mantaj[1], A. Mahnert[1], C. Moissl-Eichinger[1,2]
[1]*Medical University of Graz, Graz, Austria*
[2]*BioTechMed-Graz, Graz, Austria*

Hospital-acquired infections are a serious problem worldwide. The risk is not only related to invasive procedures or inadequate hygiene, but infection can also be transferred from patient to patient, via personnel, surfaces or equipment. Despite strict disinfection procedures resistant pathogenic bacteria are frequently found on hospital surfaces, and these microorganisms can influence the patient recovery and outcome. However, hospital indoor environment carries also diverse microbial communities, consisting mainly of human associated bacteria, and interaction between these communities, pathogenic bacteria, and resistance elements is not well understood.
The aim of this study is to deepen our understanding about the hospital microbial community dynamics and development in newly opened hospital wards as well as in departments of different confinement level. Particularly, we strive to determine the interplay between microbial diversity and distribution and transfer of (antibiotic) resistance genes.
We analysed the microbiome (16S rRNA amplicons) and resistome (metagenomics, analysis of specific resistance genes) of different areas and locations at the intensive care unit as well as in operating room at thorax surgery department. The samples were taken from various regularly cleaned or disinfected surfaces, such as floor, bed, windowsill, sink, keyboard, and door opening buttons, and subjected to molecular analyses. We correlate the resistome information and 16S rRNA gene-based microbial community composition with degree of confinement, cleaning and disinfection procedures and microbial diversity. Additionally, we followed the development (1 year) of microbiome dynamics in newly opened surgery wards.
Analysis of microbiome data showed significant differences in microbial community diversity and structure depending on confinement level, and a strong influence of human skin associated bacteria and potential pathogens was detected. The microbial community structures in particular sampling sites were highly specific. Genes coding for relevant resistances, including tetracycline, betalactamase, class 1 integron and quaternary ammonium compound were detected in most studied environments, even in extremely low biomass samples.
Ultimately, we aim to identify the bacteria carrying these resistances, track the horizontal gene transfer of resistance elements, and define the hotspots of resistance development within the hospital indoor microbiome.

P 14

Plasmid-based tools to facilitate studies of polar *Psychrobacter* spp

R. Lasek[1], A. Sówka[1], M. Krolikowski[1], D. Bartosik[1]
[1]*University of Warsaw, Faculty of Biology, Institute of Microbiology, Department of Bacterial Genetics, Warsaw, Poland*

Introduction: The study of cold-adapted bacteria is a promising branch of microbiology, especially given their huge biotechnological potential. However, both basic and applied research on psychrophiles is hindered by the lack of specific genetic tools. Having studied in detail a pool of Arctic strains of the genus *Psychrobacter*, we decided to use selected genetic modules of their native plasmids in the construction of novel *Psychrobacter*-specific vectors.
Objective: Our aim was to create a series of new genetic tools functional in DAB_AL43B, a well-characterized strain of *Psychrobacter* sp. with a completely sequenced genome.
Materials and Methods: Standard genetic manipulations were used in the construction of plasmid vectors. The obtained shuttle vectors were introduced into *Psychrobacter* sp. cells via triparental mating. The *E. coli*-derived *lacZ* gene was used as a reporter gene or a counterselection marker.
Results: Several new genetic tools were obtained: (i) protein expression vectors (pExPsy), (ii) vector for testing promoter activity in vivo (pRSPsy), and (iii) a suicide plasmid-based system for introducing deletions in the DAB_AL43B genome.
The pExPsy vectors carry a strong promoter P_{SLF} induced by sodium dodecyl sulfate and laurylaldehyde, which allows for the inducible expression of His-tagged proteins. The RSPsy vectors are used to conduct high throughput LacZ activity assays. In the new gene deletion system the lacZ gene serves as a counterselection marker (the product of X-gal hydrolysis being deleterious for DAB_AL43B).
The constructed genetic tools were verified to be functional in DAB_AL43B as well as in a pool of other polar *Psychrobacter* sp. strains. We subsequently used them for the study of restriction-modification systems carried by plasmid pP62BP1 of *Psychrobacter* sp. DAB_AL62B.
Conclusion: Using genetic modules derived from the native plasmids of the cold-adapted bacteria we were able to enlarge the available repertoire of genetic tools facilitating the study this group of prokaryotes.

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Anthropogenic impacts on the environmental resistome in the coastal water of South China Sea

S. Lau[1], X. P. Koh[2], M. Tang[1], F. Chen[2], J. Gan[1]
[1]*Hong Kong University of Science and Technology, Department of Ocean Science, Clear Water Bay, Hong Kong*
[2]*Hong Kong University of Science and Technology, Division of Environment and Sustainability , Clear Water Bay, Hong Kong*

Introduction: The enrichment of clinically important antibiotic resistance genes (ARGs) in the environment is an emerging public health threat, due to their capability to be horizontally transferred to the environmental resistome and contaminate the food chain. The impacts of anthropogenic pollution on the environmental resistome in the coastal water of South China Sea was investigated.
Materials and methods: Bacterioplankton collected from the surface water of 3 sampling sites (F101, F103 and F107 in the order of reducing human impacts) was analyzed using shotgun metagenomic sequencing. The contribution of different sources (human or animal faeces, wastewater treatment plants, natural environments) to the ARG profile of each site was predicted using a source-tracking platform.
Result: In total, 6226 ARG subtypes belonging to 16 ARG types were detected. ARGs associated with wastewater pollution (e.g., trimethoprim, sulphonamide and bacitracin) were predominant at F101 and their abundance decreased progressively at F103 and F107. An opposite trend was observed for the genes encoding multidrug efflux pumps. Importantly, the relative abundance of non-efflux ARGs over total ARGs increased rapidly from F107 (42 %) to F103 (66 %) and F101 (84 %) in association with the magnitude of anthropogenic pollution. Non-efflux-mediated resistance poses greatest public health risks as the genes have higher chance of transfer to pathogenic bacteria in comparison to the intrinsic, non-transferable multidrug efflux pump.
Conclusion: Resistome characterization coupled with source identification provided the foundation to predict the public health risks associated with anthrpogenic pollution of environmental resistome and helped identify critical control points to mitigate ARG spread. The relative abundance of trimethoprim, sulphonamide and bacitracin resistance genes may be used as signature of anthropogenic pollution.

Horizontal gene transfer, mobile genetic elements and antibiotic resistance

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Following the mobile resistome through the path of reclaimed wastewater – from treatment plants to irrigated crops

R. Marano[1,2], E. Jurkevitch[2], E. Cytryn[1]
[1]*Agricultural Research Organization, Rishon LeZion, Israel*
[2]*Hebrew University of Jerusalem, Rehovot, Israel*

Urban wastewater reuse is a viable solution for sustainable agriculture in arid and semi-arid geographic areas. However, this practice poses questions regarding potential epidemiological risks, including the discharge of antibiotic resistance genes and bacteria (ARGs, ARB), which can mobilize through food webs impacting public health. In particular, plasmid- and integron-associated antibiotic resistance genes (PI-ARGs) can significantly contribute to the ultimate spreading of ARG to anthropogenic environments via the consumption of products that have been irrigated with treated wastewater (TWW). To assess the relevance of this risk, we monitored the abundance and distribution of a set of PI-ARGs, by means of quantitative-PCR, complemented with culture-based methods, in six Israeli wastewater treatment plants (WWTP) and model (lysimeters) and real-scale agricultural fields in a TWW-to-soil-crop continuum. The objective of this study is to provide a wide overview of their distribution, abundance, and persistence in agriculture systems that entirely rely on reclaimed wastewater irrigation. Wastewater use management and soil textures were included as additional surveyed parameters to the study. TWW effluents were a significant point source of the tested genes, however, their distribution in the surveyed fields was below limits of detection. In tandem, we observed an increased distribution of *intI*1 in lysimeters, which we ascribe to a soil-borne effect rather than a direct transfer from TWW. Altogether, our findings highlight the importance of ecological barriers to ARGs dissemination in the path of reclaimed wastewater and shed light on the dynamics of targeted PI-ARGs within these agroecological settings.

P 17

Tracking antibiotic resistance genes in the treated wastewater irrigated soil and crops

M. Mihiret[1], O. Obayomi[1], N. Bernstein[2], A. Vonshak[1], O. Gillor[1]
[1]*Ben_Gurion university of the Negev, Environmental Microbiology, Midreshet Ben-Gurion, Israel*
[2]*Institute of Soil , Water and Environmental Sciences, Volcani Center, Rishon LeZion, Israel*

The use of treated wastewater (TWW) for irrigation has been suggested as an alternative to use of freshwater (FW) because of the increasing scarcity of FW in arid and semi-arid regions around the world. However, there is a growing concern for that irrigating with TWW may enhance antibiotic resistance transfer from the water to the soil and crops. We hypothesized that TWW irrigation would increase the level of antibiotic resistance genes (ARGs) in the irrigated soils and crops due to selective pressure generated by residual antibiotic compounds, and to the introduction of ARGs by antibiotic resistant bacteria. To test our hypotheses, culture independent qPCR techniques were used to monitor the abundance and dissemination of seven ARGs, class 1 integron and 16S rRNA encoding genes in 127 samples of water, soil, and crops. Water qualities (TWW and FW), soil subjected to different treatments (drip irrigation or plastic cover), different soil types (clay, loam or loamy-sand) and different crops (cucumber or melon) were monitored in a field study conducted over the course of two years. The results showed that TWW contains a diverse and abundant array of ARGs, that were absent from FW. Yet, there was no significant difference in the levels of ARGs in the irrigated soils or crops, regardless of the irrigation water quality or soil treatment ($p>0.05$). However, soil types had a significant effect on the levels of ARGs ($p<0.05$). These findings suggest that ARGs harbored in TWW may not persist in the irrigated soil and thus do not transfer to the crops, suggesting that the impact of TWW on the irrigated soil resistome might be negligible in the short term. Long term experiments would determine whether TWW has lasting effect on the soil resistome.

P 18

The moss microbiota – a natural reservoir for antibiotic resistance genes

C. A. Müller[1], M. M. Obermeier[1,2], J. Taffner[1], G. Berg[1]
[1]*Graz University of Technology, Institute of Environmental Biotechnology, Graz, Austria*
[2]*Austrian Centre of Industrial Biotechnology, Graz, Austria*

The expanding antibiotic resistance crisis makes necessary to gain more insights into the origin of resistance development. Our knowledge of the availability of antibiotic resistance genes in natural reservoirs, for example in microhabitats like plants, is still very limited. The moss *Sphagnum magellanicum* is known for its antimicrobial properties and metagenomic analysis revealed abundant genetic signatures supporting pathogen defence. By combining *in silico*, *in vivo*, and *in vitro* screenings we investigated the antibiotic resistome of the moss microbiota. mThe metagenome was analysed *in silico* using BLAST against the Comprehensive Antibiotic Resistance Database. This search revealed a high occurrence of antibiotic resistance determinants targeting 21 different drug classes. The most abundant genes were associated to resistance against β-lactams and glycopeptide antibiotics. Ten antibiotics belonging to the identified drug classes were used for further *in vivo* screening of 275 moss isolates. More than 50% of the tested isolates showed resistance against ampicillin, vancomycin and sulfadiazine. Eleven multi-resistant strains (>6 resistances) belonging to the genera *Rouxiella*, *Serratia*, *Pandorea* and *Pseudomonas* were identified as well. *In vitro* screening of a metagenomic library (90,000 clones) against the same antibiotics allowed identification of ampicillin resistance clones. Sequencing of one selected clone resulted in identification of a novel β-lactamase gene. The enzyme was purified and biochemically characterized. Based on substrate kinetics and sequence homology, the enzyme was classified as a new type of carbenicillinase.
Using this holistic screening approach, the obtained results expand our knowledge on the natural occurrence of antibiotic resistances in the moss microbiome. The implications of this investigation also pave the way to a deeper understanding of the prevalence and evolution of resistances in less disturbed environments like the bog ecosystem.

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Fungal highways – novel dissemination routs to differentially enrich and selectively disperse the antibiotic resistance genes (ARGs) in environment

R. Nazir[1], D. Shua[2], J. Shen[2], H. Hu[3], W. JunTao[2], J. He[3,2]
[1]*CUI, Abbottabad, Environmental Sciences, Abbottabad, Pakistan*
[2]*Eco-Environmental Sciences, RCEES (CAS), Beijing, China*
[3]*University of Melbourne, FVAS, Melbourne, VIC, Australia*

As evidenced in recent years, environmental antibiotic resistance genes (ARGs) and their potential transfer to other bacterial hosts of clinical importance are of serious concern. However, the dissemination strategies are largely unknown for such ARGs. As fungal highways have earlier been reported to transfer specific bacteria and even chemical substances, we hypothesized that saprotrophic soil fungi could differentially enrich the antibiotic resistant bacteria (ARBs) and consequently contribute in spatial distribution of selective ARGs. Wafergen qPCR array of 295 different ARGs was conducted for manure treated pre-sterilized soil incubated or not with selected bacterial-fungal consortia. *Pseudomonas aeruginosa* strains were also included in the experiment (as an independent treatment) to evaluate if the pathogenic invasion contributes in ARGs diversity and abundance in polluted environment.
The qPCR assay detected unique ARGs specifically found in the mycosphere of ascomycetous and basidiomycetous fungi. Both fungi exerted potentially different selection pressures on ARBs, resulting in different patterns of ARGs dissemination (to distant places) along their respective growing fungal highways. The relative abundance of mobile genetic elements (MGEs) was significantly decreased along fungal highways compared to the respective inoculation points. Moreover, the decrease in MGEs and ARGs (along fungal highways) was more prominent over time which depicts the continuous selection pressure of growing fungi on ARBs for enrichment of particular ARGs in the mycosphere. Noticeably, at fungal migration front i.e. along fungal highways of *Trichoderma atroviride* nfc5, *P. aeruginosa* inoculation with manure significantly increased the abundance of some ARGs (i.e. Aminoglycosides, tetracycline and mobile genetic elements (MGEs) in this case).
Such data also indicate the potential role of saprotrophic soil fungi to facilitate horizontal gene transfer within mycospheric environmental settings. Our study, therefore, advocates to emphasize the future investigations for such (bacteria-fungal) interactive microbial consortia for potential (spatial) dissemination of resistance determinants which could ultimately increase the ARGs exposure risk to human beings.

P 20

Following plasmids and their host genomes in urban wastewater communities by an integrative sequencing approach

J. Nesme[1], Z. Yu[1], R. Pinilla Redondo[1], J. Stenløkke Madsen[1], D. Pellow[2], I. Mizrahi[3], A. Dechesne[4], B. Smets[4], S. J. Sørensen[1]
[1]*University of Copenhagen, Section of Microbiology, Copenhagen, Denmark*
[2]*Tel-Aviv University, Blavatnik School of Computer Science, Tel Aviv, Israel*
[3]*Ben-Gurion University of the Negev, Department of Life Sciences and the National Institute for Biotechnology in the Negev, Be'er Scheva, Israel*
[4]*Technical University of Denmark, Department of Environmental Engineering, Kongens Lyngby, Denmark*

Plasmids play a crucial role in bacterial evolution and the dissemination of antibiotic resistance genes, especially in lineages such as Enterobacteriaceae. However, studying plasmid dynamics in complex environments like soil or wastewater remains a challenge. Wastewater treatment plants constantly mix environmental and gut bacteria at high cell densities together with residual pharmaceutical compounds, making them highly relevant systems to study plasmid-mediated dissemination of antibiotic resistance.
To follow the abundance, diversity and dynamics of plasmids and their hosts in such environment we combined direct shotgun metagenome and plasmidome sequencing of samples spanning the different compartments of an urban wastewater system.
We assembled sequence reads into contigs and binned those based on sequence composition and differential coverage. When possible, specific chromosome and plasmid markers genes were employed to identify the contigs" taxonomical origin and molecule type (*i.e.* plasmid or chromosomal). Furthermore, we recovered complete plasmid sequences by extracting circular paths from the metagenomic assembly graph. Importantly, using HiC crosslinking, we were able to probe plasmids-hosts range *in situ* in a sludge sample. Through this integrative approach, we reconstructed Bacterial, Archaeal and plasmid genomes and assessed their abundance at all locations, from the sewer inlet to the receiving water body.
Overall, we recovered hundreds of genomic bins, including several almost complete and novel genomes from various taxa and complete plasmid sequences. Plasmidome sequencing effectively complemented direct shotgun sequencing by providing better coverage of the plasmid molecule pools.
In the face of a global antibiotic crisis driven by the spread of resistance genes, we present promising combinatorial methods for revealing plasmid-host interactions. Antibiotic resistance plasmids can be successfully recovered from urban wastewater samples and linked to their different hosts, providing proper assembly and binning curation, thus shedding light on their potential mobility and host range.

Horizontal gene transfer, mobile genetic elements and antibiotic resistance

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The impact of plasmid host range on the dissemination of antibiotic resistance genes to enterobacteriaceae in the urban wastewater microbiome

A. K. Olesen[1], J. Nesme[1], R. Pinilla-Redondo[1], J. S. Madsen[1], S. J. Sørensen[1]
[1]*University of Copenhagen, Biology, Copenhagen, Denmark*

Horizontal transfer of genetic material between bacteria is a large contributor to the dissemination of antibiotic resistance genes (ARGs). In this way pathogenic bacteria can acquire resistance to multiple antibiotics. Specifically, the microbial community of urban wastewater treatment plants (WWTPs) is believed to be permissive to conjugational transfer of plasmids. This permissiveness of the microbial wastewater community, combined with a strong selective pressure towards drug tolerance and resistance in the WWTP, may result in an accelerated propagation of ARGs to pathogenic bacteria. Members of the Enterobacteriaceae family are well represented among those pathogens of the highest global concern. Dissemination of ARGs encoding such as extended spectrum beta lactamases and resistances to carbapenems are often found to be mediated by plasmids within this family. We aimed to elucidate the influence of plasmid host range on the dissemination of plasmid borne ARGs within the Enterobacteriaceae fraction of the microbial community in the influent of WWTPs. To achieve this, we constructed a dual fluorescent reporter gene system coupled with fluorescence activated cell sorting, to track plasmid dissemination. We found that the microbial community of the influent from three Swedish municipal WWTPs were permissive towards the plasmids R27::*gfp*, R64::*gfp* and pB10::*gfp*. The broad host range plasmid pB10::*gfp*, were disseminated significantly more compared to the two Enterobacteriaceae specific plasmids R27::*gfp*, R64::*gfp*. Additionally, the permissive fraction of the wastewater community was revealed to be highly potent donors of R27::*gfp* and pB10::*gfp*, to models of potential pathogenic Enterobacteriaceae strains. By post sorting 16S rRNA gene sequencing analysis, we furthermore showed that though R27::*gfp* was preferentially transferred to members of Enterobacteriaceae, R27::*gfp* was additionally transferred to non-Enterobacteriaceae Gammaproteobacterial genera. Additionally, several Enterobacteriaceae genera, which includes clinically relevant pathogenic members, were found in the transconjugant pools. Even though the transconjugant pool of pB10::*gfp* was ~700 times larger, R27::*gfp* was significantly more, or as, transferred to two of these genera. Thus, both narrow and broad host range plasmids should be considered, when assessing the risk of ARG dissemination among and to pathogenic Enterobacteriaceae in the WTTPs.

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Detection and quantification of antibiotic resistance genes along the wastewater treatment process by *TaqMan* multiplex qPCR

M. Oliveira[1,2], M. Nunes[1], M. T. Barreto Crespo[1,2], A. F. Silva[3]
[1]*Instituto de Biologia Experimental e Tecnológica, Food Safety and Microbiology Lab, Oeiras, Portugal*
[2]*Instituto de Tecnologia Química e Biológica Antonio Xavier, Microbiology of Man-made Environments, Oeiras, Portugal*
[3]*University of Copenhagen, Department of Biology - Section of Microbiology, Copenhagen, Denmark*

Question: Water scarcity is a worldwide problem and agriculture alone accounts for 70% consumption of the available fresh water on Earth. This scenario makes wastewater reuse for agriculture irrigation a valuable and sustainable alternative. However, the safety of this practice is not yet assured, since the conventionally applied treatments in the wastewater treatment plants (WWTPs) appear to be inefficient towards the removal of some emerging pollutants, such as antibiotics, antibiotic resistant bacteria and antibiotic resistance genes.
Therefore, the present study aims to accurately assess and quantify the presence of resistance genes towards carbapenems, a group of last-line β-lactam antibiotics, and quinolones, a group of widely used antibiotics, in different wastewater samples, using *TaqMan* multiplex qPCR protocols.
Methods: One duplex and two triplex *TaqMan* qPCR protocols were developed and optimized for the identification and quantification of five carbapenem resistance genes (duplex: *bla*KPC and *bla*OXA-48 genes; triplex: *bla*NDM, *bla*IMP and *bla*VIM genes) and three quinolone resistance genes (triplex: *qnr*A, *qnr*B and *qnr*S genes) at different points of two WWTPs.
Results: *TaqMan* multiplex qPCR protocols showed efficiencies between 90% and 110% and detection limits of 10 genome copies/µL. These protocols were applied to wastewater samples collected at different points along two WWTPs, in order to assess the efficiency of the conventional treatments on the removal of these antibiotic related pollutants. Preliminary results showed that both carbapenem and quinolone resistance genes were found in all samples collected in the WWTPs, before and after the applied treatments.
Conclusions: The obtained results point out the inefficiency of the conventionally applied treatments in the WWTPs in removing different antibiotic resistance genes, reinforcing the hypothesis that WWTPs have a role as hotspots in the spread of different antibiotic related pollutants to the environment.
Keywords: Antibiotic resistance in WWTPs; Carbapenems and quinolones, *TaqMan* multiplex qPCR protocols

Fig. 1

P 23
Cancelled

P 24
Transfer of tetracycline-resistance genes from manure to soil depends on soil indigenous microorganisms

E. Pérez-Valera[1], M. Kyselková[1], A. Engy[1], F. X. Jiří Sládeček[2,3], M. Goberna[4], D. Elhottová[1]
[1]*Biology Centre of the Czech Academy of Sciences, Institute of Soil Biology, České Budějovice, Czech Republic*
[2]*Biology Centre of the Czech Academy of Sciences, Institute of Entomology, České Budějovice, Czech Republic*
[3]*University of South Bohemia, Faculty of Science, České Budějovice, Czech Republic*
[4]*Instituto Nacional de Investigación y Tecnología Agraria y Alimentaria (INIA), Department of Environment, Madrid, Spain*

Introduction: Antimicrobial agents used in livestock select resistant microorganisms threatening both environmental and human health. Manure, frequently used as a fertilizer in soils, represents a relevant vehicle in spreading resistance genes and resistant microorganisms into the environment. However, our knowledge of the mechanisms involved in the dissemination and persistence of risky genes in the environment is still limited.
Objectives: Here we experimentally investigated the role of microorganisms and mineral nutrients on the transfer and persistence in soil of tetracycline-resistance genes originated from animal manure.
Material and Methods: We used laboratory soil microcosms to which cattle manure containing tetracycline-resistance genes was applied on the top layer, to simulate natural deposition of animal excrements in the soil. The treatments included control soil, mineral nutrients + soil, manure + soil and manure + γ-irradiated soil. Microcosms were sampled after 7 and 84 days.
Results: Manure improved soil properties such as water content, pH or levels of N and P but altered the composition of bacterial communities and increased the abundance of tetracycline-resistance genes such as *tet*(Y), *tet*(W) or *tet*(M). Changes in the soil resistome persisted with time while those in the composition of tetracycline-resistant soil bacteria partially recovered after 84 days. By exposing soil to γ-radiation, we further detect lower N and P content but a higher abundance of *tet*(Q) or *tet*(Y) in soils lacking indigenous microorganisms.
Conclusion: Our results suggest that antibiotic-resistance genes could persist in the soil regardless of the primary host viability. In addition, soil native bacteria may hinder the transfer of tetracycline-resistant genes to soil and improve the fertility after manure amendments.

Horizontal gene transfer, mobile genetic elements and antibiotic resistance

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Investigating the secretory mechanism of a beta-lactamase producing *E.coli* ST131

S. Rangama[1], I. D. Lidbury[1], R. S. James[1], J. M. Holden[1], C. Borsetto[1], P. M. Hawkey[2], E. Wellington[1]
[1]*The University of Warwick, School of Life Sciences, Coventry, United Kingdom*
[2]*University of Birmingham, Birmingham, United Kingdom*

Infections caused by antimicrobial resistant bacterial pathogens are a major global public health concern. Whilst Waste Water Treatment Plants (WWTPs) have been reported to remove up to 99.9% faecal coliforms, previous work in our lab has shown that anaerobic digestors and the water treatment process at WWTPs positively select for antimicrobial resistance genes/bacteria. Specially, strains of *E. coli* ST131 carrying multiple resistance genes including $bla_{CTX\text{-}M\text{-}15}$ (encoding extended spectrum beta-lactamase, ESBL) were isolated from rivers downstream of the WWTPs. Previous studies have shown certain ESBLs, but not CTX-M-15, can confer a protective effect to nearby neighbouring susceptible cells, via enzyme secretion.
In our study, we aimed to determine if an *E. coli* strain ST131, harbouring $bla_{CTX\text{-}M\text{-}15}$, bla_{OXA} and bla_{TEM} in its genome, also provided a protective effect to susceptible bacteria.
Our data revealed that this strain expressed and secreted an ESBL that provided a protective effect against cefotaxime for susceptible cells. Exoproteomics and whole-cell proteomics of the ESBL-producing *E. coli* identified CTX-M-15 as the major secreted ESBL. We heterologously expressed the $bla_{CTX\text{-}M\text{-}15}$ gene in another *E. coli* strain and confirmed that this gene was responsible for the observed protective effect against cefotaxime. Furthermore, through conjugation with a donor strain we isolated and sequenced the environmental plasmid, harbouring the $bla_{CTX\text{-}M\text{-}15}$, using the Oxford Nanopore MinION. Such assembly has been previously unachievable using short read sequence data set, such as Illumina. The *de novo* assembly of the plasmid showed the $bla_{CTX\text{-}M\text{-}15}$ was the only resistance genelocated on this plasmid, which also contained the entire transfer region responsible for efficient horizontal transfer. $bla_{CTX\text{-}M\text{-}15}$ was found in close proximity to a transposase element suggesting it may have been acquired through a mobile genetic element.
In conclusion, we identified the gene responsible for providing community resistance against a third-generation antibiotic and demonstrated the horizontal mobility of the plasmid harbouring this gene.

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Phenotypic and genomic features of *Klebsiella pneumoniae* isolates from clinical and environmental sources – does the origin matter?

J. Rocha[1], C. Ferreira[1], M. Brito[2], C. Lameiras[3], I. Henriques[4,5], M. Gomila[6], C. M. Manaia[1]
[1]*Universidade Católica Portuguesa, CBQF - Centro de Biotecnologia e Química Fina – Laboratório Associado, Escola Superior de Biotecnologia, Rua Arquiteto Lobão Vital, 172, 4200-374, Porto, Portugal*
[2]*Serviço de Virologia IPOP-FG, Porto, Portugal*
[3]*Serviço de Microbiologia IPOP-FG, Porto, Portugal*
[4]*Department of Life Sciences, Faculty of Science and Technology, University of Coimbra, Coimbra, Portugal*
[5]*CESAM, University of Aveiro, Aveiro, Portugal*
[6]*Microbiologia, Departament de Biologia, Universitat de les Illes Balears, Palma de Mallorca, Spain*

Introduction: Extended-spectrum β-lactamase producing *Klebsiella pneumoniae* are widespread in the environment and clinical settings. This is a serious problem since third-generation cephalosporins and carbapenems are frontline antibiotics to mitigate infections caused by Gram-negative bacteria.
Objectives: Since the relationship between environmental and human bacteria is still unclear, although crucial for understanding the paths of transmission to humans, we aimed to assess whether clinical isolates of *K. pneumoniae* maintain clinically relevant traits once thriving in the environment.
Materials and Methods: A group of 59 wastewater (n=25) and clinical (n=34) isolates of third-generation cephalosporin-resistant *K. pneumoniae* was characterized based on their antibiotic resistance phenotype and genotype and horizontal gene transfer capacity. In addition, part of these isolates and others, whose genomes were available in public databases, in a total of 73 environmental and 78 clinical, were subjected to comparative genomic analyses.
Results: Most wastewater (80%, 20/25) and clinical isolates (94%, 32/34) were multidrug resistant. ESBL genes were more prevalent in clinical (53%, 18/34) than in wastewater isolates (24%, 6/25) and the *bla*KPC gene was only found in clinical isolates (5/34). Conjugative capacity was more prevalent in clinical (76%, 26/34) than in wastewater isolates (40%, 10/25). A screening of the whole genome sequences genes identified 6 groups of genes related to antibiotic and heavy metal resistance, virulence, efflux systems, oxidative stress and quorum sensing, corresponding to a total of 1406 gene variants. Eleven genes related to oxidative stress or quorum sensing were found in all isolates, while 485 and 460 genes related to resistome or virulence were found exclusively in clinical and environmental isolates, respectively.
Conclusion: These results suggest a certain degree of *K. pneumoniae* specialization for environmental or clinical niches, although highlight that putative clinically relevant traits may persist in environmental bacteria.

P 27

Oral administration of antibiotics increased the potential mobility of bacterial resistance genes in the gut of the fish *Piaractus mesopotamicus*

J. S. Saenz[1], T. Valim Marques[2], R. S. Coelho Barone[3], J. E. P. C. Possebon Cyrino[3], S. Kublik[1], J. Nesme[1,4], M. Schloter[1,5], S. Rath[2], G. Vestergaard[1,4]

[1]*Helmholtz Zentrum Muünchen, Comparative Microbiome Analysis, München, Germany*

[2]*University of Campinas, Institute of Chemistry, Campinas, Brazil*

[3]*University of São Paulo, Departamento de Zootecnia, Piracicaba, Brazil*

[4]*University of Copenhagen, Department of Biology, Copenhagen, Denmark*

[5]*Technical University of Munich, ZIEL - Institute for Food & Health, Freising, Germany*

Question: Antibiotic resistance dispersion is a big concern for human health. Several studies suggest that the use of antibiotics comes at the price of increased antibiotic resistance. However, the use of antibiotics is a common practice during intensive aquaculture production but the effect of the oral administration of antibiotics on the mobility of microbial resistance genes in the fish gut is not well understood.

Methods: In the present study, *Piaractus mesopotamicus* was used to evaluate the effect of the antimicrobial florfenicol, usually use in aquaculture, on the diversity of the gut microbiome as well as antibiotic resistance genes (ARGs) and mobile genetic elements (MGEs) using a metagenomic approach. Pre-exposition, exposition and post-exposition phase to the antibiotic were included.

Results: Our results showed that the total relative abundance of ARGs and MGEs significantly increased during the antibiotic exposure. Additionally, phage integrases, transposases, and transposons co-occurring with ARGs accumulated in the gut microbiome because of the antibiotic exposure. Interestingly, total MGEs co-occurring with ARGs showed a significant positive correlation with the total ARGs found. Furthermore, shifts in the gut microbiome towards well-known putative pathogens such as Salmonella, *Plesiomonas*, and *Citrobacter* were observed following florfenicol treatment. Besides, *Plesiomonas* and *Citrobacter* harbored genes that code for multidrug and phenicol efflux pumps. Further, several genes related to RNA processing and modification, cell motility, SOS response, and extracellular structure were enriched due to the antibiotic application. The observed effects were visible during the complete application phase and disappeared at the post-exposure phase.

Conclusions: Our findings suggest that while the prophylactic use of antibiotics in aquaculture intends to contribute to its management, it might actually introduce several risks. In that way, oral administration of antibiotics increases the potential for MGE mediated exchange of ARGs in the gut of fish and could contribute to the enrichment and dispersion of ARGs in aquaculture systems. Importantly, this increase in the potential for ARGs exchange could be an effect of changes in community structure and/or ARG mobilization. This opens the opportunity to explore the gene synteny of ARG, using emerging technologies as long-read sequencing, during the exposition of antibiotics in aquaculture.

P 28

Social interactions influence biofilm formation among wastewater bacteria

A. F. Silva[1], Q. Wang[1], M. Burmølle[1], S. Johannes Sørensen[1]

[1]*University of Copenhagen, Department of BIology, Copenhagen, Denmark*

Question: Bacteria in Nature thrive mainly in heterogeneous biofilms, where they interact and alter the overall microbial community organization and function. Currently is widely recognized the impact of the environmental framework on the dissemination of antibiotic resistance. However, in wastewater, the influence of social interactions in the ability of different species to form biofilms and share genetic traits is underexplored.

The aim is to study the interactions between co-occurring wastewater bacteria in multi species biofilm, as well as their ability to share antibiotic resistance genes encoded in conjugative plasmids.

Methods: The wastewater bacteria were isolated in previous studies. The identification of the isolates was by Sanger sequencing of the 16S rRNA gene. Combinations of 1 to 4 different species were screen for biofilm formation in 96 wells microtiter plates with peg lids and by crystal violet assay. To evaluate the ability of the wastewater isolates to share an Incp1 plasmid (pKJK5) filter-mating assay will be performed. For the distinction of the transconjugant cells, donor and recipients strains were screened for antibiotics (AB) resistance to 9 different AB.

Results: Sequence analysis identified the bacterial isolates as belonging to 7 different genera. The majority of the isolates were resistance to more than 3 different antibiotics. It was possible to define each isolate's AB resistance profiles to further select transconjugants from the donor cells in the filter-mating assay. The comparison between the 4 different species consortiums, and with the corresponding combinations of 1 up to 3 strains, shows that wastewater bacteria' interactions are mostly biofilm induction, although they have preference towards its partners to produce biofilms. Also, isolates that poorly produce biofilm when alone, can still be included in biofilms formed by others.

Conclusions: although all bacteria were isolated from wastewater, there are differences in the production of biofilm related to the different isolates composing the consortium. This indicates that different niches within the wastewater biofilms might occur, where specific bacteria interact preferentially. Next, these consortia will be used to study the transfer of antibiotic resistance genes by conjugation between different species in wastewater.

P 29

Physiological and genomic characterization of clinical and environmental strains of an opportunistic pathogen *Paracoccus yeei* (*Alphaproteobacteria*)

M. Szuplewska[1], R. Lasek[1], D. Sentkowska[1], M. Hałucha[1], Ů Funk[1], D. Bartosik[1]
[1]*Institute of Microbiology, Faculty of Biology, University of Warsaw, Department of Bacterial Genetics, Warsaw, Poland*

Introduction. Comparative sequence analyses of bacterial genomes enable to predict the directions of evolution of individual groups of microorganisms, including pathogenic species. This is especially important considering the increasing number of highly virulent, multiresistant strains, as well as new emerging pathogens.
Objectives. *Paracoccus yeei* (*Alphaproteobacteria*) stands as a very interesting model organism for studying the acquisition of virulence genes and emergence of new pathogenic bacteria. *P. yeei* is the only species, within the genus *Paracoccus*, associated with opportunistic infections in humans. Our collection of *P. yeei* strains comprises six clinical and two environmental isolates, which significantly differ in their physiological properties. The main goal of this project was to conduct complex comparative analyses of genomic nucleotide sequences of clinical and environmental isolates, which allowed to bring valuable data on mechanisms of transition from non-pathogenic strains into emerging pathogens due to the acquisition of exogenous DNA.
Methods. A comprehensive comparative analysis of the genomes were performed using different bioinformatic tools. Analyses of physiological potential of *P. yeei* strains were conducted using standard methods of molecular biology and microbiology.
Results. The results of a genomic analysis showed (i) the similarities and differences between the genomes of clinical and environmental isolates, (ii) defined the genes unique to each *P. yeei* strain, as well as genetic information unique to the pathogenic strains, including virulence modules, (iii) defined the pangenome of *P. yeei*, the conserved genetic core of the chromosomes (housekeeping genes) and a pool of exogenous DNA that determines the phenotype of the host.
Conclusions. The analysis revealed that *P. yeei* strains have a composite genomes containing many extra chromosomal replicons (ECRs) of diverse structure and properties and numerous genetic elements integrated within the chromosome: transposable elements (TEs) as well as putative genomic islands (GIs). Interestingly, the ECRs carry more than 50% of the genes considered to be specific for *P. yeei*, which points to a significant role for these replicons in the evolution of this species. Moreover, the majority of predicted virulence-associated genes are carried within GIs and ECRs, and therefore their presence is likely to be the result of horizontal transmission.

P 30

Comparative genomic analysis in gut microbiomes of broiler and house hold chickens for evalauating the status of selected ARGs and MGEs in small scale slaughter houses

H. Waseem[1], M. I. Ali[2]
[1]*Michigan State University, Environmental Engineering, East Lansing, MI, United States*
[2]*Quaid-i-Azam University, Microbiology, Islamabad, Pakistan*

Questions: The importance of environmental reservoirs as a source of emergence and transfer of antimicrobial resistance (AMR) is well established. Antibiotic resistant genes from different environmental reservoirs are contributing in the ever increasing global problem of antimicrobial resistance. The analysis of presence and dissemination of ARGs in small scale poultry slaughter houses was the main objective of the study for which a comparative genomic analysis of antimicrobial resistance genes (ARGs) and mobile genetic elements (MGEs) in gut microbiome of broiler and household chicken was performed.
Methods: An array of 52 qPCR primers targeting 16S rRNA gene, ARGs and MGEs was used in the experiment to analyze the abundance and diversity of genes in collected samples. The clustering and correlation among ARGs and MGEs was also checked to determine the dissemination of ARGs to other non-resistant environmental and/or clinical bacteria.
Results: A total of 22 ARGs and 7 MGEs were detected in all samples. The detection frequency of specific gene classes and diversity of genes was found to be higher in broiler chickens than household chickens. *Sul1* was the most abundant ARG with the highest percent relative abundance (2.4 %). Percent relative abundance of selected ARGs in broiler chickens was found to be significantly ($p<0.05$) greater than household chicken samples. A significant linear correlation ($R2=0.89$) was found between relative abundances of *int1* and total ARGs.
Conclusion: The study has provided an effective assessment and potential insight in the role and potential risk of small scale poultry slaughter houses with the perspective of antimicrobial resistance. Broiler chicken mostly raised in large scale commercial farms are usually located in outskirts of communities. Their transport pose a risk in the introduction of exogenous ARGs in the environment since such small scale poultry slaughter houses are distributed widely throughout the country.
Figure 1: Heat Map showing the ARGs and MGEs detected in 6 tested samples. Relative abundance (log transformed) was used for the formation of heat map. Complete linkage clustering is showing that the sul genes are clustered with many MGEs including int1.
Figure 2: Sum of percent relative abundances of ARGs and MGEs in two chicken types. (*) shows that the difference is significant at 95 % confidence of interval. Error bars are showing the standard deviations of the three biological replicates.

Fig. 1

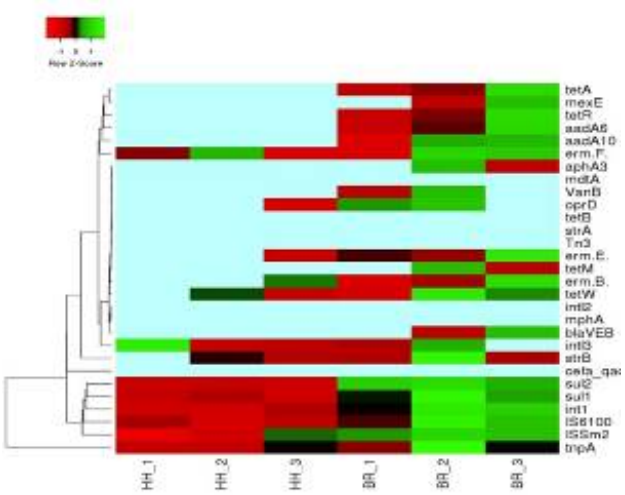
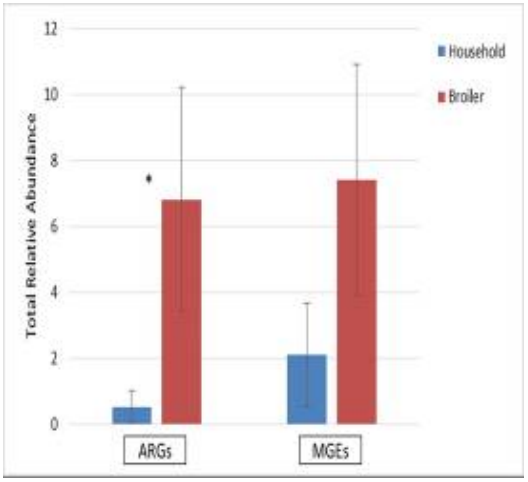


Fig. 2



P 31

Bloodstream bacterial pathogens and their antibiotic resistance patterns in hospitalised patients at the Yaounde University Teaching Hospital

B. William Abange[1], H. Gonsu[1], N. Anna Longdoh[1], C. Betbeui Anicette[1], T. Kwenti Emmanuel[1], A. Aminkeng[1], A. Essomba[1]
[1]Yaounde University Teaching Hospital , Yaounde, Cameroon

Background: Rapid and accurate diagnosis of bacterial bloodstream infection is strategic for the survival of the patient.
Methods: We carried out a cross sectional study on hospitalised patients suspected of bloodstream infections from May to June 2017 at the Yaounde University Teaching Hospital. Blood blood culture were collected from 154 patients in four different units; neonatal unit (58), pediatric ward (56), surgical ward (22) and emergency ward (18). We used the conventional classical gallery to identify the different positive culture/isolates then later using the Kirby-Bauer disc diffusion method for antimicrobial susceptibility testing.
Results: Out of the 154 patients enrolled, 45(29.22%) were positive for blood culture. 37 (82.22%) of these bloodstream infections were hospital acquired, with higher prevalence at neonatal unit 19/45 (42.22%). We identified more Gram positive cocci 30(66.67%); coagulase negative Staphylococcus 18(40%), and *Staphylococcus aureus* 12(26.67%) and Gram negative bacilli 15(33.33%); *Klebsiella pneumonia* 7(15.56%), *Enterobacter spp.* 6(13.33%), *Escherichia coli* 1(2.22%) and *Citrobacter spp.* 1(2.22%). Gram positive were resistant to cefoxitine 53.33%, amoxicillin (50%), tobramycine (33.33%), gentamycin (26.67%), vancomycin (23.33%), erythromycin (20%), clindamycine (20%), and lincomycine (20%), amox + clav (16.67%). No D-zone detected. Our study registered 53.33% MRSA and 33.33% VRSA. Gram negative bacilli-enterobacteriaceae were more resistant to fluoroquinolones; (ofloxacine (53.33%), ciprofloxacine (40%), nalidixic acid (33.33%)), the beta lactamines (Cefotaxime (40%), amox + clav (33.33%), cefuroxime (26.67%) and imipenem (6.67%)) and the aminoglycosides (gentamicine 20%).
Conclusion: Our study therefore revealed that bloodstream infection was hospital acquired, and caused by Gram positive cocci. Neonatal unit was mostly implicated. MRSA remain a big problem in our context and emergence of VRSA. Enterobacteriaceae produced increased resistant rate to fluroquinolones and few beta lactamases producing strains and one registered strain producing carbapenemases.
Key words: Bloodstream infections, hospital acquired infection, bacteria pathogens and antibiotic resistance.

Horizontal gene transfer, mobile genetic elements and antibiotic resistance

P 32

Spatial patterns of antibiotic resistance during bacterial range expansions

A. Worrich[1], T. Banitz[2]
[1]*UFZ - Helmholtz Centre for Environmental Research, Environmental Microbiology, Leipzig, Germany*
[2]*UFZ - Helmholtz Centre for Environmental Research, Ecological Modeling, Leipzig, Germany*

Antibiotic resistance accounts for hundreds of thousands of deaths each year and is recognized a major global health threat (WHO 2014). Conventionally, antibiotic resistance was a concern mainly in clinical settings, but more recently also the role of the environment as an important source and dissemination route of antibiotic resistance has been taken into account. However, our understanding of the mechanisms behind persistence and dissemination of antibiotic resistance within bacterial communities is still limited. Resistance is often coupled to increased fitness costs. Hence, there should be selection against such deleterious mutations in antibiotic-free environments, unless other factors favor their maintenance or evolution makes the fitness costs negligible. Empirical and theoretical findings show that the fate of deleterious mutations depends strongly on their position in expanding populations. Especially at the expansion front, they can reach high densities. Using experimental model systems free of antibiotics, we found that resistant bacteria persisted only in spatially-structured systems allowing for range expansions. Under well-mixed conditions, however, they rapidly went extinct. The underlying mechanism is the accumulation of antibiotic-resistant bacteria at the front, but not in the core, of expanding populations. Our findings suggest that spatially structured, dynamic habitat conditions, in which bacterial populations repeatedly spread into vacant areas, can be a key driver of the overall persistence of antibiotic resistance in the environment.

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No publication allowed.

P 34

Dominance of an unclassified Betaproteobacteria lineage in the spring waters of the Buda Thermal Karst System (Hungary)

D. Anda[1,2], A. Szabó[1], T. Felföldi[1], A. Borsodi[1,2]
[1]*ELTE Eötvös Loránd University, Department of Microbiology, Budapest, Hungary*
[2]*MTA Centre for Ecological Research, Budapest, Hungary*

The Buda Thermal Karst System (BTKS) is part of the carbonate reservoir system of the Transdanubian Central Range. One of the main discharge zones of the BTKS regional fluids can be found in Budapest where thermal waters with different origins and temperatures outcrop. This thermal karst environment provides diverse habitats for microorganisms having planktonic or biofilm forming lifestyles. Recently, microbiological studies have been carried out with respect to the BTKS which is part of the Hungary"s largest karst reservoir. These studies have already led to several new results, however, these studies focused mainly on the formation of biofilm in connection with the hypogenic karstification. Between 2015 and 2018, water samples were collected in the Buda Thermal Karst System to explore the community structure of the discharging waters. For the identification of bacteria primers specific for the V3-V4 region of the 16S rRNA gene were used and amplicons were analyzed by pyrosequencing. The members of the unclassified Betaproteobacteria were found almost exclusively during the analysis of the water samples belonging to the Southern System of the BTKS. It was even more interesting that only one OTU was present in the samples. The OTU showed the highest sequence matching with bacterial species characterized by sulfur oxidation metabolism.
The work of Dóra Anda was supported by the ÚNKP-18-3-III-ELTE-597 New Excellence Program of the Ministry of Human Capacities. This research was supported by the Hungarian Scientific Research Fund (NKFI) Grant NK101356.

P 35

Effects of precipitation regime and N-availability on the dynamics of plant-microbial and soil biogeochemical cycling responses to rewetting

I. Engelhardt[1], P. Niklaus[2], L. Philippot[1], F. Bizouard[1], M. C. Breuil[1], D. Bru[1], F. Deau[1], R. Barnard[1]
[1]*INRA Dijon, Agroecology, Dijon, France*
[2]*University Zurich, Zurich, Switzerland*

Introduction. Shifts in the frequency and magnitude of rain events (precipitation regime) associated with climate change may affect plant morphological and physiological strategies as well as soil microbial activity.
Objectives. The objective of this study was to determine i) how precipitation history shapes the response dynamics of soil bacterial and fungal communities to rewetting, as well as plant-microbial competition for N, and ii) how the N status of the system may modulate the effect of precipitation regime.
Materials & methods. The legacy effects of 12 weeks of contrasting precipitation (frequent or infrequent watering, equal total water input) and N inputs was assessed in soil mesocosms on wheat plants, soil microbial communities and N cycling. The legacy effects on the response to rewetting of potentially active (rRNA-based) bacterial and fungal communities was then documented over 29h, by sequencing phylogenetic marker genes, and following the dynamics of plant-microbial coupling and competition for N using 13C-CO2 and 15N-NH4 labeling.
Results. Precipitation and N input history had contrasting effects on plant physiology, fungal:bacterial ratio, microbial community composition and plant-microbial coupling (reduced C transfer to microbes), but showed no effect on microbial response to rewetting. After an initial short-term response, potentially active soil microbial communities changed little, regardless of precipitation or N input history. Upon rewetting, microbes outcompeted plants for N but plant competitiveness increased over time, in particular after a history of more favorable conditions. Soil CO2 efflux upon rewetting was higher in systems with a history of frequent precipitation inputs and was not modulated by N availability.
Conclusion. We suggest that by altering C and N dynamics between plants and soil microbes and reducing soil C sequestration potential, more extreme fluctuations in soil moisture may affect future ecosystem functioning.

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Carbon source concentration shifts bacterial interactions in snow microcosms

B. Bergk Pinto[1], A. Holland[2], A. Anesio[3], M. Tranter[2], T. M. Vogel[1], C. Larose[1]
[1]*Université de Lyon, Laboratoire Ampère - Environmental Microbial Genomic Group, Ecully, France*
[2]*University of Bristol, Bristol, United Kingdom*
[3]*Aarhus University, Department of Environmental Science, Roskilde, Denmark*

Carbon sources in snow, such as organic acids, vary considerably in concentration during seasonal changes in the Arctic. Our previous field observations led to the concept that these organic acid concentration changes have feedbacks with the bacterial community structure and interactions. In order to test this hypothesis, snow microcosms were initiated under laboratory conditions at -5°C. These microcosms varied only in their initial organic acid concentrations. The first set of microcosms were amended with water as a control and the second set was amended with acetate. Over one-month, destructive sampling occurred every two days. We tracked the bacterial composition of snow communities by 16S rRNA gene sequencing and the potential biological functionality by metagenomics sequencing. In parallel, the acetate concentration in the snow microcosms was measured. We assessed a surrogate level of competition within these communities by following changes in antibiotic resistance gene abundance. We also tracked plasmid backbone genes and built positive co-variance networks of gene expression for the 16S rRNA gene based taxonomy to evaluate the level of bacterial cooperation. We observed a significant difference between the control and amended time series samples in terms of surrogates of bacterial interactions. These results suggest that bacterial interactions are influenced by changes in the availability of acetate (and possibly other organic acids) concentrations in the snow.

P 37

Do bacteria shape the soil structure by producing EPS?

A. Bettermann[1], J. Zethof[2], C. Vogel[2], B. Rühmann[3], V. Sieber[3], K. Kalbitz[2], K. Smalla[1]
[1]*Julius Kühn-Institut - Federal Research Centre for Cultivated Plants (JKI), Institute for Epidemiology and Pathogen Diagnostics, Braunschweig, Germany*
[2]*Technische Universität Dresden, Institute of Soil Science and Site Ecology, Dresden, Germany*
[3]*Technical University of Munich, Chair of Chemistry of Biogenic Resources, Straubing, Germany*

Question: Extracellular polymeric substances (EPS) are considered to be important drivers of soil aggregation and stability. Our aim was to identify relationships between soil bacterial communities, vegetation and spatial distribution of EPS and their influence on soil aggregation.
Methods: At two semi-arid field sites in Spain (Almería Province), soil was sampled in a distance gradient around the stem of *Anthyllis cytisoides* and *Macrochloa tenacissima* tussock. Soil suspensions were plated onto R2A medium to determine colony forming units and to isolate randomly picked bacterial colonies that were screened for exopolysaccharide production. Positive strains were identified by sequencing of their 16S rRNA genes. The composition of the bacterial communities was assessed by denaturing gradient gel electrophoresis (DGGE) and Illumina sequencing of 16S rRNA genes amplified from total microbial community DNA.In addition, stability of microaggregates and wettability of the soil samples were assessed.
Results: Independently of the site, DGGE revealed differences in the bacterial communities depending on plant species and distance to the stem. The cultivation-based approach yielded 45 exopolysaccharide producers belonging to the phyla Actinobacteria, Firmicutes, Bacteroidetes and Proteobacteria. First results show, that *Macrochloa* enhanced microaggregate stability independently of lithology. Also soil wettability around *Macrochloa* vegetation was higher compared to *Anthyllis* at either field sites. Furthermore, wettability was positively linked to the EPS – saccharide content.
Conclusions: In summary our data suggest that the plant species influences the soil bacterial community composition as well as aggregation. Currently ongoing Illumina sequencing will provide further insights into the composition of soil and rhizosphere bacterial communities which will help to better understand the complex soil-microbiota-vegetation relationship.

P 38

Dynamics of methane-cycling microbial communities across seasons in Amazonian floodplains sediments

J. Brandão Gonitjo[1], F. da Silva Paula[1], A. Monteiro Venturini[1], S. Mui Tsai[1]
[1]*Center of Nuclear Energy in Agriculture, University of São Paulo, Cell and Molecular Biology Laboratory, Piracicaba, Brazil*

The Amazonian floodplains are dynamic ecosystems of great importance for the regional hydrological and biogeochemical cycles, and with a possible significant contribution for the global carbon balance. Although some similarities in key functional guilds may be shared across floodplains areas, unique geochemical factors may drive the microbial community composition and, consequently, affect CH_4 emissions. Here we investigated the diversity and abundance of archaeal and bacterial communities linking to CH_4 emissions and consumptions potentials in sediments from three Amazonian floodplains and a primary forest soil, in the Eastern Amazon region, Brazil. To this end, we performed samplings in the wet and dry seasons and used high-resolution sequencing of archaeal and bacterial 16S rRNA genes, as well as real-time quantitative PCR of *mcr*A and *pmo*A genes, targeting methanogens and methanotrophs, respectively. In both seasons, floodplains sediments showed higher diversity of Archaea and Bacteria compared to forest soils. There was a significant difference in the composition of methane-cycling archaeal and bacterial communities among the sites, but no difference between wet and dry seasons, indicating the importance of the environmental characteristics of each area. Euryarchaeota and Crenarchaeota (Bathyarchaea class) archaeal phyla, which contain methanogenic taxa, were dominant in the floodplains, and Methanobacteriaceae was the main methanogenic family. In contrast, the Beijerinckiaceae family was the most abundant group of methanotrophic bacteria. The Rokubacteria phylum (NC10 class), related to the bacterial anaerobic oxidation of CH_4, were found to have a significantly higher relative abundance during the wet season in floodplain areas. In both seasons, the sediment samples presented higher abundance of methanogens, as assessed by *mcr*A copy number, when compared to methanotrophs (*pmo*A). However, *mcr*A:*pmo*A ratio was higher in the wet season. The results suggest that CH_4 oxidation of can be potentially performed by anaerobic processes in the wet season, although in the dry season the drainage of the floodplains may promote an increase in the methanotrophs by methanogens ratio, which might be related to the aerobic CH_4 oxidation in Amazonian floodplains.

Support: FAPESP, CNPq and CAPES.

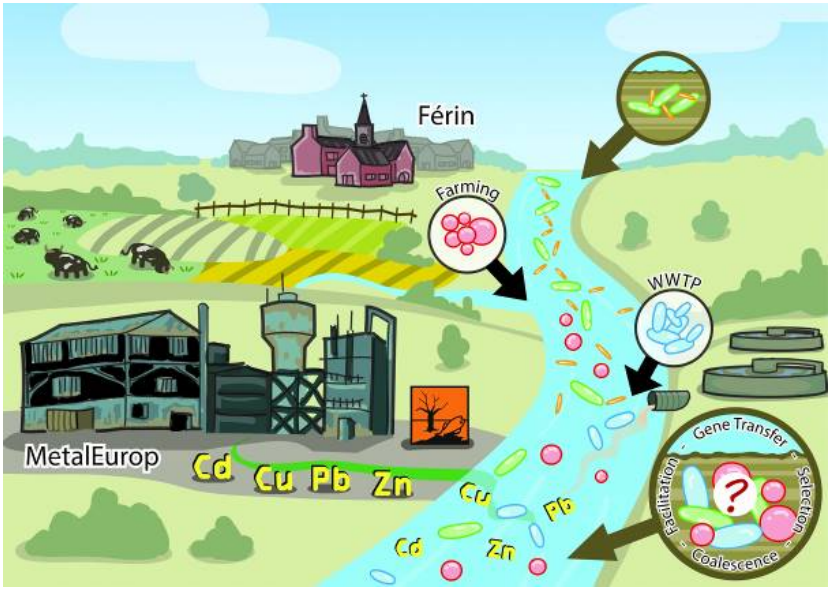
P 39

Deciphering strategies of a river-sediment microbial community to cope with anthropogenic metal contamination

V. Cyriaque[1], S. Jacquiod[2], L. Riber[3], A. Géron[1,4], W. Abu Al-Soud[5], D. Gillan[1], S. J. Sørensen[5], R. Wattiez[1]
[1]*UMONS, Proteomics and Microbiology, Mons, Belgium*
[2]*INRA Centre Dijon, UMR 1347, Agroécologie, Dijon, France*
[3]*University of Copenhagen, Section for Functional Genomics, Copenhagen, Denmark*
[4]*University of Stirling, Division of Biological and Environmental Sciences, Stirling, United Kingdom*
[5]*University of Copenhagen, Section of Microbiology, Copenhagen, Denmark*

Metal contamination poses biotoxicity and bioaccumulation issues, affecting both abiotic conditions and biological activity in ecosystems. For a century, the MetalEurop foundry released zinc, copper, cadmium and lead directly into the river "la Deûle", resulting in a 30-fold increase in metal concentrations in downstream sediments compared to upstream. We used an integrative approach coupling *in situ* sequencing of both DNA and RNA, Horizontal Gene Transfer analysis (HGT), and microcosm monitoring in order to fully understand the mechanisms driving community adaptation to metal pollution. We applied the ecological concept of Functional Response Groups (FRGs) to decipher the adaptive tolerance range of the sediment communities through characterization of microbial strategists, revealing *in situ* differences in diversity and composition. Deeper HGT analysis using qPCR and *in vitro* conjugation assays coupled to Fluorescence Activated Cell Sorting (FACS) revealed the presence of an enriched native pool of conjugative plasmids in the polluted sediments, highlighting their importance for long-term adaptation of the community facing metal contamination. Furthermore, *in vitro* microcosms analysis with upstream non-polluted sediments challenged with metals and daily supplied of fresh river water allowed to monitor the short-term impact of metal pollution on the microbial community over 6 months in controlled condition using 16S rDNA amplicon sequencing and the ecological concept of Time Response Groups (TRGs). Altogether, our integrative study reinforced the notion that microbial strategies were established to face the metal pollution, such as facilitation processes, microbial community coalescence and HGT, leading to an unexpected higher microbial diversity.

Fig. 1



P 40

Bacterial communities from extreme environments – Vulcano Island (Aeolian Islands, Sicily, Italy)

S. Del Duca[1], C. Fagorzi[1], C. Chiellini[2], S. Venturi[3], F. Tassi[3], L. M. Castronovo[1], S. Chioccioli[1], R. Fani[1]
[1]*University of Florence, Department of Biology, Sesto Fiorentino, Italy*
[2]*University of Pisa, Department of Agriculture Food and Environment, Pisa, Italy*
[3]*University of Florence, Department of Earth Science, Florence, Italy*

Introduction: Volcanoes represent extreme environments for life. Nonetheless, they harbour interesting bacterial communities. Vulcano Island (Aeolian Islands, Sicily) presents an intense fumarolic activity and widespread soil degassing, fed by variable amounts of magmatic gases (dominant at La Fossa crater) and hydrothermal fluids (dominant at Levante Bay).
Objectives: The aim of this study is to analyse the microbial communities from the different environments of Vulcano Island and to evaluate their possible correlation with the composition of the gas emissions.
Materials and methods: Microbial analyses were carried out on (i) soils from La Fossa crater and Levante Bay, (ii) waters and sediments collected near a fumarole in Levante Bay and in a non-emissive zone on the other side of the island, (iii) pioneer plants from both La Fossa crater, where high CO_2 emissions occur, and Levante Bay, in a non-emissive soil. Cultivable bacteria have been isolated and identified, and total DNA was extracted from all the samples and sequenced through Illumina MiSeq platform.
Results: Preliminary analyses revealed that all the volcanic soil samples are characterized by a lower bacterial titer respect to non-volcanic ones (about five orders of magnitude). The tissues of the pioneer plants showed higher bacterial titer when the plant was sampled close to the emission respect to plants grown in non-emissive soil. RAPD screening was performed on all the isolates, revealing the presence of different strains. The 16S rRNAs of members of different RAPD haplotypes were sequenced, revealing a predominance of the genus *Bacillus* in the emissive area.
Conclusion: Correlation between data obtained from the analyses of microbial communities and the composition of gas samples collected from the same sites will be evaluated. A positive correlation could suggest a possible role of the microbial communities in affecting the composition of the gas compounds.

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Eco-evolutionary dynamics of ATP-producing pathways in microbial communities

J. Dolinsek[1,2], D. Johnson[1]
[1]*Swiss Federal Institute of Aquatic Science and Technology (Eawag), Department of Environmental Microbiology, Dübendorf, Switzerland*
[2]*Kompetenzzentrum Holz GMBH (Wood K plus), Department for Wood Chemistry and Biotechnology, Linz, Austria*

Introduction: Multi-step ATP-producing pathways occur across all domains of life, where a series of transformations converts a primary substrate via one or more intermediates into end products. We hypothesize that the extracellular accumulation of intermediates from these pathways could promote biodiversity within microbial communities. If the intermediates accumulate outside the cell to growth-supporting concentrations, than this creates a niche for another genotype to invade into, where the new genotype specializes at transforming the intermediates. Distinct genotypes could then coexist and engage in a cross-feeding interaction. Further, the emergence of cross-feeding may also modify the environment and thus the selection pressures. This could result in eco-evolutionary dynamics that affect the fate of emerged interactions.
Objectives: Our goal was to establish such cross-feeding in a synthetic microbial community. If successful, our next task was to test its fate in the face of the eco-evolutionary forces.
Materials and Methods: We used a previously established experimental cross-feeding microbial community to test whether a specialist (*Pseudomonas stutzeri*, Δ*narG*) can indeed invade into and coexist with a generalist (*P. stutzeri*, wild type) via a cross-feeding interaction, where the generalist primarily transforms nitrate into a growth-supporting intermediate nitrite that accumulates outside the cell while the specialist transforms the intermediate.
Results: In our synthetic communities, both genotypes converged toward stable frequencies, regardless of the starting frequencies. Over time, the generalist evolved a streamlined metabolism where intermediates no longer accumulated and outcompeted the specialist. This response only occurred when the specialist was initially absent or rare.
Conclusions: Our results indicate that during colonization, early transient conditions can have major impacts on diversity, and that in some cases competition may slow evolutionary responses.

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Building up and growing a river model bacterial community in different media suggests the existence of positive interactions within the community

L. Goetghebuer[1], M. Bonal[1], K. Faust[2], P. Servais[1], I. George[1]
[1]*Université Libre de Bruxelles, Ecology of Aquatic Systems, Brussels, Belgium*
[2]*KULeuven, Laboratory of Molecular Bacteriology, Leuven, Belgium*

Twenty heterotrophic bacterial strains were isolated from the same river and characterized for their metabolic potential in BIOLOG plates. Despite partly overlapping metabolism among the strains, 43% of the carbon sources tested were better or solely used by the 20-strain mixed community. Then the dynamic of the community was followed in R2 broth and in autoclaved river water medium for 27 days in batch experiments. At an early stage of incubation, a fast-growing specialist strain, Acinetobater sp., dominated the community in both media. Later on, the community composition in both media diverged but was highly reproducible across replicates. In R2, several strains previously reported to degrade multiple simple carbon sources prevailed. In autoclaved river water, the community was more even and became dominated by several strains growing faster or exclusively in that medium. Those strains have been reported in the literature to degrade complex compounds. Their growth rate in the community was 1.5 to 7 fold greater than that observed in monoculture. Furthermore those strains developed simultaneously in the community. Together, our results suggest the existence of cooperative interactions within the community.

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Illuminating the black box – metabarcoding reveals divergent patterns of biological and functional diversity across the soil biosphere

P. George[1,2], D. Lallias[3], S. Creer[1], F. Seaton[1,2], J. Kenny[4], R. Eccles[4], R. Griffiths[2], I. Lebron[2], B. Emmett[2], D. Robinson[2], D. Jones[1]
[1]Bangor University, School of Natural Sciences, Bangor, United Kingdom
[2]Centre for Ecology & Hydrology, Bangor, United Kingdom
[3]INRA, GABI, Jouy-en-Josas, France
[4]University of Liverpool, Liverpool, United Kingdom

Soil biota accounts for nearly a quarter of global biodiversity and is vital to ecosystem services such as nutrient cycling, soil fertility and primary production. Yet life in soil has commonly been considered a "black-box" until recent advenaces in molecular ecology, such as metabarcoding. There is a growing push to study the entirety of belowground biodiversity across large ecological scales to understand how habitat and soil properties interact to shape these communities. There is growing evidence that microbial and animal components of soil communities respond differently to shifts in soil properties and land uses. However, such evidence comes from largely homogeneous landscapes and often involves mixing modern molecular and traditional taxonomic methodologies to assess complete soil communities. We compared richness and community composition of major fractions of soil biota using a metabarcoding analysis of 436 locations across diverse temperate ecosystems representative of the UK. We also assessed functional diversity of soil fungi using complementary ITS1 and 18S datasets. Our results indicate that belowground richness of animal and microbial (bacteria, archaea, fungi, and protists) cohorts follow divergent trends across an intensity gradient of heterogeneous land uses, whereas β-diversity does not. Richness of soil animals was governed by intense land use and unaffected by soil properties, whereas microbial richness was largely driven by abiotic environmental properties across land uses. In addition, we demonstrate that richness of archaea does not conform to the same trends as other microbes. We also found divergent trends of richness of fungal functional groups, across land uses between the ITS1 and 18S datasets. Richness of saprotrophs and symbiotriophs was most affected by primer choice. Our findings demonstrate the effectiveness of a standardized metabarcoding technique in capturing meaningful shifts soil biodiversity. However, primer choice may impact results in the context of assessing functional diversity. In sum, our findings have extended our understanding of how soil communities are shaped by and respond to environmental factors and land use at the regional-scale, thereby providing a clearer understanding of macroecological processes governing the belowground biosphere.

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The lingocellulose degrading potential of a thermophilic microbial consortium isolated from a thermal springs field in northern Greece

D. Hatzinikolaou[1], P. Glekas[1], A. Galanopoulou[1], P. Avramidis[1]
[1]National and Kapodistrian University of Athens, Department of Biology, Zografou, Greece

Introduction: Production of biofuels from residual lignocellulosic substrates has a particularly high potential as an alternative source of energy. The process involves the prior enzymatic decomposition of the recalcitrant lignocellulosic matrix, followed by the fermentation of the resulting sugar monomers. Industrial deconstruction of biomass, however, is an exothermic process, the cost of which could be decreased significantly by the use of hyperthermophilic enzymes and microorganisms.
Objectives: Within the above framework, the aim of this study was to isolate and evaluate novel thermophilic microorganisms with lignocellulose degrading potential from an environment where the temperatures remain above 60°C all year round.
Materials and Methods: An active thermophilic lignocellulose degrading microbial consortium was bred from high-temperature, lignocellulose rich, sediments by successive subcultivation in cellulosic substrates under aerobic conditions. Strains were phenotypically/molecularly characterized and their cellulolytic and xylanolytic enzymatic system was evaluated.
Results: A stabilized microbial consortium was obtained, that showed efficient degradation activity on potential biorefinery cellulosic substrates, including alkali pre-treated corn stover and phosphoric acid swollen cellulose. The consortium was structurally stable with the co-existence of two major aerobic bacterial genera, namely *Rhodothermus* and *Geobacillus*, along with anaerobic and uncultured bacteria. The major lignocellulolytic activities including endo-glucanase, xylanase, and exo-glucanase were present in the crude supernatant of the microbial cultures. Electrophoretic and zymogram analysis on the corresponding activities provided indications of horizontal gene transfer among the consortium members.
Conclusion: Our study was able to characterize the culturable aerobic thermophilic microbial diversity related to biomass degradation of a pristine thermal springs field in Central Greece. Using two rationally selected lignocellulosic substrates, microbial community analysis has demonstrated that the corresponding microbial consortia are composed of a few dominant phylotypes that consist of both well-studied and novel biomass-deconstructing bacteria. These consortia are amenable to detailed genomic and proteomic investigations in order to elucidate the bacterial glycoside hydrolases involved in lignocellulosic biomass deconstruction.

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From pine to pasture – investigating changes in microbial composition and functional potential associated with land use conversion

S. M. Hermans[1], M. Taylor[2], G. Grelet[3], F. Curran-Cournane[4], K. Handley[1], G. Lear[1]
[1]*University of Auckland, School of Biological Sciences, Auckland, New Zealand*
[2]*Waikato Regional Council, Waikato, New Zealand*
[3]*Manaaki Whenua – Landcare Research, Lincoln, New Zealand*
[4]*Auckland Regional Council, Auckland, New Zealand*

Bacterial communities have long been appreciated as crucial members of the soil ecosystem and are known to be sensitive to environmental changes and perturbations. However, there is a limited understanding of how changes in land use alter the composition and functional roles of bacterial communities, and to what extent current communities reflect historic land uses. Through 16S rRNA gene amplicon and shotgun metagenomic sequencing, we characterized the structure and functional potential of soil bacterial communities after land use conversion. Sites in New Zealand, converted from pine plantations to dairy pasture, were sampled five and eight years post-conversion. The bacterial community composition and functions at these sites were compared to long term dairy pasture sites and plantation (*Pinus radiata*) forest sites. The bacterial communities at the converted sites differed significantly in composition and functional potential from the long-term dairy and plantation sites (P = 0.001). This indicates that the conversion events have resulted in distinct bacterial communities. These converted pine-to-diary communities on average were significantly more similar in composition and function to the long-term dairy sites, and showed increased similarity to the long-term communities as time increased since conversion (P = 0.001). Differences in composition between the three sample groups were related to several environmental variables, including pH, C:N, Olsen P, NO3-N, and heavy metal concentrations. Functional categories related to nutrient metabolism, stress response and antibiotic resistance were found to differ between the three types of land. Overall, the results from this study provide important revelations about how changing anthropogenic pressures alter the composition of microbial communities, and the functions they provide. Understanding these interactions will allow us to better determine how major land use changes alter soil ecosystems.

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Meta-omics investigation of soil communities along a natural climatic gradient in the Finnish Arctic tundra

J. Hultman[1], S. Viitamäki[2], I. Stelmach Pessi[2], A. M. Virkkala[1], M. Luoto[1]
[1]*University of Helsinki, Department of Geography, Helsinki, Finland*
[2]*University of Helsinki, Department of Microbiology, Helsinki, Finland*

Climate change is affecting the arctic dramatically as the warming is fastest in higher latitudes. Previously frozen ground is thawing and releasing substantial quantities of carbon which microbes can decompose. Microbes mineralize the carbon fraction and convert it to carbon dioxide and methane. Climate change models estimate that C released from thawing arctic permafrost can represent the largest future transfer of C from the biosphere to the atmosphere. In addition to temperature, also oxygen and moisture, among many others, affect the microbial activity. Understanding the drivers of arctic soil communities and especially how the microbial activity changes with warming and resulting changes in soil characteristics is needed. However, this kind of information in lacking for the arctic soil microbial communities.
We have analyzed over 100 soil plots from a large field site in Scandinavian low arctic with environmental gradient of microclimatic conditions. Metagenomic approach was used to create a database of microbial genes and metagenome assembled genomes (MAGs) found within this gradient. As DNA can originate from dead or dormant cells, we utilized RNA sequencing to create comprehensive understanding on the metabolic activities of microbes within this fine-scale climatic variation.
Communities were dominated by Proteo-, Actino- and Acidobacteria. Recovered genomes were related to common soil taxa such as the nitrogen-fixing *Bradyrhizobium* (Alphaproteobacteria) and *Granulicella* (Acidobacteria), while others appear to represent distinct lineages distantly related to Candidatus *Koribacter* and Candidatus *Solibacter* (Acidobacteria).
The metatranscriptomic results show activity of genes involved in the breakdown of amino acids, carbohydrates and lipids. In addition, stress related functions were actively expressed in these harsh arctic conditions. Further phylogenomic and functional analyses are being currently carried out. These will allow us to investigate the metabolic potential of the soil communities in more detail, for example, regarding the importance of carbon, nitrogen and sulphur cycles in the tundra ecosystem along fine-scale climatic variation.

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Two Chloroflexi lineages independently evolved the ability to persist on atmospheric hydrogen and carbon monoxide

Z. Islam[1], P. Cordero[1], J. Feng[1], Y. J. Chen[1], S. Bay[1], R. Gleadow[1], T. Jirapanjawat[1], C. Carere[2], M. Stott[3], E. Chiri[1], C. Greening[1]
[1]*Monash University, School of Biological Sciences, Clayton, VIC, Australia*
[2]*University of Canterbury, Department of Chemical and Process Engineering, Christchurch, New Zealand*
[3]*University of Canterbury, School of Biological Sciences, Christchurch, New Zealand*

Most aerobic bacteria exist in dormant states within natural environments. In these states, they endure adverse environmental conditions such as nutrient starvation by decreasing metabolic expenditure and using alternative energy sources (Lennon and Jones 2011). In this study, we investigated the energy sources that support persistence of two aerobic thermophilic strains of the environmentally widespread but understudied phylum Chloroflexi (Delgado-Baquerizo et al 2018, Mehrshad et al 2018, Thompson et al 2017). A transcriptome study revealed that *Thermomicrobium roseum* (class Chloroflexia) extensively remodels its respiratory chain upon entry into stationary phase due to nutrient limitation. Whereas primary dehydrogenases associated with heterotrophic respiration were downregulated, putative operons encoding enzymes involved in molecular hydrogen (H_2), carbon monoxide (CO), and sulfur compound oxidation were significantly upregulated. Gas chromatography and microsensor experiments showed that *T. roseum* aerobically respires H_2 and CO at a range of environmentally relevant concentrations to sub-atmospheric levels. Phylogenetic analysis suggests that the hydrogenases and carbon monoxide dehydrogenases mediating these processes are widely distributed in Chloroflexi genomes and have probably been horizontally acquired on more than one occasion. Consequently, we confirmed that the sporulating isolate *Thermogemmatispora sp.* T81 (class Ktedonobacteria) also oxidises atmospheric H_2 and CO during persistence, though further studies are required to determine if these findings extend to mesophilic strains. This study provides axenic culture evidence that atmospheric CO supports bacterial persistence and reports the third phylum, following Actinobacteria and Acidobacteria (Greening et al 2015, Liot and Constant 2016), to be experimentally shown to mediate the biogeochemically and ecologically important process of atmospheric H_2 oxidation. This adds to the growing body of evidence that atmospheric trace gases are dependable energy sources for bacterial persistence.

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Novel alkaliphilic and moderately halophilic bacteria harbored by bayonet grass rhizosphere soil in Hungarian soda ponds

J. M. Aszalós[1], A. K. Borsodi[1], T. Szili-Kovács[2], E. Tóth[1]
[1]*ELTE Eötvös Loránd University, Department of Microbiology, Budapest, Hungary*
[2]*Hungarian Academy of Sciences, Institute for Soil Sciences and Agricultural Chemistry, Agricultural Research Center, Budapest, Hungary*

In biotechnology, there is a growing interest in novel species of extremophilic bacteria, due to their ability to produce unusual metabolites that can be utilized in various fields of application. Although several novel species are described every year, there is a great diversity of hitherto uncultured prokaryotes in environments with extreme circumstances. Soda ponds in the Kiskunság National Park, Hungary (including Böddi-szék and Zab-szék) among other soda lakes and soils located usually in warm semi-arid areas, are typical habitats for extremophilic bacteria. Due to the alkaline pH and the low water activity, mainly halophilic and alkaliphilic organisms are present in these environments. Microbial communities associated with different types of vegetation (e.g. *Bolboschoenus maritimus, Puccinellia limosa* or *Tripolium pannonicum*) have been studied in these soda ponds, and the dominance of phyla Firmicutes, Actinobacteria, Proteobacteria and Bacteroidetes was shown. Members of genus *Bacillus* and closely related genera e.g *Anaerobacillus* are common in these environments, and although more than 330 species in *Bacillus* were described to date, only 5 species of *Anaerobacillus* are known, nevertheless several novel lineages could be harbored in nature. Alkaliphilic and moderately halophilic bacterial strains were described from rhizosphere soil collected from Kiskunság National Park and examined by polyphasic taxonomic methods.
The work of Júlia Aszalós was supported by the ÚNKP-18-3-IV-ELTE-94 New Excellence Program of the Ministry of Human Capacities

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Addressing the impact of temperature on *Vibrio harveyi* adaptation in the time of climate change

V. Kaberdin[1], I. Montánchez[1], E. Ogayar[1], A. H. Plágaro[1], A. Esteve-Codina[2], J. Gómez-Garrido[2], M. Orruño[1], I. Arana[1]
[1]*University of the Basque Country, Immunology, Microbiology and Parasitology, Leioa, Spain*
[2]*CNAG-CRG, Centre for Genomic Regulation (CRG), Barcelona Institute of Science and Technology (BIST), Barcelona, Spain*

Vibrio species represent one of the most diverse genera of bacteria known for their ubiquitous presence in natural aquatic systems. Although recent studies have suggested a causal link between the ongoing spread of Vibrio-associated diseases and global warming, the adaptation mechanisms underlying the ability of *Vibrio* species to cope with climate change and successfully spread and colonize new habitats are still poorly understood.
The main objective of this study was to use *Vibrio harveyi* as a model organism and learn more about temperature-dependent responses and putative mechanisms this marine bacterium uses to survive in its natural habitats under limitation of nutrients.
Towards this goal, we employed a combination of microbiological assays (survival and viability tests), advanced imaging techniques (high-resolution epifluorescence and electron microscopy) along with omics approaches to analyze adaptive changes that occur in *V. harveyi* during its time-dependent persistence in seawater microcosms at 4°C [**1,2**] , 20°C [**3**] and 30°C [**4**]. While 20oC represented the average temperature of the coastal water of the Biscay Bay during the summer season, 4°C and 30°C closely mimicked the lower and upper limits of sea surface temperatures recorded around the globe.
Analysis of *V. harveyi* adaptation revealed that deprivation of nutrients (starvation) can trigger profound morphological changes leading to reduction of cell size and conversion of rod-shaped bacteria into their coccoid-like variants over a wide range of temperatures (i.e. 4 °C to 30 °C). Moreover, the use of quantitative proteomics and transcriptomics tools enabled to reveal the nature of regulatory factors (e.g. putative transcriptional regulators, essential metabolic enzymes and regulatory sRNAs) playing the major role during *V. harveyi* adaptation.
Our results indicate that, although elevated temperature (i.e. 30 °C) and shortage of carbon potentially limit the cell"s capacity to cope with the stress, they readily enhance expression of many known virulence factors. The latter likely enable *V. harveyi* to escape from adverse environments to nutrient rich, host-pathogen associations, and therefore may facilitate the spread of *Vibrio*-associated diseases in response to global warming.

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Microbial community of the meromictic arctic Lake Bolshie Hruslomeny, North European Russia

V. Kadnikov[1], A. Savvichev[1], I. Rusanov[1], A. Beletsky[1], N. Ravin[1], N. Pimenov[1]
[1]*Research Center of Biotechnology, Moscow, Russian Federation*

Microorganisms are critical to maintaining stratified biogeochemical characteristics in meromictic lakes. The meromictic Lake Bolshie Hruslomeny is located on the shores of the Kandalaksha Bay of the White Sea, North European Russia. This lake, formed from the sea bay and still retaining the subsurface connection with the sea, is meromictic, with a fresh oxygenated upper layer and an anoxic monimolimnion. The monimolimnion of the lake (below 5 meters) is brackish (up to 22 ‰) due to infiltration of seawater and has high concentrations of methane (up to 1.8 mM) and hydrogen sulphide (up to 17.8 mM). The highest rates of methane oxidation and anoxygenic photosynthesis were observed in the chemocline zone. To characterize microbial communities involved in the methane and sulfur cycles, we analysed the compositions of microbial communities at different depths using high-throughput 16S rRNA gene sequencing. Water samples were collected from an upper oxic mixolimnion (0.5–3.25 m), a chemocline layer (3.75 - 4.25 m), and a lower monimolimnion zone (5.25–18.0 m). Microbial community of the mixolimnion contained phototrophic Cyanobacteria, aerobic methanotrophs of the genera Methyloprofundus and Methylobacter, and various groups of heterotrophic bacteria assigned to Actinobacteria, Bacteroidetes, Verrucomicrobia, Alpha- and Gammaproteobacteria. In the chemocline zone green sulfur bacterium Chlorobium phaeovibrioides was the dominant microorganism, and sulfate-reducing lineages of Deltaproteobacteria were found as well. Sulfate-reducers were also found in the monimolimnion layer, but the majority of microorganisms here belonged to various uncultivated lineages of bacteria and archaea. Methanogenic archaea were identified in minor amounts only in the deep layers of the monimolimnion. Known groups of microorganisms capable to perform anaerobic methane oxidation were not found. The obtained results provided insight into the biogeochemical processes in the Lake Bolshie Hruslomeny and arctic lakes in general. The work was supported by the Russian Science Foundation (project 16-14-10201).

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Microbially induced mineral precipitation in serpentinite-hosted alkaline springs of the Voltri Massif, Italy

A. Kamran[1], M. Hoppert[1], K. Sauter[1]
[1]*Universität Göttingen Institut f. Mikrobiologie und Genetik, Göttingen, Germany*

Biomineralization has a wide application, e.g., in the removal and recovery of various metals under the shadow of environmental biotechnology. Biologically induced mineralization such as carbonate precipitation also contributes to the preservation of organic compounds in marine and freshwater sediments**.** These biominerals may be also useful as biomarkers. Microbially induced calcification in terrestrial, highly alkaline springs, however, is not well understood. In this study we investigate the role of complex microbial communities in alkaline (pH 10 -12) serpentinite springs of the Voltri Massif (Italy) and their participation in mineral precipitation. Along with analysis of chemical and mineralogical parameters, environmental DNA was extracted and subjected to analysis of microbial communities based upon next generation sequencing. Mineral precipitates were observed and analyzed by light and transmission electron microscopy. Metagenomics studies, based on analysis of 16S rRNA amplicons were used for the identification of the microbial communities involved in the biomineralization within samples. Multidimensional scaling was used to illustrate differences in taxonomic composition of communities taken from various mineral phases. These investigations point to the relevance of cyanobacteria in for carbonate precipitation in serpentinite springs.

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Functional signatures of the microbiomes of soft corals in health and disease states

T. Keller-Costa[1], R. Toscan[2], J. P. Saraiva[2], A. Lago-Lestón[3], J. Gonçalves[4], U. Nunes da Rocha[2], R. Costa[1]
[1]*Instituto Superior Técnico (IST), Insitute for Bioengineering and Biosciences (iBB), Lisbon, Portugal*
[2]*Helmholtz Centre for Environmental Research (UFZ), Leipzig, Germany*
[3]*Centro de Investigación Científica y de Educación Superior de Ensenada (CICESE), Ensenada, Mexico*
[4]*Centro de Ciências do Mar (CCMAR), Faro, Portugal*

The sustainability of coral reefs bears fundamental implications to the functioning of coastal ecosystems and the biogeochemistry of our planet. Coral-microbiome interactions presumably play a key role in host resilience in a climate change scenario. However, contrary to hard corals, in soft corals, the functional relationship between host health and its symbiotic consortium is yet to be unveiled. Here, we employ a comparative metagenomics approach to uncover the distinct phylogenetic and functional features of the microbiomes of healthy *versus* necrotised soft coral tissue. High-throughput, Illumina metagenome sequencing of seawater (*N*=4), sediments (*N*=3) and of the symbiotic consortium of healthy (*N*=3) and necrotised (*N*=3) *Eunicella gazella*, healthy *Eunicella verrucosa* (*N*=4) and *Leptogorgia sarmentosa* (*N*=3) was performed. 16S rRNA gene-based multivariate analysis revealed separate clustering of healthy corals from diseased corals, seawater and sediment. The genera *Endozoicomonas*, *Pseudoalteromonas* (*Gammaproteobacteria*) and *Mycoplasma* (*Tenericutes*) displayed sharply enriched abundances in healthy soft corals in comparison with diseased specimens and surrounding environments. We found the microbiomes of healthy soft corals enriched with eukaryotic-like repeat motifs (ankyrin and WD40 repeats), transposases and restriction endonucleases as well as detoxification-related glyoxalases and inorganic ion transporters. Genes encoding for heat-shock proteins and enzymes involved in nitrogen fixation and in the Entner-Doudoroff pathway – all more abundant in healthy corals - contributed further to the dissimilarity between the microbiomes of healthy and diseased soft corals. On the contrary, motility and chemotaxis encoding genes augmented strongly in the microbiomes of diseased soft corals, while a prevalently sedentary lifestyle characterized the microbiome of healthy hosts. The analysed microhabitats also showed distinct clustering according to the presence of biosynthetic gene clusters (BGCs). While homoserine lactone BGCs were enriched in the microbiome of necrotic soft corals, non-ribosomal peptide synthases characterised the microbiomes of healthy *Leptogorgia sarmentosa* specimens. In conclusion, this study highlights diverse nutrient cycling pathways, heat-stress response and anti-viral defence mechanisms as well as amenability to microbe-microbe and host-microbe gene transfer as signatures of the microbiome of healthy soft corals.

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Impact of ecosystem changes due to permafrost thaw on potential greenhouse gas emission

C. Keuschnig[1], M. Björkman[2], C. Larose[1], A. Pesqueda[2]
[1]*Ecole Centrale de Lyon, University of Lyon, Environmental Microbial Genomics, Ecully, France*
[2]*University of Gothenburg, Department of Earth Sciences, Gothenburg, Sweden*

Question: Carbon pools in perennially frozen soils have the potential to cause a positive feedback on the global climate. This stored carbon will likely be emitted as greenhouse gases due to microbial activity, as a result of thawing due to currently increasing temperatures. The majority of studies on permafrost thawing focus on carbon-rich organic soils, although 87% of these potentially affected soils are described as mineral soils. We studied the ecosystem change that occurred after irreversible permafrost thaw in some of these mineral soils and its effect on carbon dioxide (CO_2) and methane (CH_4) emissions.
Methods: The two Tussock tundra sites (Latnjajávri = L and Corrvosjávri = C) studied have not had their former permafrost soil frozen for about 15 and 40 years, respectively. Net fluxes of CO_2 and CH_4 and ecosystem respiration were measured bi-weekly during summer 2017, using pre-installed soil collars. Soil samples for DNA extraction and chemical analysis were taken at the same time from surface and deep soil layers. Bacterial, fungal, methanogenic and methanotrophic community structures were analyzed by amplicon sequencing (16S-V4, ITS2, mcrA and pmoA amplicons). Their abundance was estimated by quantitative PCR.
Results: Our data shows a core methanogenic community in deep layers of the L-site, where high emissions of methane were measured. In contrast, the C-site had low methane emissions and a more diverse methanotrophic community in the surface layer than the surface layer at the L-site.
Conclusions: A conceptual model of the ecosystem changes was developed for tundra soils that no longer experience permafrost. The model describes greenhouse gas emission potential and, thus, provides data for the estimation of a feedback effect in climate models.

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Long-term warming impacts moss-associated bacterial community composition and competition in Icelandic heathlands

I. Klarenberg[1,2], I. S. Jónsdóttir[2], O. Vilhelmsson[1]
[1]*University of Akureyri, Akureyri, Iceland*
[2]*University of Iceland, Reykjavík, Iceland*

Mosses contribute substantially to biomass and vegetation cover in northern ecosystems. They play an important role in terrestrial carbon storage, due to their recalcitrant tissues and via interactions with microorganisms. These moss-associated microorganisms also contribute to nitrogen fixation, providing major soil nitrogen input. Mosses can be studied at the scale of the bryosphere, defined as the combined complex of living and dead moss tissue and associated organisms. Current knowledge of the moss associated microbiomes and their role in biogeochemical cycles is sparse. Addressing this knowledge gap is especially important in the light of climate change. Here we investigated the diversity of and network interactions within moss associated bacterial communities under conditions of a warming climate. Samples of the moss *Racomitrium lanuginosum* were collected in warmed and control plots in an Icelandic dwarfshrub heathland. DNA and RNA were co-extracted and 16S rRNA and 16S rDNA amplicons were sequenced with Illumina MiSeq. Multivariate statistical techniques and DEseq2 were used to analyse the resulting OTUs for changes in structure and abundances. Spearman rank correlations between the abundances of OTUs were used to build correlation networks. The richness of the potentially metabolically active (16S rRNA based) communities increased under warming. Within the Cyanobacteria, *Nostoc* decreased in abundance while *Stigonema* became more abundant. Overall, the rRNA:rDNA ratio decreased. Network analysis showed that the amount of negative correlations (mutual exclusions) between OTUs increased with warming. OTUs indicative for either warming or controls, and not indicative OTUs for any treatment were more likely to be connected with OTUs indicative for the same treatment. While the effect of warming on the taxonomic composition of the moss microbiome was subtle, we observed changes in the nitrogen fixing communities and potential metabolic activity. The increase in richness might lead to an increase in niche-overlap and thus increased competition. Connections between OTUs with similar changes in abundance under warming were more likely to be connected in the networks. These OTUs co-oscillate with warming. The increase in negative correlations under warming can counteract these co-oscillations and promote stability. Climate change might thus affect the way bacterial communities interact within the bryosphere in the northern ecosystems.

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At the playground of evolution in the presence and absence of rivals

K. Korzeniowski[1], C. Souffreau[1], J. Gevers[1], S. Mukherjee[1], A. Van Assche [2], B. Lievens[2], L. De Meester[1]
[1]KU Leuven, Biology, Leuven, Belgium
[2]KU Leuven, Microbial and Molecular Systems (M2S), Sint-Katelijne-Waver, Belgium

Introduction: Theory has indicated that absence of species may be a key factor increasing a species" evolutionary divergence to other niches, a process known as "ecological release". While the ecological processes shaping community assembly in bacteria have been extensively studied, we lack a clear understanding of evolutionary mechanisms in this context. Due to the short generation time of bacteria substantial evolutionary change can occur within few generations raising an interest on interactions between evolutionary and ecological processes.
Objectives: The main objective of this study is to get insight into how and to what extent ecological release stimulates rapid evolution of C resource use in bacteria.
Materials and methods: Using a common garden experiment and manipulating community composition (strains/genera in isolation i.e. ecological release, or in the presence of other strains/genera) on an artificial medium composed of 10 carbon sources, we assessed the effects of competitive release on the evolution of C resource use of bacterial strains. We used a total of 25 isolates (5 strains each for 5 genera) that were isolated from ponds in Belgium. This experiment was carried out for 6 weeks, and sampling was performed every week for plating (presence/absence of strains), DNA isolation (community composition) and cryopreservation (for quantifying evolution in re-isolated strains).
Results and conclusions: After 3 weeks of incubation only 3 genera were present on plates, subsequent plating showed only 2 genera present indicating the competitive exclusion of some strains. We noticed a change in colony morphology of *Bacillus* and *Deinococcus* strains after 3 weeks. To investigate potential evolution we will revive bacteria from glycerol stocks and phenotype them to check whether they show changes in growth rates on the same medium compared to the ancestral strains. These results will help us determine the importance of biotic interactions and the degree to which evolution of resource-use can structure bacterial community composition.

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Uncovering the microbiota of the deep-sea sediments from the Southern Gulf of Mexico

A. Lago-Lestón[1], H. López[1], C. Barcelos[1], J. Chong-Robles[1], D. Magdaleno[1], K. Sidón[1], B. Covarrubias[1]
[1]CICESE, Medical Innovation, Ensenada, Mexico

Marine sediments are home to highly abundant and diverse microbial communities, however, due to its vastness, this environment remains still poorly studied and, the Gulf of Mexico (GoM) is not an exception to this reality. GoM is an ocean basin characterized by both shallow and deep-water areas, which can reach depths up to 4,000 m. Although, there are studies describing biological diversity in the GoM, very few of them involve microbial communities from the sediments, and even less from the deepest areas. Furthermore, they are focused mostly in the Northern American area and not in the Mexican exclusive economic zone despite its ecological and economic importance.
In order to establish a baseline of the diversity of microorganisms inhabiting the Southern GoM and to shed some light into the unknown microbial communities of the area, several oceanographic campaigns have been conducted since 2015, as part of a sampling effort doing by the CIGoM consortium. Sediment samples were collected over different years, in different campaigns and at different depths, ranging from 550 to more than 3500 m. Sediment DNA was extracted from different sections of the sediment column and the 16S ribosomal RNA gene was amplified and sequenced on our MiSeq (Illumina) platform and data analyzed with a set of bioinformatic programs including QIIME.
Our results show an unusual archaeal abundance not reported to date, reaching up to 40% of the total prokaryotes community, in particular, those from the deepest locations. This abundance seems to be correlated with depth since it is increased from shallow to deeper zone, but it is stable at the abyssal plain (1500 to 3500m). Besides, it was observed a strong shift on the microbial assemblages along the sediment core. The archaeal diversity was higher and distinct than those from the upper part of the core and, the bacterial abundances changed. Lokiarchaea replaced Thaumarchaea and represented 20% of the total. And in the bacterial group, Planctomycetes and Chloroflexi increased their abundances while Acidobacteria almost disappeared from the bottom of the core (30cm).

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Denitrification in *Agrobacterium fabrum* C58 – genes, regulation and role in fitness

S. Lecomte[1], X. Nesme[1], F. Hommais[2], F. E. Haichar[1]
[1]*Laboratoire d'Ecologie Microbienne, Villeurbanne, France*
[2]*Laboratoire Microbiologie Adaptation Pathogénie, Villeurbanne, France*

The rhizosphere is the interface between plant roots and soil where intense, varied interactions between plants and microbes influence plants" health and growth1. The rhizosphere is also a changing environment where oxygen can be rapidly limited and anaerobic zones can be established. Microorganisms successfully colonize the rhizosphere when they possess adaptative traits referred to as anaerobic respiration flexibility contributed to rhizosphere competence2. During anaerobic conditions, a wide range of rhizospheric compounds can serve as alternative terminal electron acceptors including nitrogen oxyanions resulting on denitrification process. Denitrification consists of four sequential reduction steps (NO3-> NO2-> NO-> N2O-> N2) catalyzed by four specific reductases. Denitrification contributes to the stratospheric ozone depletion due to N2O emission, a greenhouse gas with a warming potential nearly 300 times that of CO2over 100 years. Among rhizobacteria that are responsible of N2O emission, *Agrobacterium fabrum*C58 is worthy of interest. Indeed, C58 is present in mostly agricultural sols and lacks genetic capacity for N2O reduction as other denitrifying bacteria3. In addition, few information is known on how denitrification works in C58. Elucidating the mechanism of denitrification of C58 and its regulation would help to propose strategies to reduce N2O emission from agricultural systems. To do so, molecular, culture and mutagenesis methods were coupled. We evidenced that *napA*(nitrate reductase) is responsible of the first step of denitrification inC58 and it provides advantage in competition assays *in vitro*and *in planta*. We also evidenced that a small non-coding RNA "NopR" is implicated in NO production and consumption and hence in N2O emission. To go further in (i) determining the role of denitrification in the fitness and (ii) identifying all regulatory proteins implicated in denitrification regulation *in planta*, Tn-Seq analyses areunder progress.

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No publication allowed.

P 59

Understanding the ecological adaptations and community dynamics of the human gut microbiome during *Clostridium difficile* infection using an *in vitro* system

B. Lengger[1], T. Gnanasekaran[1], C. S. Ausejo[1], A. D. Brejnrod[1], M. Arumugam[1]
[1]*University of Copenhagen, The Novo Nordisk Foundation Center for Basic Metabolic Research (CBMR), Copenhagen, Denmark*

The human gut microbiome is a vibrant community, consisting of more than 1000 bacterial, archaeal, fungal and viral species and an overall metagenome of 2,000,000 genes. The diversity of species means intense competition, but also potentially symbiotic relationships, likely including the production of a variety of secondary metabolites and high-value compounds. We look at this underexploited but promising microbial community by culturing gut microbiome communities in a specialized *in vitro* system that has several interconnected fermentation compartments with controlled environmental parameters.

One of our experiments involves *Clostridium difficile* which is a common gut pathogen that causes nosocomial gastrointestinal disorders by invading the human colon when the gut microbiota is disturbed after antibiotics administration. Recent studies in animal models have shown that probiotic treatment is effective in combating *C. difficile* infection. Using short chain fatty acid (SCFA) analysis, qPCR and colony counts specific to *C. difficile*, preliminary experiments in our *in vitro* system have confirmed the stability of the *C. difficile* infected community as well as the probiotics strains. We currently investigate the microbial community responses and ecological adaptations to colonization of *C. difficile* using longitudinal sampling of the microbial community followed by bioinformatic analysis.

P 60

Climate changes and land use effects on methane cycling of eastern amazonian soils

F. Mancini Nakamura[1,2], J. L. Mazza Rodrigues[2], T. Siu Mui[1]
[1]Center for Nuclear Energy in Agriculture, Laboratory of Cellular and Molecular Biology, Piracicaba, Brazil
[2]University of California, Davis, Land, Air and Water Resources, Davis, CA, United States

Soil conditions influence microbial growth, such as temperature, moisture, and soil nutritional composition. Based on climate changes predictions for the Amazonian environment, the experiment was designed to determine the temperature, moisture and soil properties dependence of methanogens and methanotrophs in a Pristine Forest soil (PF2) and a Pasture soil (PT3) from Eastern Amazon, Brazil. Samples were incubated under moistures 17%, 35% or 70% v.v. at f.c. and factorial temperatures of 25°C or 30°C. Gases and molecular traits were measured to detect the influence of factors in total Bacteria and Archaea, as well as the marker genes for methane cycle, the methanotrophs by *pmoA* gene and methanogens by *mcrA* gene, the most harmful GHG in soils due to its chemical activity and amount released from a biotic activity. It was possible to visualize that soil biological GHGs cycle is sensitive to modulations of moisture and temperature. Total *Bacteria* and *Archaea*, methanotrophs, and methanogens are positively benefited by an increase in moisture and temperature. GHG emissions are specifically altered by the combination of the abiotic factors. At 25°C and drought, Forest soils present the highest CH4 emission with no significant change in any populations abundance, CH4 emissions maybe from non-biotic or a previous amount stocked inside clayey aggregates. However, microbial populations are altered by the additional moisture on soil which raises all populations in a directly proportional increase. Regarding CH4 emissions, at dry Forest`s soil and 35% of moisture, there is an equilibrium of population where CH4 emission is retarded and restarts to increase at higher moisture of 70% within the increase of bacterial and archaeal populations. Forest`s soils under drought and 30°C have bacterial and archaeal populations increase within the increase in moisture, however with no changes to GHG emissions. For Pasture, under 35% moisture and 25oC there was an increase in the total number of *Bacteria* and *Archaea* with a slight increase in emissions for all gases. Both drought and wetness with an increase in temperature in Pasture lead to significant increase in CH4 emission. This study highlights the influence of changes that Amazonian biome could face on the next decades, which would impact negatively and worse the CH4 emissions by natural and anthropical Amazonian lands.

P 61

CDOM causes shifts in the microbial community in shallow lakes

A. Mentes[1,2], A. Szabó[1], N. Tugyi[3], B. Somogyi[3], L. Vörös[3], B. Csitári[1,4], A. J. Székely[4], T. Felföldi[1]
[1]ELTE Eötvös Loránd University, Department of Microbiology, Budapest, Hungary
[2]Hungarian Academy of Sciences RCNS, Institute of Enzymology, Budapest, Hungary
[3]Hungarian Academy of Sciences CER , Balaton Limnological Institute, Tihany, Hungary
[4]Uppsala University, Department of Ecology and Genetics/Limnology, Uppsala, Sweden

Colored dissolved organic matter (CDOM) is composed of small macromolecules (proteins, carbohydrates) and polymers (lignin, cellulose) and is originated
from living and decomposing plants. According to previous studies, CDOM, despite its persistent properties, can be used as energy and carbon source by bacteria and it can contribute up to 80% to the dissolved organic matter (DOC) pool. Nevertheless, CDOM has important effects on ecosystem productivity, has a negative impact on primary production while has a positive impact on secondary production in aquatic environments.
This research aimed to investigate ten water samples from the Carpathian Basin, which differ from each other in the amount of CDOM (from clear to dark brown water) and to reveal the effect of CDOM on the planktonic microbial community. The sampling was carried out during the summer period when the bacterial communities apparently are most active. We were looking for connections among the limnological parameters and the production (tritium-labeled leucine technique), the metabolic potential (Biolog EcoPlate™) and the composition of microbial communities (NGS).
Our results showed that differences appeared already at the phylum level in the bacterial communities of the investigated samples. Representatives of Proteobacteria (5-65%), Actinobacteria (7-72%) dominated among all samples. Metabolic potential and composition of bacterioplankton did not show any correlation with CDOM concentration since changes can occur simultaneously in the metabolism and community composition with changing CDOM composition (even in the case of samples with similar CDOM amount).
We found a positive correlation between CDOM concentration and the production of bacterial communities, nevertheless, CDOM is not the only factor that can be responsible for the formation of different microbial communities: the type of lakes, water quality, salt concentration and the interaction between organisms need to be also considered.
The work of Anikó Mentes *was supported by the ÚNKP-18-3-III-ELTE-184 New National Excellence Program of the Ministry of Human Capacities (Hungary).*

P 62

Microbial screening of subsurface radioactive water springs in the Czech Republic

G. Nováková[1], T. Šmrhová[1], M. Strejček[1], J. Šuman[1], O. Uhlík[1]

[1]University of Chemistry and Technology Prague, Department of Biochemistry and Microbiology, Prague, Czech Republic

Unique localities, such as radioactive water springs, are a promising source of biotechnologically interesting microorganisms. Generally, though, most such microorganisms are rarely obtained in a pure culture. The aim of this study is to perform microbial screening of radioactive water springs in order to describe the community structure and find microorganisms with biotechnological potential. In an attempt to increase cultivation yields, we investigate a combination of classical cultivation approaches with factors imitating sampling source conditions. In this study, four radioactive water springs were sampled in the Czech Republic. Microorganisms were collected by filtering 30 litres of spring water. Total community of each water spring was determined by the isolation of total DNA and subsequent sequencing of the 16S rRNA gene on an Illumina MiSeq platform. Parallel to sequencing, traditional media as well as non-traditional approaches were used for cultivation under laboratory conditions. After collection, the isolates were clustered by their MALDI-TOF mass spectra similarity and identified using BioTyper database. The isolates not identified by MALDI-TOF MS were identified by 16S rRNA gene sequencing. The most frequent identifications belong to members of the families *Pseudomonadaceae*, *Bacillaceae*, *Moraxellaceae* or *Dietziaceae*. Our results show that, underground water springs contain diverse bacterial populations, including many bacterial clades of different phylogenetic origin. Furthermore, this study suggests that, improved cultivation techniques increase the efficiency of cultivation and thus increase a chance of capturing microorganisms with special properties.

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P 63

Comparative genome analysis reveals habitat-specific adaptations of two *Methylocystis* species from wetlands

I. Oshkin[1]

[1]Research center for biotechnology, Winogradsky Institute for Microbiology, Moscow, Russian Federation

Introduction: Methanotrophic bacteria represent a unique group of microorganisms able to utilize methane as a sole source of carbon and energy. Among all currently known methanotrophs members of *Methylocystis* genus are most frequently detected in culture-independent studies. They inhabit different soils, rice paddies, peatlands, landfills, and freshwater sediments. Recent genome analysis of peat-inhabiting *M. bryophila* S285 by Han *et al*. revealed habitat-specific environmental adaptations.

Objectives: In order to identify similar or different trends in environmental adaptations compared to *M. bryophila* S285, we sequenced and analyzed the genome of *M. heyeri* H2^T which was isolated from a similar habitat, i.e. a *Sphagnum*-dominated wetland.

Materials and methods: Genome sequencing of strain H2^T was performed using the PacBio RSII platform. De novo assembly was done using the combination of Canu and SPAdes. The genome was annotated using RAST v. 2.0 and PROKKA.

Results: The genomes of strains H2^T and S285 encode the full set of known methane oxidation enzymes: pMMO1, pMMO2, and sMMO. Moreover, they also possess *pxmABC*-like gene clusters which are rarely identified in type II methanotrophs. Strains S285 and H2^T share V–Fe type of nitrogenases which are present only in a limited number of microorganisms. Both methanotrophs contain genes encoding alcohol- and aldehyde dehydrogenases. Presence of these enzymes coupled with complete EMC pathway provides the possibility for growth on ethanol or acetate. Despite the high proportion of shared genes, we managed to identify a few unique features in the genome of strain H2^T. The presence of endo-1,4-β-xylanases may provide metabolic potential for hydrolytic activity towards xylan. Moreover, this strain has broader pool of CAZymes. CAZy family GH5 is present only in *M. heyeri* H2^T. GH5 enzymes could also be responsible for hydrolysis of xylan. A number of 16S rRNA gene sequences closely related to that of *M. heyeri* H2^T (98% similarity) were retrieved in several environmental studies of peat soil, freshwater lake sediment and permafrost. Strain H2^T possesses fatty acid desaturases which may be an important adaptation to low-temperature conditions.

Conclusions: Strains S285 and H2^T share a number of common adaptations, which enable their survival in peat ecosystems. However, strain H2^T has a broader range of CAZy enzymes and encode endo-1,4-β-xylanases which may indicate a potential hydrolytic activity towards xylan.

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Ammonia-oxidizing Archaea and Bacteria in Amazonian Forest Soils and Floodplain Forest Sediments

F. Paula[1], J. B. Gontijo[1], B. Ferrari[1], A. M. Venturini[1], L. Lemos[1], F. Asselta[1], S. M. Tsai[1]
[1]Universidade de São Paulo, Centro de Energia Nuclear na Agricultura, Piracicaba, Brazil

The Amazon rainforest has an important role in the global carbon budget, and therefore the dynamics of the biogeochemical cycles in this ecosystem are of great interest. Nitrogen availability controls the activity of microorganisms and consequently affects ecosystem processes. The availability of mineral N in the soils is directly affected by the activity of ammonia-oxidizing archaea (AOA) and bacteria (AOB), which are suggested to respond differently to environmental changes. Assessing the distribution of these communities across Amazonian landscapes, as well as their responses to seasonal patterns may contribute to understand the dynamics of the regional N cycle. Here we investigate the diversity and abundance of AOA and AOB in pristine areas at Tapajos National Forest. We used qPCR targeting ammonia monooxygenase subunit A (*amoA*) genes and sequencing of 16SrRNA genes to investigate the ammonia-oxidizing communities in forest soils, as well as in floodplain sediments, in both dry and wet seasons. We observed a higher abundance of AOA *amoA* genes (10^3 to 10^4 copies/ng DNA), compared to AOB (10^1 to 10^3 copies/ng DNA), in all soils and sediments analyzed. The two groups responded differently to the seasonal changes: while no significant variation was observed for AOA, AOB abundance decreased sharply during the wet season in both environments. AOA abundance was significantly higher in forest soils, compared to floodplain sediments. Similar patterns were observed in the 16SrRNA sequencing data, which indicated no seasonal changes and higher relative abundance of the archaeal phylum Thaumarchaeota in the forest. Nitrososphaeraceae was the most abundant family among the Thaumarchaeota groups and it represented above 80% of the archaeal community in the forest soils. Bacteria from the families Nitrosomonadaceae and Nitrospiraceae were also detected in low relative abundance (<0.8%), but did not show a clear response to the seasonal changes. AOA has been suggested to be the most active nitrifying group in low pH environments. Their dominance in the acidic Amazon soils and sediments may be an indication of their importance for the regional N cycle. Additionally, despite being essentially aerobic organisms, AOA seems to have higher resistance to the flooding events in comparison to AOB, which could also confer advantage. Studies targeting the active communities may offer further understanding of their dynamics and contributions for the nitrification process.

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Impact of Ag_2S NPs on soil bacterial community – a terrestrial mesocosm approach

S. Peixoto[1], Z. Khodaparast[1], I. Henriques [2], S. Loureiro[1]
[1]University of Aveiro, Department of Biology & CESAM, Aveiro, Portugal
[2]University of Coimbra, CESAM & Department of Life Sciences, Faculty of Science and Technology, Coimbra, Portugal

Most of silver nanoparticles are transformed to silver sulfide (Ag_2S NP) within wastewater treatment plants, being discharged into agricultural soils as part of sludge-derived fertilizers. Due to this exposure route, we aimed to investigate the effects of Ag_2S NP on the structure and function of soil bacterial community (SBC).

A replicated terrestrial indoor mesocosm was performed using a single dose of silver treatment (10 mg Ag kg^{-1} soil). The treatments included Ag_2S NP-spiked soil, soil spiked with ionic silver ($AgNO_3$) and non-exposed soil. Besides, plants (*Triticum aestivum L.*), mealworms (*Tenebrio molitor*), isopods (*Porcellio scaber*) and earthworms (*Lumbricus rubellus*) were included in each mesocosm. The SBC was analyzed to determine SBC function-related parameters (enzymatic activity and community level physiological profile, CLPP) and SBC structure (denaturing gradient gel electrophoresis and culture-based analysis). Sampling was done at 0, 14 and 28 days.

Our results showed that the presence plants and invertebrates increased the abundance of specific bacterial groups (e.g. phosphate solubilizing bacteria) and substrate consumption (CLPP). After 28 days, SBC exposed to Ag_2S NP presented significantly lower richness and diversity (structural effects), and a reduced beta-glucosidase activity and *L-arginine* consumption (CLPP). Strong effects were also observed when SBC was exposed to $AgNO_3$, although these effects were detected at earlier times of exposure.

This study indicates that using terrestrial mesocosm, including biota, was a suitable method to analyze the impact of Ag_2S NP on SBC. Observed Ag_2S NP effects on the SBC might cause the imbalance of soil functions, namely those related to the carbon and phosphorus cycles. These effects were only observed at 28 days, highlighting the importance of long-term exposure experiments. Regarding the silver form, later effects induced by Ag_2S NP seem to be dependent on Ag dissolution in time.

P 66

The multi-level relationship between heterotrophic bacteria and nutrients

E. Perrin[1], P. Turano[2], V. Ghini[2], M. Giovannini[1], B. Cardazzo[3], L. Carraro[3], E. Parrilli[4], M. L. Tutino[4], R. Fani[1], M. Fondi[1]
[1]*University of Florence, Department of Biology, Sesto Fiorentino, Italy*
[2]*University of Florence, CERM, Sesto Fiorentino, Italy*
[3]*University of Padua, Dipartimento di Biomedicina Comparata e Alimentazione, Legnaro, Italy*
[4]*University of Naples, Dipartimento di Scienze chimiche, Naples, Italy*

Introduction: A detailed comprehension of the functioning of heterotrophic bacteria metabolism is crucial for deciphering the complex processes that regulate nutrients assimilation and recycling in the oceans and the possible effect of external perturbations on such a system.
Objectives: We want to disclose the molecular details of two main features in the relationship between marine microbes and nutrients: 1) the formation of microbial aggregates in response to external nutrients concentration, 2) the regulation of nutrient assimilatory pathways when facing a nutritionally rich environment.
Materials & methods: We combined a series of transcriptomics and metabolomics experiments in the model heterotrophic bacterium *Pseudoalteromonas haloplanktis* TAC125. We made use of mathematical modelling to generate predictive models and to integrate -omics data.
Results: Nutrients concentration in the medium deeply influences cell aggregation and (partially) cell motility in *P. haloplanktis* TAC125. Cells grown in a nutrient-rich medium appeared mucoid, non-motile and overall displayed a greater tendency to aggregate in respect to cells grown in a nutrient poor medium. Transcriptomics analysis was carried out in such conditions, revealing the gene clusters likely involved in such processes and a possible regulatory circuit accounting for the switch between planktonic and aggregated cells. Time-course transcriptomics on cells grown in a nutritionally complex environment revealed a time-resolved hierarchy in the metabolism of available nutrient sources, with metabolic switches marking the boundaries of each of the assimilated set of compounds.
Conclusion: The combination of targeted metabolomics and mathematical modelling allowed the formulation of a computational model capable of explaining and predicting such metabolic phenotypes.

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Linking litter microbial communities to substrate quality, environmental conditions and decomposition dynamics – insights from two studies using tea bags

S. Pioli[1], L. Montagnani[1], J. M. Sarneel[2], L. Beneduce[3], L. Brusetti[1]
[1]*Free University of Bolzano, Science and Technology, Bolzano, Italy*
[2]*Umeå University, Landscape Ecology Group, Department of Ecology and Environmental Science, Umeå, Sweden*
[3]*University of Foggia, Department of Agricultural, Food and Environmental Science, Foggia, Italy*

Leaf Llitter decomposition is a key process in carbon dynamics and nutrient cycling in forest soils. The interaction between substrate properties, climatic conditions and microhabitat attributes, influences the activity of microorganisms responsible for litter mineralization. So far, few studies used tea bags as standard litter to investigate the response of microbial communities to changes in the environmental conditions at local and European scales.
The tea bags of rooibos and green tea leaves were used in two different experimental set up with the aim of:
- Disentangling the relative importance of selected environmental variables on microbial communities' diversity, stressing the variation among litter types and across 12 study sites along a European transect from Spain to Finland.
- Quantifying the changes in bacterial biomass during the decomposition process in relation to substrate mass loss and along an altitudinal gradient in South Tyrol (Italy).
ARISA fingerprinting and qPCR were used to characterize the microbial communities in the first and second experiment respectively. Soil and litter chemical traits were determined to assess the influence of local substrates on microbial diversity. Mass loss was used as a proxy for microbial activity.
Our results indicated that bacteria were better differentiated across litter types compared to fungi. Moreover, the tea bags were characterized by specialized communities that only in part reflect the local litter assemblages. Green tea degraded faster than rooibos and accounted for higher bacterial biomass. The effect of altitude on mass loss and bacterial abundances is less clear, although we found total bacterial biomass to differ significantly ($p<0.05$) between study sites.
Our findings suggest that the microbial community in each litter type reflects the complex interplay of climatic variables, species interactions and soil/litter attributes. Litter mass loss in part reflects the abundance of bacteria, which showed fluctuating dynamics over time.

P 68

Plasmids in gut environments

M. Probst[1], N. Stertzer[1], D. Bogumil[1], G. Sasson[1], E. Jami[2], D. Svetlitsky[1], M. Ziv-Ukelson[1], I. Mizrahi[1]
[1]Ben Gurion University of the Negev, Be'er Scheva, Israel
[2]Volcani Center, Rishon LeZion, Israel

Introduction: Plasmids are extrachromosomal genetic elements, which occur in all kinds of different organisms, mainly microbes, and ecosystems. Plasmids contribute to genetic diversity and consequently, participate in microbial adaptation and evolution. Their relevance is reinforced by the plasmids" ability to move horizontally from one microbe to another, sometimes even crossing long taxonomic distances. For the organisms involved, this horizontal gene transfer (HGT) means access to a shared pool of genetic material. Consequently, the plasmidome might have a big impact on its ecosystem. Still, the plasmidome is poorly researched and there is little knowledge about the occurrences and characteristics of plasmids in ecosystems.
Objectives: What is the gut's plasmidome? Is it being selected for?
Material ans Methods: Plasmids were wet-lab extracted from safari animals" gut samples, represented by their feces. The animals belonged to different species and could be categorized according to their digestive system into foregut, hindgut and simple gut animals. The extracted plasmidomes were sequenced on the Illumina Hiseq sequencing platform. From the very same samples, the microbiome was profiled by Illumina Miseq sequencing of 16S libraries. Using the resulting data, we studied the plasmidome composition of the animals and researched its dependency on the animals" digestive category.
Results: In all animals" gut samples, we found a high number of different plasmids. Plasmids differed in their length, the coding of accessory functions and the presence/absence of genes enabling HGT. Despite a high individual variability, we found an effect of the digestive strategy on the plasmidome. Clustering analysis clearly showed that both the plasmidome and the microbiome composition depended on the animals" digestive category. The digestive system also determined what kind of accessory functions were coded on plasmids.
Conclusion: Our finding that different digestive strategies co-occur not only with different microbiomes but also with different plasmidomes underline the relevance of plasmids in terms of bacterial evolution and adaptation. Furthermore, our results suggest that environmental selection might act on a scale smaller than the living cell itself.

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Diversity of Brazilian Soda Lakes' associated microbiome

S. Raposo Cotta[1], T. A. Pellegrinetti[1], A. P. Dini Andreote[1], J. S. Costa[1], M. F. Fiore[1]
[1]Cena, Piracicaba, Brazil

Soda lakes are widespread around the world (North America, Africa, and Central Asia), being characterized by a high concentration of sodium carbonates resulting in elevated salinity and pH. Despite harsh conditions, they are inhabited by abundant, mostly prokaryotic, microbial communities, that are responsible for sustain the ecological processes and environmental productivity. Albeit its importance, biological information of these systems is still poorly understood. At this work, we used metagenomic sequencing to evaluate the composition and functional patterns of the microbial communities of five soda lakes (lakes with a gradient of Cyanobacteria bloom) from the sub-region Nhecolândia, state of Mato Grosso do Sul, Brazil. Lakes with intense Cyanobacteria bloom showed high diversity and richness of microbial groups and realized functions, followed by the lake with absent Cyanobacteria bloom. Lakes with moderate Cyanobacteria bloom showed lowest diversity and richness of functions. Prevalent bacterial groups for all evaluated areas were Cyanobacteria, Proteobacteria, Firmicutes, Bacteriodetes and Actinobacteria. As expected, lakes with absent Cyanobacteria bloom showed lowest identification of Cyanobacteria while lakes with intense Cyanobacteria bloom showed a high prevalence of this group. Lakes with moderate and absent Cyanobacteria bloom showed prevalence of Actinobacteria while Lakes with intense Cyanobacteria bloom showed a prevalence of Bacteriodetes. It is imperative that Cyanobacteria bloom directly impact on microbiota associated with Soda Lakes and the next steps comprise to understand what is the impact on environment functionality and ecological interactions.

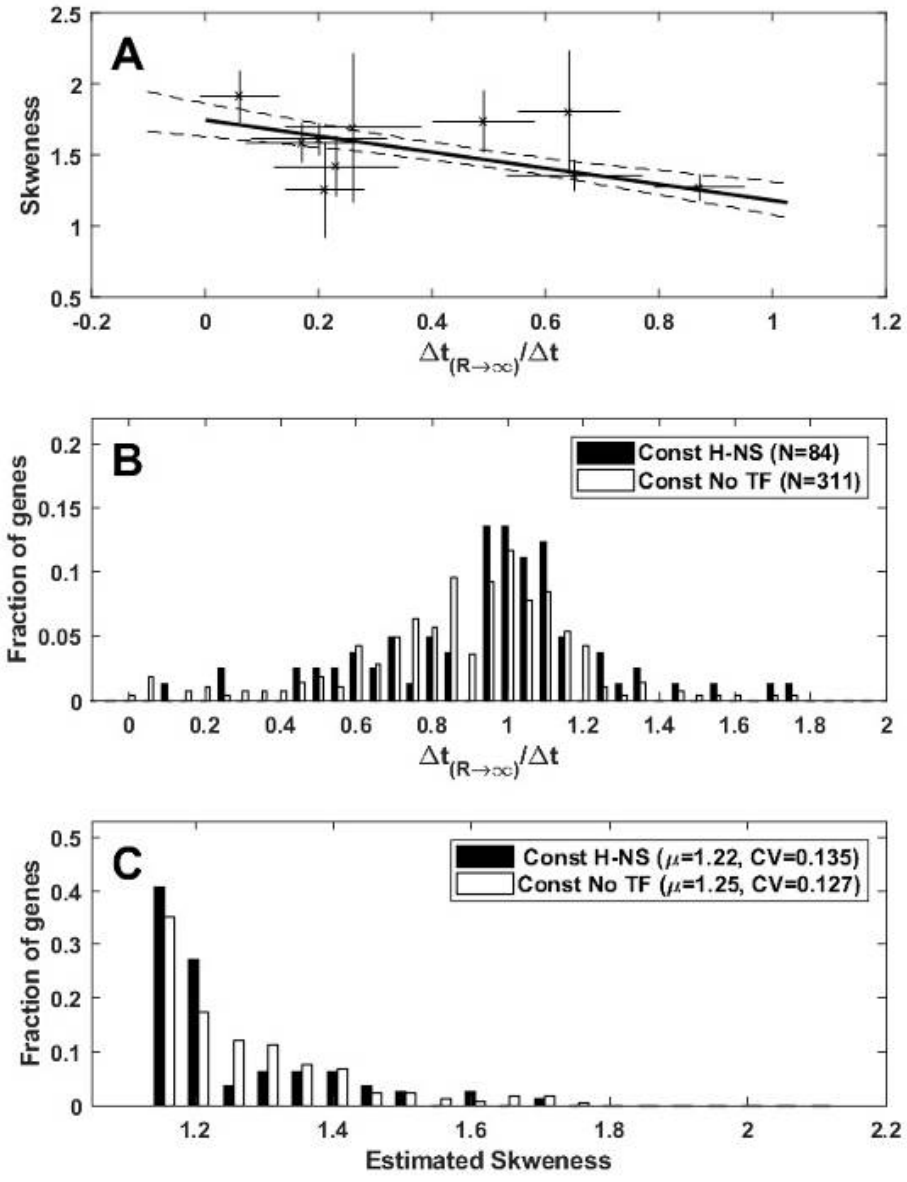
P 70

Asymmetries in gene expression dynamics as a potential enhancer of microbial resilience

B. Almeida[1], S. Oliveira[1], A. Ribeiro[1]
[1]Tampere University, Faculty of Medicine and Health Technology, Tampere, Finland

Introduction: Environmental stress can affect the health of microbial populations. Phenotypic diversity is advantageous for isogenic populations to survive such stresses. One known source of phenotypic diversity in isogenic populations is noise in gene expression. A recent study [1] suggested that another potential source of this diversity are asymmetries in the RNA and proteins numbers of cell populations, which were shown to be adjustable by regulation of the kinetics of the multi-step transcription initiation process.
Objectives: We investigated the influence of the global transcription regulator H-NS on asymmetries in RNA numbers of constitutively expressed genes in *Escherichia coli*.
Materials and Methods: Using a single RNA tagging system, we first quantified RNA numbers of various genes in single *E. coli* cells as well as RNA polymerase concentration in various media conditions. From this data, we estimated the correlation between the kinetics of transcription initiation of a promoter and the consequent asymmetry of the single-cell distribution of RNA numbers, as quantified by skewness (Fig. 1A). Next, we used RNAseq data to dissect the kinetics of transcription initiation of constitutively expressed genes (Fig. 1B) which, based on the correlation established above, we used to estimate the asymmetry in RNA numbers of each constitutively expressed gene.
Results: We show that the set of constitutively expressed genes known to be affected by H-NS exhibit a more diverse set of values of asymmetry in the single-cell distribution of RNA numbers (Fig. 1C), as quantified by the coefficient of variation.
Conclusion: The results suggest that H-NS may affect the asymmetry in RNA numbers of constitutively expressed genes, likely enhancing the phenotypic diversity, which could contribute to the resilience of *E. coli* populations to stressful environments.
[1] S Startceva, VK Kandavalli, A Visa, and AS Ribeiro (2019) *BBA - Gene Regulatory Mechanisms.* 1862(2), 119–128.
Fig. 1. (A) Linear-fit of the correlation between $\Delta t(R=\infty)/\Delta t$ and skewness in RNA numbers for multiple promoters and conditions as measured by single-RNA detection and microscopy [1]. (B) Distribution of the genome-wide $\Delta t(R=\infty)/\Delta t$ ratios as measured by RNAseq. (C) Distribution of the estimated skewness for constitutive genes regulated either by σ factors (Black bars) or by σ factors and global regulators (White bars), with μ representing the mean and CV the coefficient of variation.

Fig. 1


A
Skweness
$\Delta t_{(R\to\infty)}/\Delta t$
B
Const H-NS (N=84)
Const No TF (N=311)
Fraction of genes
$\Delta t_{(R\to\infty)}/\Delta t$
C
Const H-NS (μ=1.22, CV=0.135)
Const No TF (μ=1.25, CV=0.127)
Fraction of genes
Estimated Skweness

P 71

Calcareous sponge holobiont in a changing ocean

B. Ribeiro[1], A. Padua[1], G. Duarte[2], H. Villela[2], A. Rossi[3], U. Nunes da Rocha[4], F. Côrrea[4], F. da Costa[5], R. Peixoto[2], M. Klautau[1]
[1]*Federal University of Rio de Janeiro, Zoology, Rio de Janeiro, Brazil*
[2]*Federal University of Rio de Janeiro, Microbiology, Rio de Janeiro, Brazil*
[3]*Brazilian Center for Physical Research, Rio de Janeiro, Brazil*
[4]*Helmholtz Environmental Research Center, Environmental Microbiology, Leipzig, Germany*
[5]*Institute of Studies of the Sea Almirante Paulo Moreira, Arraial do Cabo, Brazil*

Ocean warming and acidification are major threats to the marine environment today and in future projections. Both stressors have already been shown to be harmful to many marine organisms, especially to calcifiers. However, despite playing important functional roles in the marine environment, little is known about the possible effects of climate change on the calcareous sponges holobiont. Thereby, the aim of the present study was to verify the response of the bacterial community of the calcareous sponges *Sycettusa hastifera* under ocean warming and acidification. To achieve this purpose, short-term experiments (10 days) were performed in a semi-open aquarium system, comprising four treatments: Control (22 ºC and pH 8.1), High Temperature (26 ºC and pH 8.1), Low pH (22 ºC and pH 7.6), and Combined Effects (26 ºC and pH 7.6) with three temporal replicas of each treatment. To assess possible shifts in the microbiota across treatments, the V4 hypervariable region of the 16S rRNA gene was sequenced in the Illumina MiSeq platform. Alpha and Beta diversities were calculated and statistically compared between treatments using two-way ANOVA and PERMANOVA, respectively. At the end of the experiment, both alpha and beta diversities of the bacterial community of the calcareous sponge *S. hastifera* remained stable and neither necrosis nor mortality of the sponges were observed. The genus *Ruegeria* spp. (class Alphaproteobacteria) dominated the bacterial core, including the field samples, and presented no significant differences between climate change treatments. Here we provide the first study on how the bacterial community of a calcareous sponge may respond to the future projections of elevated temperature and low pH. Our results indicate that the host-microbe interaction has a wider threshold of temperature and pH, as it seems to be resistant to the ocean warming and acidification stressors, at least during the 10 days of the experiment. However, long-term studies are needed to reinforce these results. *Ruegeria* strains were already related to quorum sensing in previous studies, and their maintenance in the bacterial community core could be an attempt to keep the holobiont healthy.

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Probing the performance of *Lactobacillus sanfranciscensis* strains in wheat and rye sourdough by using CRISPR-locus length polymorphism

E. Rogalski[1], G. Celano[2], F. Minervini[2], R. F. Vogel[1], M. A. Ehrmann[1]
[1]*Technische Universität München, Technische Mikrobiologie, Freising, Germany*
[2]*Università degli Studi di Bari Aldo Moro, Dipartimento Di Scienze Del Suolo, Della Pianta E Degli Alimenti (Di.S.S.P.A.), Bari, Italy*

Introduction: *Lactobacillus sanfranciscensis* is known as a competitive key species in sourdough fermentations. Frequently, different strains occur in a single fermentation. A fast and reliable differentiation of single strains and monitoring of these strains during fermentation are rather limited by a narrow phylogenomic diversity within this species. In this contribution we evaluated a strain specific PCR-based typing method by using a length heterogeneity of the CRISPR-locus found in their genomes.
Objectives: Develop and test a strain specific detection method for *L. sanfranciscensis* in sourdough.
Material and methods: *In silico* analysis of genomes with CRISPR finder tool allowed for the design of a primer set to detect the length heterogeneity of the CRISPR-locus. Based on these differences in length, strains were chosen for the characterization of their assertiveness in sourdough. Rye or wheat flour was mixed with water to a dough yield of 200. Each dough was inoculated with four different *L. sanfranciscensis* strains (TMW 1.1150, TMW 1.392, TMW 1.2142, TMW 1.2138). With back slopping of 5% to the flour mass the sourdough was propagated for five days after 24 h at 28 °C. Each day samples were taken, and DNA was isolated. Dynamics of strains was detected in the sourdoughs with PCR followed by agarose gel electrophoresis.
Results: CRISPR-loci were found in 91 % and length heterogeneity in 67 % of strains tested. Thus, 14 CRISPR-genotypes could be differentiated within 23 tested strains. Tracking of strains in a lab-scale model sourdough was possible. *L. sanfranciscensis* TMW 1.392 was dominant after two days in the sourdough and remained the dominant strain for the remaining sampling period.
Conclusion: By using CRISPR-locus length polymorphism-based PCR it is possible to investigate the assertiveness of different strains of *L. sanfranciscensis* in rye and wheat sourdoughs.
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Towards characterization of microbial interactions – functional annotation by clustering orthologous groups

J. P. Saraiva[1], M. Gomes[2], O. Dias[2], U. Nunes da Rocha[1]
[1]*Helmholtz Center for Environmental Research, Environmental Microbiology, Leipzig, Germany*
[2]*University of Minho, Center of Biological Engineering, Braga, Portugal*

Introduction. An important step to predict microbial interactions is the characterization of the functional capacity of individual species. Functional annotation of genomes can be performed via detection of orthologue genes. The development of functional annotation in a timely manner and with low computational costs is essential to determine potential microbial interactions in complex communities.
Objectives. In the current study, we developed a pipeline to perform rapid functional annotation of various organisms based on detection of orthologues. Reactions involved in the transformation of benzoate to acetyl-CoA (BTA) were used to evaluate orthologue detection.
Methods. OrthoMCL and CD-HIT were used to assemble different clusters of orthologous groups (COGs) using 11 bacteria (previously sequenced) retrieved from Uniprot. COG annotation relied on the alignment of different representative sequences (consensus, largest and smallest sequences, and randomly selected) from each cluster to those of proteins that actively participate in BTA. Alignments of all sequences of a cluster to the BTA were used as controls.
Results. Each generated cluster represented a group of proteins with the potential to share the same function. From the analysis of 61122 proteins from the 11 isolates, OrthoMCL originated 10985 clusters and CD-HIT 41960 clusters. Further steps were only performed using the results from OrthoMCL as preliminary results showed higher accuracy in functional annotation of unknown protein sequences. The use of consensus sequences showed a high degree of incorrect associations between COGs and BTA. The use of all other sequences as representatives of COGs presented high accuracy when compared to the controls.
Discussion. OrthoMCL generated a lower number of clusters which might facilitate analysis due to the need for fewer alignments. This number could be further decreased if COGs associated with the same groups of known protein sequences were to be merged. However, its feasibility warrants further study. The speed and accuracy, when selecting a random sequence of the cluster, appears to be a much better option and less computationally demanding. COG annotation allows inferring which reactions of a pathway each species has the potential to perform. Cases where all required reactions are only possible through the combination from different species could identify potential microbial interactions.

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Nitrification by archaea fuels high nitrous oxide emissions from arctic peatlands

H. Siljanen[1], R. Alves[2], J. Ronkainen[1], R. Lamprecht[1], H. Bhattarai[1], A. Bagnoud[2], M. Marushchak[1], P. Martikainen[1], C. Schleper[2], C. Biasi[1]
[1]*University of Eastern Finland, Department of Environmental and Biological Sciences, Kuopio, Finland*
[2]*University of Vienna, Division of Archaea Biology and Ecogenomics, Vienna, Austria*

Bare peat surfaces located on permafrost peatlands and created by frost and wind erosion are the only known areas in subarctic and arctic ecosystems showing high nitrous oxide (N2O) emissions. With global warming, emissions of this highly potent greenhouse gas are likely to increase from arctic permafrost peatlands. In natural unamended soils with low atmospheric nitrogen deposition, nitrification is the main source of nitrite and nitrateand thus directly or indirectly a key driver of N2O production. Here, we studied nitrification and N2O production in both vegetated and bare permafrost peat soils at four distant arctic locations. Through a combination of molecular studies and specific inhibitors we show that ammonia oxidation, the first step in nitrification, is mainly performed by ammonia-oxidizing archaea (AOA). All the high N2O emitting bare peat as well as low emitting vegetated peat soils contained only two AOA phylotypes, including an organism closely related to *Ca*. Nitrosocosmicus spp.. This indicates that high N2O emissions from these ecosystems are primarily fueled through ammonia oxidation by very few archaeal keystone species. Any changes in archaeal nitrification induced by global warming will have a key role for future N2O emissions from the arctic ecosystems.

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Farming practices shape the structural and functional diversity of rhizospheric microbial community of *Cajanus cajan*

U. Singh[1], S. Sharma[1], A. K. Choudhary[2]
[1]*Indian Institute of Technology Delhi, Department of Biochemical Engineering and Biotechnology, New Delhi, India*
[2]*Indian Agricultural Research Institute, Division of Agronomy , New Delhi, India*

Agriculture is one of the foremost anthropogenic activities responsible for the alteration of soil physiochemical and biological properties. These activities affect the diversity, relative abundance and activities of soil microorganisms. Various agriculture practices have been reported to diminish the soil microbial structure and function. Therefore, the farming system needs alternative approaches that can preserve the microbiome and decrease the susceptibility of agriculture to climate variation. So, the purpose of the study was to understand the effect of farming practices agriculture on rhizosphere microbial community structure and function in *Cajanus cajan*. Nine different modules were set under three farming approaches, viz. conservational, convention and organic agriculture, which included various types of tillage, beds, crop rotation, fertilizers dosages, and fertilizers. Rhizospheric bacterial dynamics was examined qualitatively and quantitatively by 16S rRNA PCR-DGGE, and quantitative analysis by q-PCR of various markers including genes involved in nitrogen cycle (16S rRNA, *nifH, amoA, narG* and *nirK*), respectively. Quantitative analysis of these markers revealed that total rhizospheric soil bacterial community and bacterial community driving the N cycle were enriched in conservational agriculture, followed by organic and convention agriculture. Qualitative analysis of the markers revealed lesser diversity of bacterial community in conventional agriculture, which can be attributed to high mechanical inputs, erosion and loss of organic matter. The study brings forth a comprehensive picture of the mechanisms involved in better performance of conservational approach in agriculture compared to conventional means. It also stringly propagates the development of alternative agricultural practices to overcome the effect of rigorous farming practices, and boost nutrient cycles driven by the soil microbial community.

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Targeted meta-genomics evidences role of IS*1071* in microbial community adaptation to degradation of organic pollutants

D. Springael[1], V. Dunon[1], E. Top[1]
[1]*University of Idaho, Institute for Bioinformatics and Evolutionary Studies, Moskow, ID, United States*

IS*1071*, an insertion element that primarily flanks composite transposons carrying organic xenobiotic degradation genes, appears to play a key role in the formation and distribution of bacterial catabolic genes. However, this is concluded from cultured isolates. In environmental settings it is not known whether IS*1071* carry accessory genes neither do we know about the identity of the genetic cargo and whether this identity corresponds to the local selective conditions. To respond, a long-range PCR (LR-PCR) that amplifies accessory genes between two IS*1071* copies from community DNA combined with amplicon next generation sequencing, was applied to a variety of environments exposed to organic contaminants including both man-made organic pollutants (xenobiotics) like pesticides and natural pollutants like polycyclic aromatic hydrocarbons. Examined envorinments included pesticide treated soils, the organic matrix of on farm biopurification systems (BPS) that treat complex agricultural wastewater, sandfilters of drinking water treatment plants receiving pesticide polluted intake waters and soil from hydrocarbon contaminated sites. The IS*1071* cargo genes related to organic pollutant degradation and their diversity mirrored the selective forces of the respective environments. Genes not directly linked to adaptive traits and unknown genes were recovered as well. Various accessory regions showed shared gene synteny with published genome sequences. Our study shows the role of IS*1071* as an important carrier of catabolic genes in organic polluted environments and in community level adaptation to degrade organic pollutants.

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Earth, air and water – oxygen availability influences comammox activity in soil

C. Thion[1], H. Daims[2], P. Pjevac[2], G. W. Nicol[1]
[1]*Ecole Centrale de Lyon, Laboratoire Ampère, Ecully, France*
[2]*Department of Microbiology and Ecosystem Science, Division of Microbial Ecology, Vienna, Austria*

For over 100 years, aerobic nitrification was considered a two-step process, with ammonia oxidised to nitrite by ammonia oxidising microorganisms (AOM) and nitrite oxidised to nitrate by nitrite oxidising bacteria (NOB). This dogma was challenged by the discovery of *Nitrospira* performing both steps, or "complete ammonia oxidation" (comammox), with representatives belonging to two phylogenetic clades (A and B). Using a model soil, our recent work demonstrated comammox *Nitrospira* clade B are more abundant than other AOM communities at neutral pH soil where they possess niches distinct from canonical ammonia oxidising archaea (AOA) and bacteria (AOB). Specifically, whereas AOA and AOB grew in aerated soil using mineralised organic N (AOA) or added inorganic ammonium (AOA and AOB), comammox *Nitrospira* only grew using ammonia derived from mineralised organic N at higher water contents. Therefore, to test the hypothesis that comammox *Nitrospira* activity increases towards microaerophilic conditions, microcosms were established with increasing water filled pore space or decreasing headspace oxygen concentration. Specifically, soil was incubated for 30 days with ^{12}C- or ^{13}C-CO_2 at 30%, 40% or 50% soil water content (w/w) (at 21% initial headspace oxygen concentration) or with an initial 21%, 10.5% or 2.1% headspace oxygen concentration at 30% water content. DNA-SIP combined with quantitative PCR of group-specific *amoA* genes was used to assess AOM growth and activity. AOA and AOB were active and inactive, respectively, in all conditions, but comammox *Nitrospira* demonstrated growth and incorporation of ^{13}C only at 40% water content or at 2.1% headspace oxygen. These results demonstrate that reduced oxygen availability within the soil matrix may be an important factor in controlling *Nitrospira* comammox activity and is consistent with predictions of *Nitrospira* growth under microaerophilic conditions (e.g. through use of the rTCA cycle for CO_2 fixation).

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Gobal airborne microbial communities controlled by surrounding earth surfaces and wind conditions

R. Tignat-Perrier[1,2], A. Dommergue[1], A. Thollot[1], O. Magand[1], T. M. Vogel[2], C. Larose[2]
[1]*Institut des Géosciences de l'Environnement, Grenoble, France*
[2]*Laboratoire Ampère, Ecole Centrale de Lyon, University of Lyon, Environmental Microbial Genomics, Ecully, France*

The atmosphere is an important route for transporting and disseminating microorganisms over short and long distances. The comprehension of how microorganisms are distributed in the atmosphere is critical due to their role in public health, meteorology and atmospheric chemistry. Many studies have emphasized the capacity of microorganisms to be transported over long distances, and some of them have even shown the aerial dissemination of human and plant pathogens between regions separated by thousands of kilometers. However, the relative contribution of long and short-range transport of microorganisms in the overall composition of airborne microbial communities remains unclear. Probabilistically, proximity should have an effect and local sources of microorganisms should contribute significantly to atmospheric microbial communities. We investigated the diversity and abundance of both bacterial and fungal populations over time at nine different meteorological stations around the world using Illumina MiSeq amplicon sequencing and qPCR analyses. Simultaneously, we measured the particulate matter chemistry and local meteorological characteristics. We found that the nearby (within 50 km) ecosystems were the main contributors to the composition of airborne microbial communities. We observed a different temporal stability in the composition of airborne bacterial populations depending on the site. This was mainly explained by the richness and evenness of the surrounding earth surfaces and the wind direction variability over time. Airborne microbial populations appear to be the result of large inputs from nearby sources with possible low and diluted inputs from distant sources. The current context of global warming and land use changes might have an important effect on the concentration and composition of airborne microbial communities.

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Moisture differently affects methane microbial communities of forest and pasture soils in the Eastern Amazon

A. M. Venturini[1], N. M. S. Dias[2], J. B. Gontijo[1], C. A. Yoshiura[1], A. G. da França[1], B. J. M. Bohannan[3], S. M. Tsai[1]
[1]Center for Nuclear Energy in Agriculture - University of São Paulo, Cell and Molecular Biology Laboratory, Piracicaba, Brazil
[2]Center for Nuclear Energy in Agriculture - University of São Paulo, Environmental Biogeochemistry Laboratory, Piracicaba, Brazil
[3]Institute of Ecology and Evolution - University of Oregon, Eugene, OR, United States

Climate change in the Amazon has the potential to alter the precipitation regime on a regional scale and, consequently, soil moisture. Moisture is a major driver of methane (CH_4) fluxes from soil, which are controlled by the balance between the activity of microbial producers (methanogens) and consumers (methanotrophs). Our study focused on the influence of moisture on CH_4 fluxes and microbial communities of the main land uses – forest and pasture – in the Brazilian Eastern Amazon. Based on the moisture and field capacity (FC) of each soil, a microcosm experiment was established with four treatments: original moisture; 60%, 80%, and 100% FC. The experiment was carried out for a 30-day period, in which gas samples were periodically collected for gas chromatography analysis. Following DNA extraction, the abundance of methanogens (*mcrA*) and methanotrophs (*pmoA* and *mmoX*) was evaluated by quantitative real-time PCR (qPCR), while their taxonomic and functional profile, by metagenomic sequencing. Cumulative carbon dioxide emissions did not change among treatments. CH_4 daily fluxes presented positive and negative results for forest and pasture, demonstrating that soil can act as a source and a sink for this gas, while its cumulative fluxes were strongly correlated with the moisture content. These results, as well as the abundance of methanogenesis genes and related groups, analyzed by qPCR and metagenomics, were influenced by land-use, moisture, and their interaction, with the highest emission and abundance values found in pasture soils under 100% FC. Although the methanotrophy results showed greater variation between the datasets, similar trends were found. The methanogenic community was composed exclusively of Archaea from the Euryarchaeota phylum and did not change in diversity and evenness after the experimental period. Proteobacteria of the alpha (72%) and gamma (21%) classes were the most dominant methanotrophs followed by Verrucomicrobia (7%). Diversity and evenness indices of the methanotrophic community were also affected by the studied factors, presenting the highest values for pasture soils under 100% FC. Taken together, the results showed that CH_4 fluxes and microbial communities responded to land-use changes so that pasture exhibited a higher emission potential than forest soils, which was more clearly observed by increasing moisture. Support: FAPESP (2014/50320-4, 2015/13546-7, 2017/09643-2), CNPq (140032/2015-0), and CAPES - Finance Code 001.

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The function and activity of microbial communities in subarctic soils

S. Viitamäki[1], I. Pessi[1], A. M. Virkkala[2], P. Niittynen[2], M. Luoto[2], J. Hultman[1]
[1]University of Helsinki, Department of Microbiology, Helsinki, Finland
[2]University of Helsinki, Department of Geosciences and Geography, Helsinki, Finland

Introduction: Soil microbial communities have critical role in biogeochemical processes on Earth, but our understanding of their response to the ongoing climate change is poor. Arctic and subarctic soils harbor approximately 50 % of Earth"s below ground carbon. Warmer climate leads to increased rate of soil organic matter decomposition in polar regions, but the overall impact to carbon and other biogeochemical cycles is difficult to predict without a deeper understanding of the soil microbial ecology.
Objectives: Our aim is to improve the knowledge of the ecology of microbial communities in subarctic soil in changing climate conditions, and to consider how this data could be used to predict the microbial feedback to environmental change.
Materials and methods: Metatranscriptomics was applied to elucidate the functions and activity of microbial communities in subarctic soils collected from Kilpisjärvi, northern Finland. The sampling site forms a climate gradient, which represents the possible scenarios of the impact of climate change to soil microbial community functions. Various metadata including pH, carbon content, soil organic matter, support the metatranscriptomic data.
Results: Our data shows differences in the composition and activity of the microbial communities along the climate gradient. Soil pH, organic matter and moisture were the main drivers of activity in organic layer, whereas pH was in the mineral layer.
Conclusions: Our results give information on how environmental factors contribute to microbial activity and again its feedback effect to warming climate.

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Exploring the culturable microbiome diversity and biotechnological potential in corals

C. Vilela[1,2], F. do Carmo[3], C. Rachid[1], H. Villela[1,2], H. Santos[4], C. Pereira[5], R. Peixoto[1,2]
[1]*Federal University of Rio de Janeiro, General Microbiology, Rio de Janeiro, Brazil*
[2]*Marine Aquarium of Rio de Janeiro - AquaRio, Rio de Janeiro, Brazil*
[3]*Innovation Agency- Federal University of Rio de Janeiro, Rio de Janeiro, Brazil*
[4]*Federal Fluminense University, Rio de Janeiro, Brazil*
[5]*Projeto Coral Vivo, Porto Seguro, Brazil*

Corals are marine animals that harbor highly variety communities of symbiotic microorganisms, that have many functions indispensable for the host survival, as adaptation, resilience, protection of pathogens and nutrient cycling. Enzymes from marine microorganisms tend to have peculiar characteristics that allow corals to adapt and develop in heterogeneous marine environments. This fact makes corals so relevant for discovery of enzymes of biotechnological interest. Any other way, the investigation of the diversity of coral"s microbiome is so important. This area needs to be more explored for discovery new microorganisms and know if different coral species living under the same environmental conditions can present distinct biotechnological potentials. Then, the aim of this study was assess the similarities of the microbiome profile (by dependent and independent cultivation techniques), the abundance of bacteria by qPCR and to investigate the production of enzymes of bacteria isolated from coral species from Recife de Fora, Brazil: *Mussismilia braziliensis* (*Mb*), *Millepora alcicornis* (*Ma*) and *Porites astreoides* (*Pa*). The results shows that the Bacteria, archaea and microeukaryote community structures indicated specific associations for each coral specie studied, corroborating the hypothesis that microbiota is host-specific, being *Exiguobacterium, Bacillus* and *Halomonas* the most abundant bacterial genera found. Between the forty-nine bacterial isolated, 8 of them were new species candidates. Enzymatic assays showed that 61% of the isolates produced lipase, 57% caseinase, 26% amylase, 16% gelatinase, 12% cellulase, 12% chitinase and for the first time related in coral microbiota, 37% produced keratinase. The ordination demonstrated that the bacterial community of the seawater is completely different that found in corals. Among the corals, *Mb* and *Pa* are closer while *Ma* is distant. The taxonomic analysis demonstrated different profiles between samples. At phylum level, Proteobacteria was the most abundant in all samples, but the second one in seawater was Cyanobacteria, and in coral *Ma* was Firmicutes, in *Mb* was Planctomycetes and in *Pa* was Firmicutes. This study demonstrates the importance of exploring the microbiome of coral for discovery new biotechnological products and microorganisms species, and use this knowledge in industries, adding economic value to the coralline ecosystem and preserving coral reefs and their diversity.

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Identification of key-players in anaerobic alkane oxidation in the diffuse venting Barite Field at the Loki´s castle

F. Vulcano[1,2], R. Stokke[1,2], H. Dahle[1,2], E. Reeves[3,2], D. Roerdink[3,2], C. Hahn[4], G. Wegener[5], I. H. Steen[1,2]
[1]*University of Bergen, Department of Biological Science, Bergen, Norway*
[2]*K.G. Jebsen for DeepSea Research, Bergen, Norway*
[3]*University of Bergen, Department of Earth Science, Bergen, Norway*
[4]*Max Planck Institute for Marine Microbiology, Department of Molecular Ecology, Bremen, Germany*
[5]*Max Planck Institute for Marine Microbiology, HGF MPG Joint Research Group for Deep-Sea Ecology and Technology , Bremen, Germany*

The Loki's Castle Vent Field (LCVF), located along the central region of the Arctic Mid-Ocean Ridge, is a sediment-influenced hydrothermal system. High-temperature end-members fluids (~ 320 degrees) emitted from this site are characterized by a high concentration of thermogenic CH_4 (12 - 15 mmol/kg) and short-chain hydrocarbons (151-180 µmol/kg of ethane C_2H_6, 0.54-0.71 µmol/kg of butane C_4H_{10} (1). The LCVF includes an area of diffuse venting known as the Barite Field, where hydrothermal fluids discharge from small barite chimneys and sediments. Here, the mixing between the main endmember hydrothermal flow and the seawater results in milder temperatures. Significant input of hydrocarbons to this latter system supports extensive populations of putative anaerobic alkane oxidizers (AAOs) like ANME-1 and GoM-Arc1 archaea (2). The objective of this study is to describe the role of AAOs and their sulfate-reducer partners in short-chain hydrocarbons consumption at LCVF. To address this topic we reconstruct the phylogeny of McrA functional genes, we analyzed metagenome-assembled genomes (MAGs) of AAOs and visualized target organisms with CARD-FISH microscopy.
Phylogenetic analyses of the alpha subunit of the methyl-Coenzyme M reductase (McrA) suggest a methane-based metabolism in ANME-1 whereas one divergent McrA is identified in the GoM-Arc1 MAG indicating use of a different alkane, most likely ethane. CARD-FISH analyses of chimneys and sediments collected from different regions of the barite field reveal the presence of AAOs obligate syntrophs, in particular SeepSRB2, SeepSRB1 and HotSeep-1. The abundances of the AAOs and their relative partners are comparable in the majority of the chimneys analysed. An interesting exception is represented by the actively venting sediments, where the fraction of syntrophs is considerably lower than the fraction of ANME-1 . Altogether our results suggest that the Barite Field in the LCVF provides an invaluable locality to study AAO, a results of the unique geological and geochemical features found in this hydrothermal vent system.
(1)Baumberger et al.(2016), Fluid composition of the sediment-influenced Loki"s castle vent field at the ultra-slow spreading Arctic Mid-Ocean ridge. Geochim. Cosmochim Acta 187:156-178
(2)Steen et al. (2016), Novel Barite Chimneys at the Loki's Castle Vent Field Shed Light on Key Factors Shaping Microbial Communities and Functions in Hydrothermal Systems. Front. Microbiol. 6

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RopB protein of the root nodule bacterium *Rhizobium leguminosarum* possesses amyloid-forming properties

M. Belousov[1,2], A. Kosolapova[1,2], M. Belousova[1], K. Antonets[1,2], O. Shtark[2], E. Vasilyeva[1,2], A. Nizhnikov[1,2]
[1]*All-Russia Research Institute for Agricultural Microbiology, Laboratory for Proteomics of Supra-Organismal Systems, Saint Petersburg, Russian Federation*
[2]*Saint Petersburg university, Genetics and Biotechnology, Saint Petersburg, Russian Federation*

Scientific question and context: Amyloids are a group of protein aggregates possessing a set of unusual features including high resistance to ionic detergent or protease treatment and ability to induce transition of some proteins from soluble to aggregated form. They play important pathogenic and functional roles and have been found in different taxonomic groups, from prokaryotes to humans and animals. Nevertheless, the observations of the last decades demonstrated the ability of amyloids to perform diverse physiological functions. The majority of functional amyloids of bacteria were identified within *Gammaproteobacteria* species including *Escherichia coli* and *Salmonella enteritidis*. Functional amyloids of *Gammaproteobacteria* play important roles in the biofilm formation, act as a storage of toxins and cause hypersensitive response in plants.
Methods: We performed screening for novel amyloid-forming proteins in the proteome of *Rhizobium leguminosarum*, an agriculturally important species of *Alphaproteobacteria*. Next, we analyzed the obtained data by various molecular genetic methods: PSIA, SDS-PAGE and C-DAG.
Results: Using previously developed Proteomic Screening and Identification of Amyloids (PSIA) approach, we identified 54 potentially amyloidogenic detergent-resistant proteins of *Rhizobium leguminosarum*. For further analysis, we selected the RopB protein that belongs to the Omp25/RopB family of the outer membrane proteins containing the trans-membrane beta-barrel domain. We produced recombinant RopB protein in the *E. coli* bacteria, and its ability to aggregate was shown. We determined the detergent-resistant properties of the obtained RopB aggregates using sodium dodecyl sulfate–polyacrylamide gel electrophoresis (SDS-PAGE). Moreover, RopB fibrils produced *in vivo* on the surface of bacterial cells in the Curli-Dependent Amyloid Generator system (C-DAG) were shown to demonstrate properties typical for amyloids including unbranched morphology and apple-green birefringence upon binding of amyloid-specific dye Congo Red.
Conclusions: Taking together, our results provide an opportunity to conclude that RopB is an amyloid-forming protein of *Rhizobium leguminosarum*. This work was supported by the Russian Science Foundation (Grant No 17-16-01100).

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Interactions between the wasp *Asobara japonica,* its endosymbiont *Wolbachia,* and the remainder of the microbiome

P. Brinker[1], M. C. Fontaine[1,2], L. W. Beukeboom[1], J. Falcão Salles[1]
[1]*University of Groningen, Groningen, Netherlands*
[2]*University of Montpellier, MIVEGEC, IRD, CNRS, Montpellier, France*

Animals commonly host specific microbes, so-called symbionts. The relationship between hosts and symbionts is not a one-to-one interaction but needs to be considered in the context of the whole microbiome, i.e. the collection of all host-associated microbes. We set out to disentangle the interactions within the complex of host-symbiont-microbiome in the parasitic wasp *Asobara japonica* infected with the endosymbiont *Wolbachia.* The *A. japonica* infection with *Wolbachia* varies geographically and infected wasps show asexual reproduction, with an all-female offspring. However, *Wolbachia*-induced reproductive manipulation is imperfect, with infected females occasionally producing sons. We propose that this is due to microbe-microbe and host-microbe interactions. To investigate this hypothesis, we collected 3 uninfected (sexual) lines from 3 islands in the south of Japan and 7 infected (asexual) lines on the main island of Japan. As a first necessary step, we analyzed the microbiome of the collected field lines and their population structure. This is currently followed by the artificial removal of *Wolbachia* with heat shock and antibiotic treatment as the first manipulation of an interacting partner. In the future, we plan to manipulate each interaction partner singularly and measure the effect on the other partners, which will provide new insights into interactions within the complex of host-symbiont-microbiome.

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Analysis of bacteria associated to the medicinal plant *Origanum vulgare L.*

L. M. Castronovo[1], S. Del Duca[1], I. Strozza[1], S. Chioccioli[1], R. Ascrizzi[2], L. Pistelli[2], M. De Leo[2], S. Biffi[3], A. Lo Nostro[4], C. Calonico[4], R. Fani[1]

[1]*University of Florence, Biology, Sesto Fiorentino, Italy*
[2]*University of Pisa, Pharmacy, Pisa, Italy*
[3]*Giardino delle Erbe, Casola Valsenio, Italy*
[4]*University of Florence, Health Sciences , Firenze, Italy*

Introduction: Medicinal plants produce a lot of bioactive antimicrobial compounds of pharmacological interest. *Origanum vulgare* L. is a medicinal plant with several properties: its essential oil is recognized for its antiseptic, antimicrobial, antiviral, antioxidant activities. Increasing antibiotics resistance and emergence of multidrug-resistant (MDR) pathogens prioritize research to discover new antimicrobials.
Objectives: The aim of this work is firstly to characterize the bacterial communities associated to the medicinal plant *O. vulgare* to individuate species-associated bacteria that produce secondary metabolites active *vs* MDR strains. Secondly, to discover if the production of antimicrobial molecules by *O. vulgare* plant could be dependent on its associated microbiota.
Materials & Methods: Sampling of four different subspecies of *O. vulgare* has been performed, followed by extraction of endophytic bacteria from four compartments (flowers, leaves, stem, soil) and production of essential oils from a pool of plants belonging to the same variety. Analyses of bacterial communities through culture-dependent and independent methods, evaluation of antibiotic resistance and antagonistic interactions were performed.
Results: The antibiotic resistance of cultivable bacteria associated to *O. vulgare* has been evaluated, highlighting a widespread resistance to commercial antibiotics; moreover, antagonistic interactions among bacterial strains (within-niche and cross-niche), tested using cross-streak method, showed a marked antagonistic force. Strains that revealed the ability to inhibit strongly other strains have been also tested against human pathogens. Total DNA of each compartment from each pool of plants has been extracted, followed by 16S rRNA sequencing through Illumina MiSeq platform. Plasmid extraction and 16S rRNA gene sequencing of each strain were performed; in order to characterize the structure of the microbial communities, RAPD profiles have been investigated too. Furthermore, essential oils and aromatic waters produced from the four *O. vulgare* subspecies have been analyzed, highlighting marked differences between the varieties.
Conclusion: In conclusion this study has allowed to characterize bacterial communities associated to the medicinal plant *O. vulgare*, to evaluate antibiotic resistance and antagonistic interactions. A correlation between bacterial communities and *O. vulgare* secondary metabolites will be evaluated.

P 86

Sex and age taxonomical and functional differences of Egyptian mongoose gut microbiota

A. Conceição Pereira[1,2,3], V. Bandeira[4], C. Fonseca[4], M. Vieira Cunha[1,2,3]

[1]*INIAV, IP - National Institute for Agrarian and Veterinary Research, Oeiras, Portugal*
[2]*Centre for Ecology, Evolution and Environmental Changes (cE3c), Faculdade de Ciências da Universidade de Lisboa, Lisbon, Portugal*
[3]*Biosystems & Integrative Sciences Institute (BioISI), Faculdade de Ciências da Universidade de Lisboa, Lisbon, Portugal*
[4]*Departamento de Biologia & CESAM, Universidade de Aveiro, Aveiro, Portugal*

Gut microbiota has been progressively acknowledged as a fundamental component of mammals' biology. Egyptian mongoose [*Herpestes ichneumon* (Linnaeus, 1758)] is a carnivore species, member of the Herpestidae family, whose distribution in Europe is restricted to the Iberian Peninsula(1,2). This species fecal microbiota has been previously studied by our group using culture-dependent methods but never using a culture-independent approach. In this work, we investigated the gut microbiota of 20 Egyptian mongoose specimens sampled in South Portugal, using Single Molecule Real-Time sequencing of the 16S rRNA gene to characterize its gut bacterial microbiota and to investigate sex- and age class-related taxonomical and functional differences. Our results show a core gut bacterial microbiota dominated by Firmicutes, Fusobacteria, Actinobacteria, and Proteobacteria. Four genera were uniquely found in females, and six in males, while eight genera were restricted to adult age class and five to juvenile'. Besides these compositional distinctions, the differential functional profile of this carnivore species was evaluated for the first time. Males showed a significantly higher abundance of amino acid and citrate cycle metabolic pathways, contrasting with females that evidenced a significant overrepresentation of galactose metabolic pathways. Also, specimens from the adult age class showed a significantly higher abundance of cationic antimicrobial peptide resistance pathways, when comparing with juveniles that exhibited a significant overrepresentation of two-component systems associated with antibiotic synthesis, flagellin production, chemotaxis control, and biofilm formation. These functional dissimilarities possibly reflect sex- and age-related differences in mongoose's diet and behavior, supporting the importance of gut microbiome characterization to fully comprehend mammal's ecology.
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P 87

Exploring the diversity of non-ribosomal peptide and polyketide synthases in rhizosphere bacteria

B. Dror[1,2], E. Cytryn[1], E. Jurkevitch[2]

[1]*ARO, Institution of Soil, Water and Environmental Sciences, Rishon LeZion, Israel*

[2]*Hebrew University of Jerusalem, Rehovot, Israel*

Introduction

The rhizosphere microbiome is known to positively affect plant health, for instance by inhibiting various plant pathogens. Non-ribosomal peptides and polyketides are secondary metabolites produced by complex enzymes encoded by large biosynthetic gene clusters (BGC) coined non-ribosomal peptide and polyketide synthases (NRPS/PKS). NRPS and PKS produced by soil bacteria are responsible for the synthesis of many siderophores, toxins and antimicrobials. However, despite their ecological importance, little is known about their diversity and dynamics within microbe-plant interactions

Objectives

Elucidate the scope, diversity and temporal dynamics and potential function of bacterial secondary metabolites in the rhizosphere, specifically focusing on NRPS and PKS BGCs.

Materials & methods

We used high-throuhput culture-independent methods, namely amplicon sequencing, metagenome and metatranscriptome, coupled with custom bioinformatic pipelines and a variety of manually-made and public secondary metabolites-related databases.

Results

The diversity and abundance of NRPS and PKS BGCs on tomato root surfaces was significantly different from that of the surrounding soil. Approximately 20% and 40% of these rhizoplane NRPS and PKS, respectively, were unique to the rhizoplane, and many of these were associated with genes encoding antimicrobials. Interestingly, the vast majority of identified NRPS and PKS amplicons shared only 70%-85% sequence identity to clusters encoding for known antimicrobials and antifungals. We also determined that the scope and diversity of these genes in culture independent analyses was much higher than in a wide collection of common cultured root-associated bacteria.

Conclusion

The rhizosphere microbiome harbors a profuse array of BGCs encoding potentially novel antibacterial and antifungal compounds that play a crucial role in bacterial-bacterial and bacterial-plant interactions and thereby shape this highly dynamic ecosystem.

Fig. 1

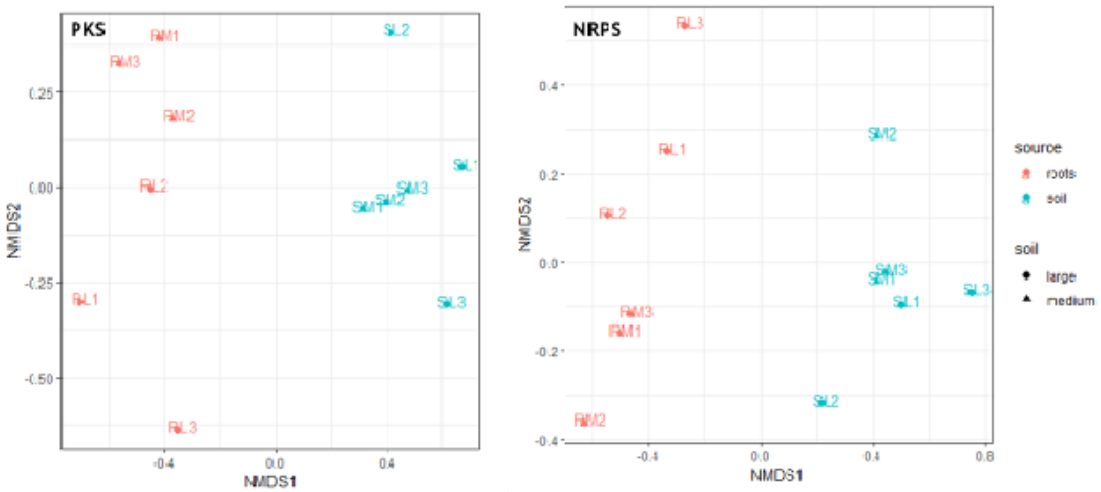
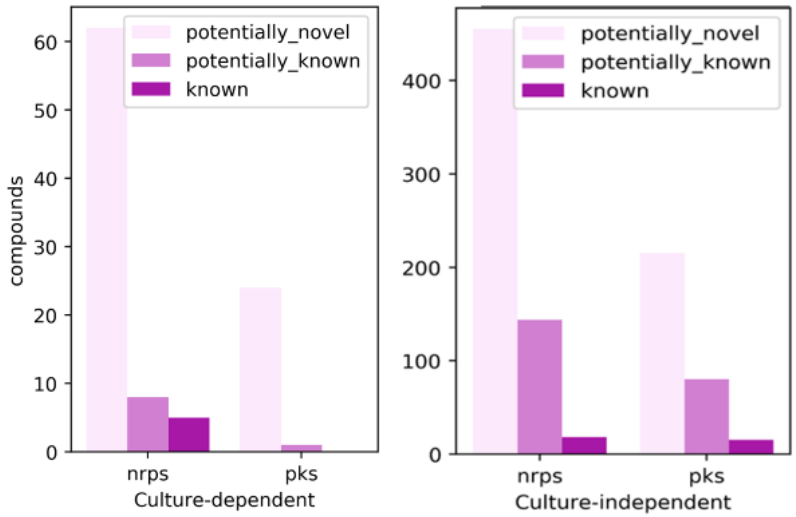


Fig. 2



P 88

The cypsela (achene) of *Echinacea purpurea* (Asteraceae) as a diffusion unit of a community of (micro)organisms?

R. Fani[1], M. Cardinale[2], M. Viola[1], E. Miceli[1], T. Faddetta[3], A. M. Puglia[3], V. Maggini[1], C. Tani[1], S. Schiff[1], P. Bogani[1], A. Mengoni[1], A. Papini[1], F. Firenzuoli[4]

[1]*University of Florence, Dept of Biology, Sesto Fiorentino, Italy*

[2]*3Dep Giessen Institute of Applied Microbiology, Research Center for BioSystems, Giessen, Germany*

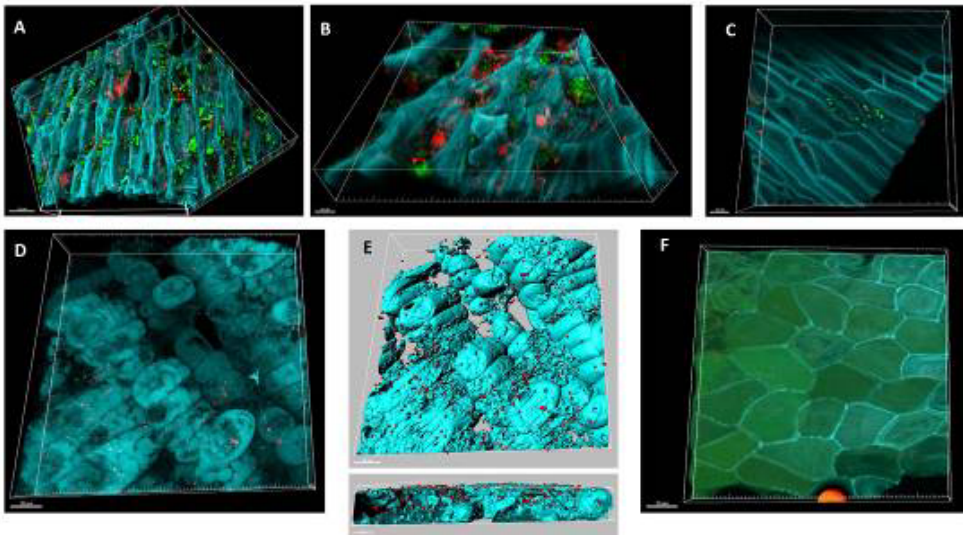
[3]*University of Palermo, Department of Biological, Chemical and Pharmaceutical Sciences and Technologies (STEBICEF), Palermo, Italy*

[4]*University of Florence, Referring Center for Phytotherapy,, Firenze, Italy*

In the last years it has been shown that the bacterial communities inhabiting different compartments (Roots, Stem/Leaves and Rizhosperic soils) of the medicinal plant *Echinacea purpurea* were very different in both structure and composition. The structuring of these communities might be driven by either antibiotic resistance and/or antagonistic activity. Moreover, using an *in vitro* system, it has also been shown that the presence of endophytes isolated from *E. purpurea* is related to the increase of plant alkilamides content and to the higher expression level of the valine decarboxylase (*VDC*) gene, involved in the biosynthesis of the amine moieties of alkylamides, strongly suggesting that the presence of endophytic bacteria might (directly or indirectly) be responsible for the biosynthesis of bioactive molecules, which are present in the medicinal plant extracts. The aim of this work was to check the presence of bacteria within the *E. purpurea* seeds using a combination of different techniques. Transmission electron microscopy of the seeds revealed the presence of bacteria inside the seeds and also at endocellular level. This finding was confirmed by Fluorescent in Situ Hybridization (Figure 1). Cultivable bacteria were isolated from the seeds and 37 of them were affiliated, through sequencing and analysis of 16S rRNA genes, to different species of *Paenibacillus* (19 isolates), *Pantoea* (16 isolates), and *Sanguibacter* (2 isolates). The Random Amplified Polymorphic DNA analysis revealed the existence of different strains belonging to the same species. The antibiotic resistance profile of each strain was also tested. Moreover, the genome sequence of endocellular strains is in progress.

Figure 1. **Confocal laser scanning microscopy images (volume-renderings) showing the bacterial colonization of *Echinacea purpurea* seeds.** Seed cryosections were stained by fluorescent *in situ* hybridization using the Cy3-labelled bacterial probe EUB338MIX. A) Microbial colonization of the seed surface; B) Three-dimensional model of panel A. C) FISH negative control (seed surface of sections stained with the non-sense probe NONEUB. D) Microbial colonization of the seed endosphere. E) Three-dimensional models of panel D. F) FISH negative control (seed endosphere of sections stained with the non-sense probe NONEUB. Red: bacteria; green: probably microalgae; blue/cyan: autofluorescence of seed tissues. Scale bars: A, B= 30 µm; C= 10 µm; D-F= 20 µm.

Fig. 1



P 89

Root microbiome changes in response to salt marsh elevation

D. E. Garcia Hernandez[1], E. Baraza-Ruiz[2], C. Smit[3], M. P. Berg[4,5], J. Falcão Salles[1]
[1]*University of Groningen, Microbial Community Ecology, Groningen, Netherlands*
[2]*Universidad de las Islas Baleares, Islas Baleares, Spain*
[3]*University of Groningen, Experimental conservation ecology, Groningen, Netherlands*
[4]*Vrije Universiteit Amsterdam, Animal Ecology, Amsterdam, Netherlands*
[5]*University of Groningen, Conservation Ecology, Groningen, Netherlands*

Salt marshes are ecosystems characterized by a strong zonation in plant communities, determined by marsh elevation and distance to the sea. Marsh elevation determines inundation frequency thus sediment input, soil aeration and soil chemical parameters, which consequently have an impact on soil microbial and plant communities. Few plant species can survive under these harsh environmental conditions. In the case of the salt marsh located on the Dutch Barrier Island Schiermonnikoog, a successful inhabitant of late successional stages is the perennial grass *Elytrigia atherica.* Till a few decades ago, this grass was distributed mainly at high marsh elevations. However, over time this species has invaded low marsh elevations, where it is exposed to a higher frequency of salt water inundation. In this study we explore whether *E. atherica* modifies the chemical conditions and microbial communities of the soil, via plant-soil feedbacks, in response to elevation. To achieve this, we explored the root associated microbial communities using High-throughput sequencing. We also measured plant traits and several soil physicochemical parameters. Our results show that elevation highly influences the soil properties (sodium, nitrates, ammonia, pH and organic matter content) driving the bacterial communities. Similarly, root microbiome (rhizosphere and endosphere) was highly influenced by the elevation, as indicated by the close association between certain bacterial families and elevation. Moreover, mycorrhizal colonization of roots was significantly higher at high elevation. The shifts in microbiome composition along the elevation gradient were correlated with differences in plant traits. Taken together, our study provides insights on how the selective forces driven by plant-microbe interactions might lead to the expansion of plant environmental range, which might be of great relevance in the context of future global changes.

P 90

Lichen microbiomes across time and space

M. Grube[1], T. Cernava[2], G. Berg[2]
[1]*University of Graz, Institute of Biology, Graz, Austria*
[2]*University of Technology, Institute of Environmental Biotechnology, Graz, Austria*

Lichen-forming fungi form unique morphologies through the association with compatible algae. By conglutination of vegetative hyphae, they form a coherent layer that covers a seemingly uniform population of algae and provide a persistent matrix for colonization of bacterial communities. These structures, known as lichen thalli, are perfectly suited to grow under oligotrophic conditions and periodic desiccation. We were interested to know to what extent bacteria contribute to the lichen symbiosis. Our previous culture-independent sequencing approaches of lichens revealed specific bacterial microbiomes in different lichens across their geographic range, usually with a dominance of alphaproteobacteria. Metagenomic and metaproteomic analyses of the lung lichen (*Lobaria pulmonaria* (L.) Hoffm.) provided first insights into the functional contributions of the bacterial microbiome to the lichen symbiosis by demonstrating bacterial nutrient supply and amelioration of stress, among other roles (1). We now retrieved metatranscriptomic datasets of lichen-associated bacteria. Lichens sampled under representative hydration stages revealed significant structural shifts and functional specialization to host conditions. Up-regulation of a specialized ketone metabolism indicated a switch to lipid-based nutrition under dry conditions, reminiscent of fasting (2). We suggest that a more comprehensive understanding of symbioses and new insights into the interplay of partners can only be gained if their mutual responses are studied over the whole range conditions encountered in the natural environment.

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P 91

Exploring the prokaryotic community associated with the Rumen ciliate protozoa population

E. Jami[1], B. Levy[1,2]
[1]*Agricultural Research Organization (ARO) Volcani center, Ruminant Science, Rishon LeZion, Israel*
[2]*Bar Ilan University, Faculty of Life Sciences, Ramat Gan, Israel*

Ciliate protozoa are an integral part of the rumen microbiome and were found to exert a large effect on the rumen ecosystem itself as well as their host animal physiology. Part of these effects have been attributed to their ability to harbor a diverse ecto- and endo-symbiotic community of prokaryotic cells. Studies on the relationship between the protozoa population and their associated prokaryotic community in the rumen mainly focused on the methanogens, revealing that protozoa play a major role in enhancing methanogenesis potential. In contrast, little is known about the composition and function of the bacteria associated with rumen protozoa and the extent of this association. In this study, we characterize the prokaryotic communities associated with different protozoa populations and compare their structure to the free-living prokaryotic population residing in the cow rumen. We show that the overall protozoa associated prokaryotic community structure differs significantly compared to the free-living community in terms of richness and composition. The methanogens proportion was significantly higher in all protozoa populations compared to the free-living fraction, while the Lachnospiraceae was the most prevalent bacterial family in the protozoa associated bacterial communities. Several taxa not detected or detected in extremely low abundance in the free-living community were enriched in the protozoa associated bacterial community. These include members of the Endomicrobia class, previously identified as protozoa symbionts in the termite gut. Our results show that rumen protozoa harbor prokaryotic communities that are compositionally different from their surroundings, which may be the result of specific tropism between the prokaryotic community and protozoa.

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Predatory bacteria and their impact on the microbiome of C. elegans

J. Johnke[1], H. Schulenburg[1]
[1]*CAU Kiel, Kiel, Germany*

The microbiome of natural isolates of the nematode *Caenorhabditis elegans* has been shown to be diverse and sample specific on the bacterial OTU level. In order to validate and expand these results we took *C. elegans* and associated substrate samples from the same location and at multiple time points in 2016 and 2017. 16S amplicon sequencing revealed changes in the worm"s microbiome over time. Substrate samples showed a higher amplicon sequence variant (ASV) richness than worm microbiome samples, with only little changes in composition over time. Co-occurrence analyses revealed that ASVs from worm microbiomes show a higher percentage of significant negative co-occurrences than ASVs from substrate samples. Additionally, networks generated from worm microbiome data were more complex then networks generated from substrate-derived data.
Worm samples show a more neutral OTU composition than substrate samples. Additionally, we found that the majority of *C. elegans* microbiome samples contained predatory bacteria that belong to the *Bdellovibrio*-and like organisms (BALOs). Worms that contained BALOs were further characterized by an overall more diverse and more neutral microbiome. Lastly, worms fed with a fluorescently labeled food bacterium showed a dramatic decrease of labeled bacteria in their gut after only three hours of incubation with *Bdellovibrio bacteriovorus* HD100. BALOs can be therefore considered as predictors and potentially drivers of microbiome diversity and thereby host fitness in cases of *C. elegans*.
As we additionally isolated a number of bacteria from these worms we can now directly test the impact of new BALO isolates on microbial community diversity in the context of the C. elegans microbiome.

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Succession of intestinal microbiota of a Eurasian wigeon while spending a winter

T. Kenzaka[1], H. Takehashi[1], S. Tsujita[1], K. Tani[1]
[1]*Osaka Ohtani University, Faculty of Pharmacy, Tondabayashi, Japan*

Understanding the transboundary movement of microorganisms is an important requirement from the perspective of public health and environmental science. Migratory birds carry pathogenic microorganisms and antibiotic-resistant bacteria when traveling over long distances to several parts of the world. In this study, we attempted to explore the stability of the intestinal bacterial communities in migratory birds, and the potential of long-distance movement of antibiotic-resistant bacteria associated with migratory birds.
We examined the succession of intestinal bacterial communities in the feces of a Eurasian wigeon, which was flying to the northern part of Osaka at the beginning of the winter season, staying in the same area when spending a winter. In December 2017, when the Eurasian wigeon flew to Japan, we examined the succession over time with monthly intestinal bacteria of Eurasian wigeon around the Ai River in north Osaka. Community analysis at the class level revealed that Clostridia constituted 64.7% in December, but the proportion decreased to 18.4% in April 2018 ($P < 0.01$). The proportion of Bacilli, Fusobacteria, Alphaproteobacteria, and Gammaproteobacteria significantly increased from 0.3% to 7.4%, 1.2% to 10.4%, 1.2% to 6.3%, and 4.1% to 29%, respectively ($P < 0.01$), while spending a winter in Japan. The intestinal bacterial community composition of Eurasian wigeon, which flew to Japan at the beginning of the winter season, significantly changed while staying in Japan for about four months.
The frequency distribution of the number of colistin-resistant *E. coli* per gram of the sample was also examined. In December 2017, the proportion of fecal samples below the detection limit was approximately 50%, and >100 CFU/g was about 50%. In March, the proportion gradually increased to approximately 70%, about 90% at the beginning of April, and 100% at the end of April 2018. For the colistin-resistant coliform, it was also found that the proportion of samples below the detection limit increased gradually while spending a winter in Japan. These results suggest that colistin-resistant *E. coli* and coliform may have been carried over to Japan after ingestion by the Eurasian wigeon in the northern area. Research on the spread of bacterial populations would led to the elucidation of the roles of migratory birds regarding human health risks.

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Host genetic selection for cold tolerance shapes microbiome composition and modulates its response to temperature

F. Kokou[1], G. Sasson[1], T. Nitzan[2], A. Cnaani[2], I. Mizrahi[1]
[1]*Ben Gurion University of the Negev, Life Sciences, Be'er Scheva, Israel*
[2]*Agricultural Research Organization, Rishon LeZion, Israel*

The hologenome concept proposes that microbes together with their hosting organism are an independent unit of selection. Motivated by this concept, we hypothesized that thermal acclimation in poikilothermic organisms is connected to their microbiome composition due to their inability to maintain their body temperature. To test this hypothesis, we used a unique experimental setup with a transgenerational selective breeding scheme for cold tolerance in tropical blue tilapias (Figure 1). We tested the effects of the selection on the gut microbiome and host transcriptomic response on individuals that were not pre-exposed to the stressor. Interestingly, we found that host genetic selection for thermal tolerance shapes microbiome composition and its response to cold. The microbiomes of cold-resistant fish showed higher resilience to temperature changes, indicating that the microbiome is shaped by its host's selection for environmental tolerance (Figure 2). These findings are consistent with the hologenome concept and highlight the connection between the host and its microbiome's response to the environment.

Fig. 1

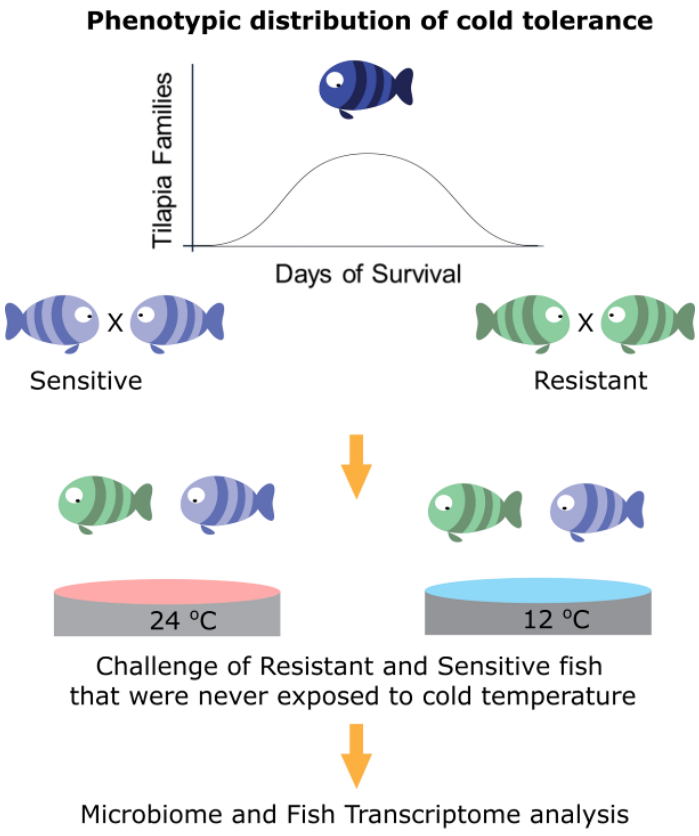

Figure 1. Experimental setup

Fig. 2

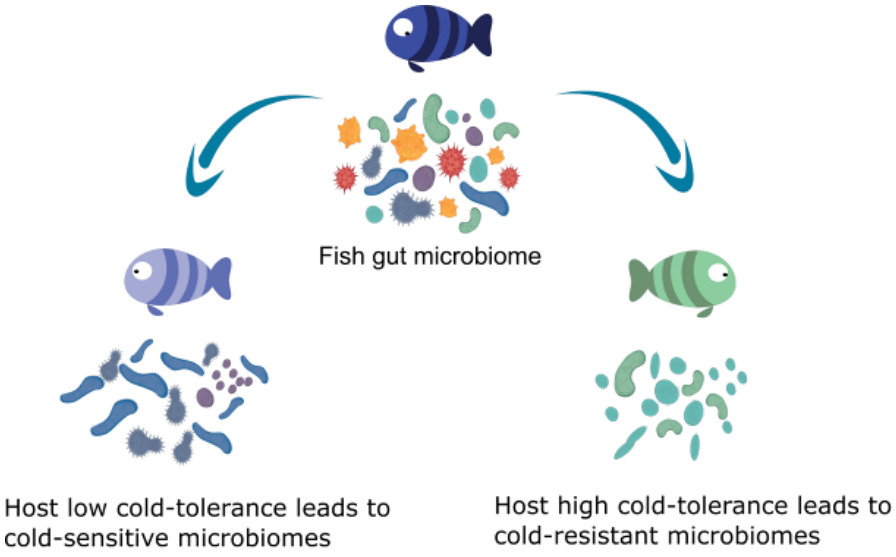

Figure 2. Host cold-tolerant phenotype selection is accompanied by a cold-tolerant microbiome

P 95

Unravelling the entomopathogenic potential of tropical multi-trait plant growth-promoting rhizobacteria *Bacillus* RZ2MS9

D. P. Longatto[1], B. D. Batista[2], J. R. P. Parra[3], J. L. Azevedo[1], M. C. Quecine[1]
[1]*University of São Paulo - College of Agriculture Luiz de Queiroz, Department of Genetics, Piracicaba, Brazil*
[2]*BASF S.A, Brazil, Brazil*
[3]*University of São Paulo, Department of Entomology and Acarology, Escola Superior de Agricultura "Luiz de Queiroz", Piracicaba, Brazil*

The Plant Growth Promoting Rhizobacteria (PGPR) inoculation has been emerging as an eco-friendly alternative to sustain agricultural yield against depletion of genetic and chemical resources and pest attack. Multi-trait growth ability is preferred but not always completely understood in screening works. Thus, in this work, we revealed the *Bacillus* RZ2MS9 ability as entomopathogenic strain and supported the correct classification of this strain as *B. thuringiensis.* A phylogenetic tree performed with sequences of pyruvate carboxylase gene (*pycA*) of RZ2MS9 plus other 105 *Bacillus cereus* sensu latu strains grouped *Bacillus* RZ2MS9 in clade along with commercial entomopathogenic *Bacillus thuringiensis* strains. Comparison of Functional COG classification of the annotated genes of the 8 strains present in the clade showed similar distribution, suggesting security to RZ2MS9 use as an inoculant. Moreover, RZ2MS9 Insecticidal Crystal Proteins (ICP) produced in vitro were cuboid and spherical consistent to entomocidal potential against Lepidoptera and Coleoptera insect orders. Furthermore, RZ2MS9 ICPs showed statistically similar mortality of respectively 90% and 92,5% comparing to 100% DiPel® (*B. thuringiensis* subsp *kurstaki* HD1) mortalities (Tukey test 5% of confidence) when feed up to major tropical lepidopterans pests sugarcane borer (*Diatraea saccharalis* F.) and old-World cotton bollworm (*Helicoverpa armigera* Hübner). Moreover, the phylogenetic and entomopathogenic findings aside remarkable plant growth promotion unfolded *B. thuringiensis* RZ2MS9 as a unique crop inoculation choice, a piece of Brazilian biodiversity potential to make plants healthier and more productive.

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Fate and impact of the antibiotic Sulfametoxazole on soil microbial communities: a spatial distribution problem

J. M. F. Martins[1], L. Spadini[1], E. François[1], E. Vince[1], M. C. Morel[1,2]
[1]*Institut Geoscience & Environemment, IGE UMR 5001, Grenoble, France*
[2]*CNAM, Laboratoire d'analyses chimiques et bio analyses, Paris, France*

The fate of Sulfamethoxazole (SMX), a sulfonamide antibiotic widely spread in natural soils and waters, was evaluated in batches and columns of a silty-loam soil under culture from Feucherolles (INRA Versailles, France). SMX revealed to be a weak sorbent since in batches only 10% of SMX sorbed at a solid/solution ratio of 0.1. It"s sorption increased strongly with soil organic matter content (addition of manure), indicating that the essential feature of OM addition is an increase in sorption sites density at almost constant sorption strength, and confirms previous results about the strong influence of sorbed complexants such as Cu(II). The mobility of SMX evaluated in water-saturated columns showed higher mobility of SMX than expected from the sorption study. Unexpectedly, this increased mobility was even higher in the OM-amended soil, suggesting that soil OM contributes to antibiotics transport in soils. Batch degradation experiments revealed that SMX removal is quite fast with half-life values ranging between 18 and 350 days in non-sterile and sterile soils. This degradation process was shown to occur principally in both the coarsest and finest soil-size fractions, while almost no biodegradation was observed in the mass-dominant silty fraction of the soil in agreement with its low microbial biomass content. The impact of SMX on the soil bacterial community, evaluated through total biomass (16SDNA), qPCR (Antibiotic Resistance Genes, ARG, *sul*1 and *sul*2), DGGE fingerprinting and high throughput sequencing revealed important impacts of SMX on soil microbial biodiversity and species richness and the emergence of specific taxons, resistant to the antibiotic. These results permitted to characterize the global fate and impact of SMX in an agricultural soil. SMX appeared quite mobile in soil with enhanced mobility in presence of added OM. SMX appeared also quite readily biodegradable, especially when in contact with coarse and fine soil size fractions, where it had the strongest impact on soil bacteria.

Keywords: Antibiotics, SMX, organic matter, impact, DGGE, Miseq, ARG, biodegradation

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Colonization strategies of the dominant bacterium *Enteroccus mundtii* in the gut of host larvae, *Spodoptera littoralis*

T. Mazumdar[1], B. S. Teh[1], A. Murali[2,1], W. Boland[1]
[1]*Max Planck Institute for Chemical Ecology, Department of Bioorganic Chemistry, Jena, Germany*
[2]*Friedrich Schiller University, Department of Microbiology, Jena, Germany*

The complex interaction amongst a higher organism and its resident gut flora is a subject of immense interest in the field of symbiosis. The larvae of *Spodoptera littoralis*, harbor a simple and dynamic bacterial community that varies along its life stages. One of the dominating gut bacteria, *Enterococcus mundtii,*produces a class IIa bacteriocin called mundticin KS that is bacteriocidal against potential pathogens[1].Bacteria in the gut of *S. littoralis* are exposed to adverse conditions, such as oxidative stress, alkaline pH, and diminished iron availability. Since *S. littoralis* larvae produce the iron chelating compound 8-HQA, their gut environment is diminished in iron. This work addresses how *E. mundtii* survive the gut environmental stresses and persist throughout the life cycle, and also throws light on the type of interaction between the host and this bacterium.

A methodology has been standardized to address the real-time conditions of *E. mundtii* in the gut. A GFP-tagged reporter *E. mundtii* has been constructed[2], sent down the guts of the larvae, and retrieved using a flow-cytometer after the reporters have aligned their gene expression profile according to the gut environment. A transcriptomic analysis of the retrieved bacteria from the host gut was performed.

RNA-sequencing of the FACS-sorted bacteria has informed us about various strategies of the symbiont's survival[3]. There are upregulated pathways for stress tolerance: alkaline stress, biofilm formation, nucleotide metabolism for colonization, two-component signaling system, resistance towards oxidative stress and quorum sensing. Although these symbionts seem to depend on the host for amino acid and fatty acids, they have an enriched lysine synthesis pathway in the hindgut of the larva, indicating their lysine contribution towards the host.

E. mundtii indeed is capable of reigning supreme by exercising a colonization resistance. This method with reporter organism reported us about their survival strategies straight from the host gut.

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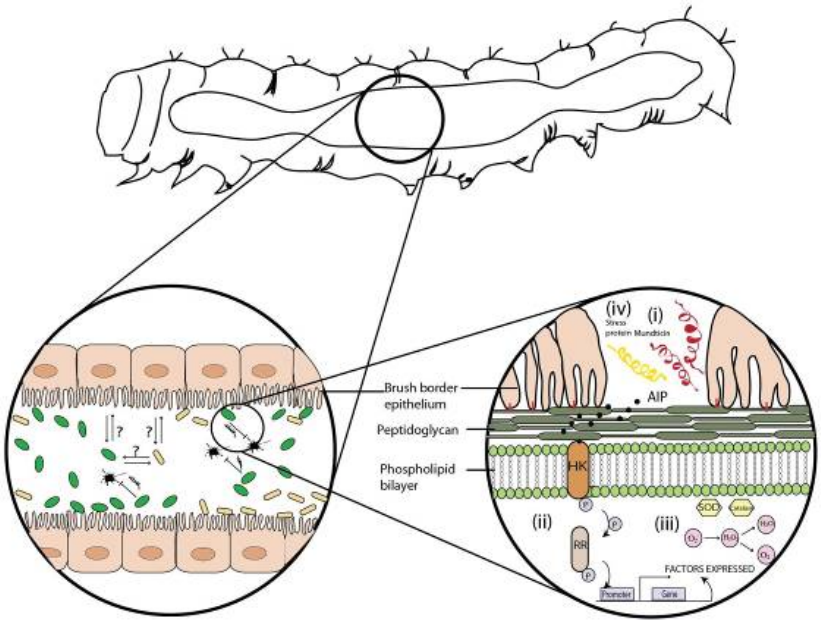
Fig. 1



The gut microbiome of *S. litoralis* dominated by *E. mundtii* and *Clostridia* sp. (A) Overview of the gut structure of fifth-instar larva of *S. litoralis*. (B) Illustration from within the gut space, which harbors major symbionts *E. mundtii, Clostridia* sp. and other bacteria. Bacteria adhere to the mucus layer of insect gut epithelium. Unknown interactions occur between microbe-microbe and host-microbe. (C) Illustration of some major expressed pathways *E. mundtii* used for survival in the gut. (i) Secretion of mundticin, an antimicrobial peptide, keeps pathogens at bay and helps the *E. mundtii* dominate the colonization process. (ii) A two-component system involving the accessory gene regulator (agr) system, which directs a histidine kinase to phosphorylate the response regulator, leads to the activation of transcription factors required for adaption. (iii) The induction of superoxide dismutase and catalase to manage oxidative stress leads to the conversion of superoxide radicals to water and oxygen. (iv) General or universal stress proteins help to overcome diferent kinds of stresses, such as oxygen starvation, heat or oxidative stress

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Evolution-mediated priority effects in the assembly of gut microbiome and consequences for host fitness

S. Mukherjee[1], M. Declerecq[1], R. Props[2], K. Korzeniowski [1], N. Boon[2], L. De Meester[1]
[1]*KU Leuven, Biology, Leuven, Belgium*
[2]*University of Ghent, Ghent, Belgium*

Introduction: Majority of research on community assembly in gut microbiome focuses on, either deterministic niche-related processes or, on neutral processes. The importance of evolution or historical contingency has received much less attention. Priority effects – i.e. the impact that an earlier arriving species can have on community assembly trajectories – can likely be very important during the colonization of gut. Although often attributed to purely ecological mechanisms (niche preemption and niche modification), priority effects might also be mediated by local genetic adaptation of earlier colonizer. In evolution-mediated priority effects or niche monopolization, local genetic adaptation augments transient numerical priority effects by accelerating the initial growth rate of the early colonist and promotes its long-term dominance by equalizing or elevating its fitness relative to later-arriving taxa. More specifically, the speed of evolutionary change of the original colonist relative to the speed of arrival of further immigrants determines the scope for eco-evolutionary dynamics.
Objectives: In this study, using *Daphnia magna* as a model host organism, we tested the hypothesis that evolution-mediated priority effects can play an important role in the assembly of gut microbiome and can exert an effect on the host fitness.
Methods: We performed reciprocal time-lag inoculations of four bacterial strains (isogenic and non-isogenic pairs) in germ-free *D. magna* juveniles. After 8 days and 12 days of inoculations, gut dissections were performed and DNA was isolated from the gut of these animals. Using digital PCR, we measured the absolute abundances of each strain in the gut of experimental animals. We also scored the life-history traits (size at maturity, age at maturity, clutch-size and grazing rate) of hosts. To test whether the priority effects were evolution mediated or not, we performed re-inoculation experiment (*in vivo* serial passage) with the evolved and original strains.
Results and conclusion: We noticed significant differences in body size and age at maturity of animals that received a sequential inoculation of strains compared to the ones that received a simultaneous inoculation indicating the consequences of priority effects for host fitness. Calculation of absolute abundances with digital PCR and genomic analyses of bacterial strains are currently underway.

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Insights into plant factors driving rhizosphere establishment by *Bacillus velezensis*

G. Hoff[1], S. Sebastien[1], A. Arguelles-Arias[1], M. Ongena[1]
[1]*Liege University, Gembloux Agro-Bio Tech, Gembloux, Belgium*

Question: *Bacillus velezensis* is a root-associated bacteria which is particularly efficient in protecting plants against a wide range of pathogens, namely by direct antagonism, competition or defense priming. The use of *B. velezensis* as biocontrol agent for a sustainable agriculture is promising but suffers from some insufficient efficacy in practice and needs to be improved. One important point that remains unclear is the molecular dialogue that exists between *Bacillus* and his host allowing bacterial establishment in the rhizosphere. Recent sequencing of several *B. velezensis* strains suggests that more than 8.5% of the genome is devoted to the synthesis of numerous secondary metabolites, which make possible a complex signaling network inside of the rhizosphere.
Methods & Results: Nevertheless, *in planta* investigations revealed that only the cyclic lipopeptide surfactin could be detected during the first days of interaction between both partners. We show that surfactin biosynthesis by B. velezensis evolving in its natural rhizosphere niche is not only guided by intrinsic regulatory rules. Transcriptional expression is actually also reprogrammed by some host plant cues such as root cell wall polymers and exudates to further boost lipopeptide production. This is obviously of benefit for the bacterium as it favors rhizosphere establishment by improving motility and settlement of multicellular population/communities. In support to such bacterial taming, our data also illustrate why surfactin has to be released in substantial amounts in order to be used as a good also shared by the host to prime its immune-related defensive capacity and better resist further pathogen ingress.
Conclusion: Through this work, we provide new insights into the intricate molecular dialogue that can be established between B. velezensis and its host plant to make from the multifunctional surfactin lipopeptide a shared good.

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Surface metabolome together with sea water temperature and trace metals contamination shape the surface microbiote of the Mediterranean brown seaweed holobiont *Taonia atomaria*

B. Paix[1], N. Layglon[2], C. Le Poupon[2], S. D'Onofrio[2], B. Misson[2], M. Peirache[3], G. Culioli[1], J. F. Briand[1]
[1]*Université de Toulon, Laboratoire MAPIEM, EA 4323, Toulon, France*
[2]*Université de Toulon, Aix Marseille Université, CNRS, IRD, Mediterranean Institute of Oceanography (MIO), UM110, Toulon, France*
[3]*Parc National de Port Cros, Hyères, France*

In marine ecosystems, macroalgal surfaces are prone to be colonized by complex communities of microorganisms. Through the evolution, seaweeds and epiphytic microbes have developed a wide range of interactions defining an integrated entity called holobiont. Especially, algal surfaces constitute a privileged area of chemical interactions with the associated microbiome. Advances in technologies for microbiome sequencing and metabolome analysis have enabled these research fields to raise, allowing a better understanding of the impact of surface metabolites released by algae on their epiphytic microbial communities.
The cosmopolitan brown alga *Taonia atomaria* (Dictyotaceae) was shown to produce surface antifoulants (1). The impact of environmental factors on surface metabolome and epiphytic microbial communities of *T. atomaria* was investigated during its occurrence period (February to July) on five contrasted sites (North-Western Mediterranean Sea). Densities of heteroprokaryotic cells and bacterial alpha-diversity indexes showed a continuous increase during survey period whatever the sites. A distinct beta- and a higher alpha-diversity were observed in comparison to communities from surrounding sea water and biofilms formed on abiotic surfaces. The increase of sea water temperature was found to drastically impact the community composition for all sites (e.g increase of Bacteroidetes). Moreover, in two sites with distinct trace metal contamination profiles, specific bacterial taxa (e.g. Rubritaleaceae) were observed at the surface of *T. atomaria*. In line with a previous work (2), strong correlations between epibacterial communities and surface metabolome were supported using a multi-omics networking approach. Thus, several surface metabolites (e.g. DMSP and proline) already reported for their ecological significance (3, 4) seemed to play a key role in the colonization process during spring and summer.
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Chitin-degrading capacities of sponge and octocoral microbiomes

R. R. Silva[1], I. Raimundo[1], L. Meunier[2], T. Keller-Costa[1], R. Costa[1]
[1]*Instituto Superior Técnico, Universidade de Lisboa, Lisbon, Portugal*
[2]*Université libre de Bruxelles (ULB), Brussels, Belgium*

Chitin is the most abundant polymer of marine ecosystems. Chitin-degrading enzymes (chitinases) have wide applications e.g. in pathogen control, food-waste processing or bio-plastic production. We hypothesize that filter-feeding marine organisms such as marine sponges and octocorals are hotspots for chitin breakdown and novel chitinases as their associated bacteria metabolize the chitin that the hosts filter-concentrate from seawater. Here, we analyze the abundance of chitin degradation-encoding genes within 30 microbial metagenomes of octocorals (endo- and ectosymbiotic microbiome), sponges (endosymbiotic microbiome only) and their surrounding environments. We further use genomics and *in-vitro* bioassays to investigate the chitin-degrading capacities of cultured symbionts. Metagenomics-based assessments revealed that the frequency of chitinase-encoding genes in octocoral, sediment, and seawater microbiomes were similar to one another and higher than those in the sponge endosymbiotic microbiome. Furthermore, in the octocoral microbiomes, we found a significant enrichment of chitin-binding domain 3 (IPR004302) that enhances the cells' binding capacity to chitin, suggesting higher chitin-processing efficiency in octocorals, especially compared to sponges. Laboratory assessments of 42 distinct bacterial symbionts from the octocoral *Eunicella labiata* and the sponge *Sarcotragus spinosulus* revealed highly efficient *in-vitro* chitin degradation for the genera *Aquimarina (Bacteroidetes), Vibrio, Enterovibrio, Shewanella and Pseudoalteromonas* (*Proteobacteria*). Inspection of fully sequenced genomes available for 23 of these strains showed strong taxon-specific segregations in the type and copy number of chitinase-encoding genes per genome, and an unprecedented within- and across-taxon diversity of *chiA*-like genes sequences. While *Gammaproteobacteria* genomes showed both the highest number and diversity of chitinase-encoding genes, *Flavobacteria* and *Alphaproteobacteria* genomes displayed the lowest diversity and copy number of such genes per genome, respectively. In conclusion, filter-feeding marine invertebrates are a valuable source of diverse chitin-degrading bacteria and potentially novel biocatalysts. The hypothesized ability of octocoral microbiomes to optimally degrade chitin suggests that octocoral forests play important roles in mediating marine nitrogen and carbon cycles.

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Cultivation dependent studies on the methylome and transcriptome of plant-growth associated bacteria

M. Reisinger[1,2], H. Müller[2], G. Berg[2]
[1]*Austrian Centre of Industrial Biotechnology, Graz, Austria*
[2]*Institute of Environmental Biotechnology, Graz, Austria*

Plants live in close association with microorganisms that provide beneficial functions. These plant-beneficial bacterial strains have long since been isolated by researchers and employed as inoculum to improve plant growth and health. In this project, we aim to shed light on the role of DNA methylation as an epigenetic regulatory factor for host - and niche specific adaptation of plant-beneficial bacteria. We focused on phylogenetically closely related strains belonging to the genera *Serratia* and *Stenotrophomonas*, which are already well known for their plant-growth promoting and stress protecting capabilities. Despite the high genotypic similarities of our sample strains, the colonization competence and the ability to exhibit beneficial effects *ad planta* were shown to be host-specific. Gene-by-gene comparison revealed that the present repertoire of genomic features alone cannot explain the strain-specificity of the plant-microbe interactions.
We observed the differences in bacterial DNA methylation patterns and gene expression profiles in response to varying culture conditions. These conditions include both complex media (liquid and solid culture) as well as minimal media supplemented with plant exudates (tomato and maize roots). All strains exhibit distinct restriction-modification systems, suggesting that the epigenotype determines the specific phenotypes. Furthermore, our results indicate that the ability to regulate their expression profile by epigenetic means, such as DNA methylation, is a significant contributing factor for plant-host specificity. The results of this study will advance our understanding of the interplay between genotype, methylotype and phenotype of phylogenetically closely related plant-beneficial bacterial strains.

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Influence of a high diversity of the microbial community on the persistence of *Salmonella enterica* in agricultural soil

J. Schierstaedt[1,2], S. Jechalke[1,3], H. J. Barkowski[1], J. Nesme[4], K. Neuhaus[5], S. J. Sørensen[4], R. Grosch[2], K. Smalla[1], A. Schikora[1]
[1]*Julius Kühn-Institut, Institute for Epidemiology and Pathogen Diagnostics, Braunschweig, Germany*
[2]*Leibniz Institute of Vegetable and Ornamental Crops, Plant-Microbe Systems, Großbeeren, Germany*
[3]*Justus Liebig University Giessen, Institute for Phytopathology, Giessen, Germany*
[4]*University of Copenhagen, Section of Microbiology, Department of Biology, Copenhagen, Denmark*
[5]*Technische Universität München, ZIEL - Institute for Food & Health, Core Facility Microbiome/NGS, München, Germany*

In the last years, food-borne diseases were increasingly associated with contaminated fruits and vegetables. *Salmonella enterica* was one of the most frequent recorded causes of food-borne diseases in Europe. Contamination of fresh produce can occur along the whole production chain, including plant growth stage. Agricultural soils might be a potential reservoir of human pathogens, delivering an essential precondition for the colonization of plants via the rhizosphere. Due to the competition for colonization sites, indigenous soil microbiota play a major role in the success of invading species. We hypothesized that the level of diversity influences the persistence of *Salmonella* in agricultural soil. We analyzed the influence of soil bacterial diversity on the survival of three *Salmonella enterica* strains (Senftenberg, Typhimurium 14028s, Typhimurium LT2) in soil microcosm experiments using culture-dependent and -independent techniques. The persistence of *Salmonella* was indeed reduced in soil with high diversity of the native microbial community in comparison to the persistence in autoclaved soil. These results gave rise to the question if *Salmonella* adapts its lifestyle in this environment. Therefore, in an additional experimental set the transcriptomes of *Salmonella enterica* exposed to different soil-related media where analyzed by RNA-Seq. In the soil environment, the regulation of amino acid biosynthesis and the glyoxylate cycle were significantly changed. The results presented here underline the necessity of an integral approach and the importance to preserve a diverse soil microbiome in order to lower the number of disease outbreaks.

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Wolbachia endosymbiont* geographical distribution in the natural populations of the cotton leafhopper, *Amrasca devastans

M. Shafiq[1]
[1]*University of the Punjab, Institute of Agricultural Sciences, University of the Punjab Lahore, Pakistan, Lahore, Pakistan*

Wolbachia and Arsenophonus is a maternally transmitted endosymbiont, which is found in the reproductive and steroidogenic tissues of arthropods and nematodes. The cotton leafhopper, *Amrasca. devastans* (Distant) (Cicadellidae: Homoptera) is one of the major sap feeding pests of malvaceous and solanaceous crops in Pakistan and is broadly distributed throughout the cotton growing provinces, Sindh and Punjab. Live field specimens of *A. devastans* were collected from 8 different cotton field locations from Punjab province pakistan and preserved in 70% ethanol for genetic studies. The presence of *Wolbachia and Arsenophonus* in leafhopper was detected by partial 16s rDNA gene amplification and sequencing. Both Arsenophonus and wolbachia endosymbiont was detected in different leafhopper. Out of four sequences (SA7, SW1, SW2and SW3) obtained from this study SA7 and SW2 were found to be most similar (99.5% sequence identity) to the *Wolbachia* strain of *Bemisia tabaci* reported from India. While SW1 showed maximum homology (79% sequence identity) with *Wolbachia* strain of *Guignotus pusillus* and SW3 showed maximum homology with *Wolbachia* strain of *B. tabaci* (99% sequence identity) reported from China. The Arsenophonus was also detected in more than 10 samples using arsenophonus specific 23S ribososal RNA primers. This study is first evidence of Arsenophonus and wolbachia endosymbiont incidence in cotton leafhopper

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Constrained chaos in the pathobiome of a new slow-spreading coral disease

M. Sweet[1]

[1]*University of Derby, Environmental Sciences , Derby, United Kingdom*

Background: Coral reefs face unprecedented declines in diversity and cover, a development largely attributed to climate-change-induced bleaching and subsequent disease outbreaks. Coral-associated microbiomes may strongly influence the fitness of their hosts and alter heat tolerance and disease susceptibility of coral colonies. Here, we describe a new coral disease found in Micronesia and present a detailed assessment of infection-driven changes in the coral microbiome.

Results: Combining field monitoring, histological, microscopic and next-generation barcoding assessments, we demonstrate that the outbreak of the disease, named "grey-patch disease", is associated with the establishment of cyanobacterial biofilm overgrowing coral tissue. The disease is characterised by slow progression rates, with coral tissue sometimes growing back over the GPD biofilm. Community networks of the corals microbiome highlighted the dominance of co-occurring members, and microbes which were identified as "disease-associated" formed highly connected "infection-complexes" in the microbiomes of apparently healthy corals.

Conclusions: Our results appear to be in contrast to that recently proposed Anna-Karenina principle, which states that disturbances (such as diseases) trigger chaotic dynamics and increases β-diversity. Here we show significantly higher community similarity in the pathobiome of diseased corals than those associated with apparently healthy tissue. A possible explanation for this pattern is strong competition between members of the pathobiome and those of the "healthy" microbiome. Further, we demonstrate that synergistic effects between multiple pathogens are seemingly required to overcome coral immune defences in the case of the onset of this disease, a result which creates a structuring mechanism that homogenises the pathobiome.

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Plant root-microbiome responses to elevated CO_2

A. Usyskin-Tonne[1,2], Y. Hadar[1,2], U. Yermiyahu[1,3], D. Minz[1]

[1]*The Volcani Center, Agricultural Research Organization, Institute of Soil, Water and Environmental Sciences, Rishon LeZion, Israel*

[2]*Hebrew University of Jerusalem, Robert H. Smith Faculty of Agriculture, Food and Environment, Rehovot, Israel*

[3]*Agricultural Research Organization, Gilat Research Center, Soil, Water and Environmental Sciences, Negev, Israel*

Atmospheric CO_2 levels had been increasing for the last 150 years, stimulating growth and photosynthesis, and among others, affecting plant root deposition. The composition and quantity of these deposits depend on plant type and age, leading to altering structure and function of root microbiome reacting to these compounds.

In the current study, the effect of elevated CO_2 on wheat-root microbiome composition and plant development was studied, with special emphasis on denitrifying communities.

Wheat plants were grown in greenhouse for six weeks under two CO_2 levels: ambient CO_2 of 400 ppm (aCO_2); and elevated CO_2 of 850 ppm (eCO2) and plant and microbial parameters were measured. Total bacterial community was quantified using qPCR with universal 16S RNA gene primers, while denitrifying genes (e.g. nirK, nirS, nosZ) were measured using specific primers constructed based on metagenome analysis. In addition, total and N_2O reducing bacterial community in soils and roots were analyzed by amplicon sequencing.

In eCO_2, wheat plants developed faster, resulting in nitrate and ammonium deficiency in the soil and consequently decreased emission of the greenhouse gas N_2O. At the 6th week, total nitrogen concentrations in plant tissues was declined and increase in relative abundance of nirK and nirS genes and decrease in nosZ gene were observed in the bacterial community. Dynamics of total and denitrifying root bacterial communities was observed during wheat growth. In both communities, a higher dissimilarity in structure was observed between weeks at eCO_2 compared to aCO_2. Even greater dissimilarity between weeks of wheat growth was shown for nosZ community compared to a total community.

In conclusion, eCO_2 resulted in increased plant growth, accompanied by increased nitrate consumption, leading to changes in community structure and diversity of total bacterial populations and even more so in N_2O reducing community on the roots.

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Exploring reproductive microbiotas of a sexually promiscuous host

L. Veerus[1], S. B. Mombrikotb[2], E. Ransome[2], T. Bell[2], T. Pizzari[1]
[1]*University of Oxford, Department of Zoology, Oxford, United Kingdom*
[2]*Imperial College London, Department of Life Sciences, London, United Kingdom*

The Human Microbiome Project has catalysed research on microbes living both in and on their hosts. While the primary focus has been on gut microbiomes, other organ systems are beginning to be explored. One of the most intriguing systems is the reproductive tract, which allows studying horizontal bacterial transmissions between sexual partners, but also vertical bacterial transfers along the mother-offspring pathway. Such host-microbe interactions may affect host life history, mating system, and mate choice. In this study, we explore microbial communities in the male and female reproductive tracts of a bird host, the sexually-promiscuous red junglefowl (*Gallus gallus*). We carry out 16S rRNA processing on functionally-distinct samples from different regions of the male and female reproductive tracts, from male ejaculates, and from cloacal wipes of individuals in sexually-interacting social groups. We: (i) characterise spatial patterns of variation in community structure along male and female reproductive tracts, (ii) analyse patterns of temporal variation in ejaculate microbiota, and (iii) test whether sexual contact acts as a potential pathway for bacterial transmission by analysing patterns of variation in the cloacal samples of individual birds in relation to their sexual activity in the group. Our results suggest that the reproductive tracts harbour spatially-structured communities that are distinguished from the gut microbiota, despite the shared outlet with the digestive system. We discover two distinct microbial niches present in the reproductive systems: one structurally-bound to the epithelial tissue and one fluid excretion-bound. We also show inter- and intra-male variation in the structure of ejaculate microbiota, and quantify scope for sexual transmission of microbial species in free-ranging groups. Collectively, these results represent one of the most comprehensive surveys of reproductive microbiota in non-human animals.

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Exploring multi-domain probiotics and chemical remediation of oil-polluted marine waters and their impacts on hydrocorals health

H. Villela[1], D. Silva [1], H. Santos [2], G. Duarte[1], C. Fazolato[1], P. Rosado[1], J. Ribeiro[1], C. Simões[1], A. Ghizelini[1], C. Vilela[1], E. Santoro Peçanha[1],
F. Lima do Carmo[1], C. Rachid[1], R. Vega Thurber[3], D. Ximenes[4], A. Soriano[4], R. Peixoto[1]
[1]*Federal University of Rio de Janeiro, Microbiology Department, Rio de Janeiro, Brazil*
[2]*NUPEM/UFRJ, Microbiology Department, Macaé, Brazil*
[3]*Oregon State University, Microbiology Department, Corvallis, OR, United States*
[4]*PETROBRAS, CENPES, Rio de Janeiro, Brazil*

The burning of fossil fuels is the largest source of CO2 emissions, contributing to the increase of seawater temperature and to ocean acidification. Oil industry activities also contribute to ocean pollution, by chronic oil contamination and incidents resulting on spills. Additionally, compounds used to clean contaminated sites, such as chemical dispersants, are more harmful to marine life than oil itself. Coral reefs are the most biodiverse ecosystems in the oceans, being responsible for the persistence of numerous marine species. Corals are known by their associations with specific Beneficial Microorganisms (BMCs), that play important roles in the metaorganism homeostasis. Here, we address two main questions: 1) How calcifying-cnidarian associated bacteria exposed to chemical dispersants are changing compared to the ones exposed to oil and putative BMCs (pBMCs)? 2) Is it possible to select a microbial consortium with pBMCs that can protect corals from impacts caused by oil water-soluble fraction (oWSF) and chemical dispersant exposure, while it degrades oil? Our results show that the selected oil degrading pBMC consortium, which included members of different groups of fungi, yeast and bacteria (belonging to genera *Halomonas, Pseudoalteromonas, Cobetia, Shewanella, Ochrobactrum, Rhodotorula, Geotrichum* and *Penicillium*), can protect the hydrocoral *Millepora alcicornis* exposed to oWSF, while controls without pBMCs started to bleach after 12 days of exposition, which was accessed by coral morphology and photosynthetic efficiency (although this last result was not significant). By the other hand, hydrocorals exposed to Corexit 9500, responded to the stress regardless pBMC application, starting to bleach in less than 24 h of exposition. For the first time, we showed the chemical dispersant impact on a bacterial community associated with a calcifying-cnidarian host. Remarkably, the phylogenetic tree of the potential bioindicator OTUs of the presence and absence of dispersant reveal an evolutionary relationship among the groups affected by the contaminant. These results suggest that the chemical compounds preferentially affect bacterial groups sharing physiological or biochemical characteristics, favoring the growth of members belonging to the Family Rhodobacteraceae, such as *Shimia sp.*, *Labrenzia sp.* and *Thalassobius sp.*, and negatively affecting the presence of the well-known coral symbiont *Endozoicomonas sp*.

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Biodegrading two pesticide residues in rice crops and growth media by a genetically engineered approach

X. N. Su[1], H. Yang[1], Z. M. Yang[2]
[1]*Nanjing Agricultural University, College of Sciences, Jiangsu Key Laboratory of Pesticide Science, Nanjing, China*
[2]*Nanjing Agricultural University, College of Sciences, Nanjing, China*

Accumulating pesticide residues in soils and crops become a seriously environmental problem. Both plants and microbial organisms can remove and catabolize their surrounding organic xenobiotics through multi-specific mechanisms, but studies in the perspectives of plant-microbia are limited. Isoproturon (IPU) is a widely used phenylurea herbicide for controlling pre- or post-emergence weed, while acetochlor (ACT) as an acetanilide herbicide is applied to killing pre-emergence weed in farmlands. Due to their intensive use for crop production, the residues of the pesticides in soils are accumulating. The widespread of IPU/ACT residues in ecosystems pose the environmental risks to wild life and human health. This study built up an engineered paddy plant overexpressing a novel glycosyltransferase that can effectively remove two widely used pesticides IPU and ACT in plants and wetland. IRGT1 conferred resistance of IPU/ACT, manifested by enhanced plant growth and attenuated cellular injure. A short-term study (4-6 d) showed that the IRGT1-transformed lines removed 33.3−48.3% and 39.8−53.5% from growth medium, suggesting that soil media would help remove the pesticides through microbial media. We also found that the rice plants contained 59.5−72.1% and 58.9−70.4% IPU and ACT of the wide type, respectively. This phenotype was well confirmed by IRGT1-expression in yeast (*Pichia pastoris*), which grew better and contained less IPU/ACT than control cells. A long-term study showed that IPU/ACT concentrations at all developmental stages were significantly lower in transformed rice, which contained only 59.3-69.2% (IPU) and 51.7-57.4% (ACT) of those in wild-type. The degradation products of IPU and ACT were characterized by UPLC/Q-TOF-MS/MS. Sixteen metabolites of IPU (or nineteen for ACT) for Phase I reaction and nine conjugates of IPU (or thirteen for ACT) for Phase II reaction in rice were identified. More amounts of IPU (or ACT) metabolites and conjugates were detected in *IRGT1*-transformed overexpression rice than in wild-type. Our work unveiled a fundamental regulatory mechanism for enhanced IPU and ACT detoxification/degradation in plant and soils, and broadens our understanding of the insightful process that would be used realistically for extracting and degrading toxicants from environments and plants, thus reducing the health risks through food chains.

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The functional repertoire encoded within the C. elegans microbiome

J. Zimmermann[1], N. Obeng[2], C. Kaleta[1], H. Schulenburg[2]
[1]*Christian-Albrechts-University and University Hospital Schleswig-Holstein, Research Group Medical Systems Biology Institute of Experimental Medicine, Kiel, Germany*
[2]*Christian-Albrechts-University, Evolutionary Ecology and Genetics, Kiel, Germany*

The microbiome is generally assumed to have a substantial influence on the biology of multicellular organisms. The exact functional contributions of the microbial community are often unclear and cannot easily be inferred from 16S rRNA genotyping, which is commonly used for taxonomic characterization of the bacterial associates. In order to bridge this knowledge gap, we here analyzed the metabolic competences of the microbiome from the nematode Caenorhabditis elegans. We integrated whole genome sequences of 77 bacterial microbiome members with metabolic modelling and experimental characterization of bacterial physiology, and found that, as a community, the microbiome can synthesize all essential nutrients for C. elegans. Both metabolic models and experimental analyses further revealed that nutrient context can influence how bacteria interact within the microbiome. Moreover, we identified key bacterial traits that are likely to determine their colonization ability (e.g., pyruvate fermentation to acetoin) and also resulting effects on nematode fitness (e.g., hydroxyproline degradation). Considering that the microbiome is usually neglected in C. elegans research, the resource presented here can help to assess the microbiome"s influence on nematode life-history traits and, more generally, provide a framework to dissect microbiome-mediated functions.

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From head to toe – PGP Bacterial endophytes in three Mediterranean terrestrial orchid species

P. Alibrandi[1], M. Cardinale[2,3], N. Lo Monaco[4], S. Voyron[1], A. M. Puglia[4], S. Perotto[1]
[1]*University of Turin, Department of Life Sciences and Systems Biology, Torino, Italy*
[2]*University of Salento, Department of Biological and Environmental Sciences and Technologies, Lecce, Italy*
[3]*Justus-Liebig-University Giessen, Institute of Applied Microbiology, Giessen, Germany*
[4]*University of Palermo, Department of Biological, Chemical and Pharmaceutical Sciences and Technologies, Palermo, Italy*

Orchids are highly dependent on symbiotic microorganisms during their life cycle. Whereas an important role has been established for mycorrhizal fungi during orchid seed germination and development, the role of plant-associated bacteria is less understood. We investigated the diversity and functions of the endophytic bacterial microbiome associated with three Mediterranean terrestrial orchid species by culture-dependent and culture-independent approaches.
Endophytic bacteria were isolated from roots, stems, leaves and capsules of three orchid species (*Serapias vomeracea, Spiranthes spiralis* and *Neottia ovata*) collected in Ligury (Italy). Bacteria were found in all plant organs, with a significantly higher density in roots and leaves, and included genera such as *Bacillus, Pantoea, Pseudomonas, Streptomyces, Staphylococcus, Luteibacter, Rahnella, Fictibacillus* and *Microbacterium*. Endophytic *Pseudomonas* were isolated from most plant organs, whereas distribution of other bacterial genera was tissue specific.
Plant growth-promoting activities were assayed for these bacterial isolates, such as: 1) organic and inorganic phosphate solubilizing activities, 2) potassium solubilizing activity, 3) growth without nitrogen, 4) production of indole acetic acid and siderophores. These traits were found in many isolates, mostly from stems and roots. In addition, some isolates showed salt and drought stress tolerance, and antimicrobial activity against *Tulasnella calospora* (the mycorrhizal fungal symbiont of *S. vomeracea*). Overall, these results suggest that endophytic orchid-associated bacteria could play a role in growth promotion and plant defense.
A metabarcoding analysis showed Proteobacteria as the dominant phylum in all orchid species, followed by Firmicutes, Actinobacteria and Acidobacteria, although their distribution is plant organ-specific. A preliminary analysis of the core microbiome suggested that the three different orchid plants store potentially beneficial bacteria.
In conclusion, this work addresses for the first time bacterial diversity in various organs of three orchid Mediterranean species and could lead to new insights into their functional roles in the orchid plant.

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Beneficial microorganisms for Coral (BMC) Growth

J. Assis[1,2], R. Valle[2], F. Abreu[1], R. Marraschi[2], G. Duarte[1,2], R. Peixoto[1,2]
[1]*Federal University of Rio de Janeiro, Rio de Janeiro, Brazil*
[2]*Aquario Marinho do Rio de Janeiro, Rio de Janeiro, Brazil*

Corals have been suffering significant declines worldwide, as a result of global and local changes and impacts, which affects a wide diversity of marine species that inhabit this ecosystem. In order to support restoration initiatives, this work aims to create an experimental system to explore coral physiology and microbial therapy to promote coral growth. The coral model used was Pocillopora damicornis, one of the world's leading reef formers and for which we have already selected an efficient BMC (Beneficial Microorganisms for Corals - or coral probiotics) consortium. For that, 16 closed system tanks, with constant circulation of water and air, 400 µmoles.m-2.s-1 of light (photoperiod of 12/12 hours) were built to work with 04 different treatments in quadruplicates. As for the tested treatments, the previously tested and published BMC consortium was improved by the addition of BMCs selected by using selective urease, calcium carbonate and siderophore producers culture media. The new isolates obtained were identified through 16S rDNA sequencing. The new tanks system has already been tested and showed its efficiency in maintaining the replicates in similar and ideal conditions and the coral fragments had their taxonomic identification confirmed by the Veron method (1976). To improve our knowledge on different carriers and strategies for BMCs inoculation, the use of saline suspended BMC is being compared with its application through feeding, by the use of BMC enriched and not enriched rotifers. Several proxies are being utilized to evaluate the different treatments (feeding, enriched feeding, BMC and controls) such as the microbiome, reach, primary productivity, respiration, Fv/Fm rates and microscopy, in addition to analyzing the water physical-chemical data for the six months of the experiment.
Keywords: coral reef, climate change, calcification, microorganisms beneficial to corals (BMC), Pocillopora damicornis

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Investigating ungulate-associated bacterial, plant and parasite communities on a common landscape through comparative fecal pellet metabarcode analysis

W. Holben[1], S. Pannoni[1], J. Adcock[1,2], P. Ramsey[2], K. Dueño[1], L. Hinze[1]
[1]*University of Montana, Cellular, Molecular and Microbial Biology Program, Missoula, MT, United States*
[2]*MPG Ranch, Florence, MT, United States*

We compared metagenomic DNA purified from elk, mule deer, whitetail deer, and wild horse feces to survey bacterial, plant and parasite communities associated with each ungulate species. To accomplish this, we amplified the 16S rRNA gene for bacteria, and the internally transcribed spacer region (ITS) of the rRNA operon and 18S rRNA gene for plants, eukaryotes, and eukaryotic parasites. We also amplified the rubisco gene (*rbcL*) for additional survey coverage of plant species. We analyzed samples from 36 individual animals for elk, mule deer, and whitetail deer (cervids), and also 10 individual wild horse samples, using PCR to generate the 4 sets of amplicons for each sample. These were subjected to Illumina paired-end sequencing to generate approximately 50 million sequence reads in total for the four markers. For bacterial microbiome comparisons between the four ungulates, Alpha diversity was similar, with numbers of OTUs in the low thousands. Beta diversity of the wild horse microbiome was the most divergent from that of the elk and two deer species. When we compared just the cervids, a bioinformatic algorithm separated the elk microbiome from that of the two deer species. Whitetail and mule deer microbiomes were similar at the genus level. Interestingly, fungal sequences made up about 40% of the total 18S rRNA reads in the cervids. We are currently filtering the 18S, ITS and rbcL data for local plant taxa to conduct an individual-based diet analysis, and are comparing individual "parasitomes" from 18S reads. Information on ungulate diet, parasite diversity, and parasite load determination between host species via molecular markers will advance non-invasive sampling methods and support greater understanding of inter-species ecological interactions.

Fig. 1

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Rhizospheric microbiome selection and enhanced activity associated to *Agave lechuguilla* Torr. from the Chihuahuan desert

G. Medina-de la Rosa[1], N. López-Lozano[1], î Alpuche-Solís[1], B. Celis-García[1], F. García-Oliva[2], L. López-Reyes[3]
[1]*IPICYT, División de Ciencias Ambientales, San Luís Potosí, Mexico*
[2]*IIES-UNAM, Morelia, Mexico*
[3]*BUAP, CICM, Puebla, Mexico*

Introduction: It is known that besides abiotic factors, root exudates are one of the main driving forces in the selection of beneficial microorganisms in the rhizosphere. However, despite its importance, little is known about root exudates released by Agaves, the differences between the components according to its phenological stage or the changes that these metabolites can induce to the microbiome. Therefore, the objective of this study was to determine the variation of the activity and abundance of nitrogen fixing bacteria (NFB) and phosphatase producing bacteria (PPB) in the rhizosphere of *A. lechuguilla*; a plant with one of the widest distributions in North America.
Methods: Sampling was carried on 4 sites in the Chihuahuan Desert, within the states of Durango and San Luís Potosí. Rhizospheric soil was obtained from adult and juvenile plants, 4 samples each one. Bulk soil was alsotaken. Firstly, 21 physicochemical properties of the soil were evaluated. Enzymatic activity of NFB was quantified with the acetylene reduction, while activity of PPB was assessed with the production of p-nitrophenol. Abundance of both groups was evaluated by means of absolute quantification of functional genes (*NifH* and *phoD*), with real time qPCR.Root exudates were extracted from the soil with a solution of methanol and formic acid. Its identification will be performed with HPLC.
Results: A backward stepwise regression, performed in R, showed that, among the physicochemical properties of soil, humidity (74.39%) and pH (18.39%) are the main factors that explain activity of NFB associated to the rhizosphere of *A. lechuguilla* ($p<0.001$). Whilst, humidity (46%), Mg (15.1%) and Fe (10.16%) content ($p<0.001$), are for PPB. A two-way ANOVA, showed that both, type (soil, adult or juvenile plants) and site are significant factors for enzymatic activity ($p<0.05$).
Preliminary conclusions: This preliminary results suggest a significant effect of the plant over microbiome, as well as the presence of other factors that explain the variation in the enzymatic activity. As this is an undergoing investigation, root exudates and bacterial abundance, are still being evaluated.

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Microbiotic diversity in the sand of four different zones of a sea turtle nesting beach in Venice Beach, Florida

M. Mota[1]

[1]*National University, Mathematics and Natural Sciences, La Jolla, CA, United States*

Florida beaches are popular, but these sandy beaches are also nesting grounds for the loggerhead sea turtle (*Caretta caretta*). However, beaches are threatened by natural erosion and hurricanes and when destroyed, they are nourished. Beach nourishment has become a common practice and changes the physical and ecological characteristics of a beach.

Changes can impact the incubation of sea turtles eggs because of their natal philopatry. When sea turtles return to the beach where they hatched, they may find a beach with different sand quality and microdiversity. This study looks at the microbiotic diversity of loggerhead sea turtles nests in Venice Beach, Florida, and investigates if specific bacteria can be correlated to beach nourishment or to the degree of usage.

We chose four different study areas. The first, low-use nourished beach; Zone 2, a high-use public beach; Zone 3, near a water outfall; and Zone 4, a dog beach. **(Figure 1).** Weekly sand samples were collected during the sea turtle nesting season. They were incubated in Trypsic Soy broth and plated. Individual colonies were identified and characterized. Data were converted into bacteria colony-forming units (CFU) and then to a Log value. Once isolated DNA was extracted, PCR (16s primer), sequenced, and BLASTed for identification.

We compared data from nests and beach zone according to bacterial diversity, beach characteristics and level of human usage. Our data show that the average biologic log of gram + microbe species is lower than gram -. When data are separated by level of public usage, there is no distinction for gram + species, but the average biologic log gram - species show a pattern.

Microbial data collected from the four zones on Venice Beach showed a change in diversity over time. DNA sequencing indicated the presence of *Pseudomonas spp., Proteus* spp., *Escherichia* spp., and *Enterobacter* spp. Several species can be correlated to beach usage, drainage, proximity to homes and dog beach. Although some species were found more prevalent in hatched or unhatched nests we cannot state they played a significant role in the fate of the clutch. Further research is needed to test how these species influence the incubation of sea turtle eggs.

Figure 1- Study sites in Venice Beach, Florida showing different sampling areas. Top is a low-use public beach; the second, a high-use public beach; third, a residential drain; and lastly, a dog beach.

Fig. 1

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Rhizosphere microbiome of cork oak under forest decline-disease disturbances

D. Pinho[1,2], C. Barroso[1], H. Froufe[1], C. S. Silva[3], N. CM Gomes[2], D. FR Cleary[2], E. Vanguelova[4], S. Denman[4], C. Egas[1]
[1]*Biocant, Next Generation Sequencing Unit, Cantanhede, Portugal*
[2]*University of Aveiro, Department of Biology and CESAM, Aveiro, Portugal*
[3]*Associação de Produtores Florestais de Coruche (APFC), Coruche, Portugal*
[4]*Forest Research, Farnham, United Kingdom*

Forest decline-diseases are damaging complex processes driven by multiple and cumulative abiotic and biotic factors which can lead to the disruption of ecosystem services and consequently to socioeconomic losses. Studies on the drivers and mechanisms behind tree decline are underway using *Quercus* species as a research platform at a range of scales spanning landscape (1) to molecule (2, 3). Moreover, emerging evidence has emphasised for a better understanding of the microbiomes across the niches of large, complex, long-lived plants like trees, as it is thought they have a crucial role in host health and fitness (2, 3, 4). The rhizosphere is a particularly important niche where nutrient exchanges occur and soil microorganisms establish close relations with trees. In an attempt to get new insights on the host-microbiome interactions that underpin health condition of trees, we set an experiment to test the hypothesis that forest and tree health conditions, rhizosphere microbiome, and soil environment are linked.
We sampled the rhizosphere soil of cork oaks (*Quercus suber* L.) in two human-managed woodlands that exhibited different levels of forest health. The rhizosphere microbiome, both bacteria and fungi, of asymptomatic ("healthy") and symptomatic trees were characterised using metagenomics approaches and rhizosphere soils were analysed for moisture, pH, total carbon, nitrogen, and C:N ratio.
Rhizosphere chemical properties and microbiome were strongly and significantly correlated, in particular moisture and pH correlated with both bacterial and fungal assemblies. Moreover, rhizosphere chemistry and microbiome composition were distinct across the sites and forest health condition, but no clear relations were detected when investigating tree health condition.
Our study provides new knowledge for developing improved forest management strategies to predict, mitigate, and monitor the re-emerging decline-disease events threatening oak ecosystems throughout Europe.

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Competition between two holobionts – a multi-omic analysis of *in situ* coral-algal interactions

T. Roach[1]
[1]*Biosphere 2/ Hawaii Institute of Marine Biology, Waialua, HI, United States*

All plants and animals host diverse symbiotic consortia of microbes, including viruses, Bacteria, Protists and Archaea, which together with the macroorganism form a holobiont. It has been shown that the holobiont plays critical roles in host development, physiology and metabolism. However, the role of the holobiont in ecological interactions between macroorganisms is less well understood. This study uses a combination of epifluorescence microscopy, metagenomic sequencing, and metabolomic profiling to investigate the role of the holobiont in coral-algal interactions *in situ* in a tropical reef ecosystem. By taking surface-associated samples and tissue biopsies we assess the surface-associated epibiont and the tissue-associated holobiont of corals in direct competitive interactions with algae at a centimeter resolution. There were higher viral and microbial abundances at the coral-algal interface, as well as significantly larger microbial cells. Besides the differential enrichment of specific functional genes, microbial taxa, and metabolites in coral compared to algal holobionts, an emergent microbial assemblage was present at the interface that was unique and not merely a mix of the coral and algal holobionts. Lastly, we found specific functions, taxa, and metabolites involved in the competitive outcomes of these interactions (i.e., whether the coral is winning or losing). Specifically the Bacteroidetes to Firmicutes ratio and genes involved in bacterial growth and cell division were elevated at the interface and in losing coral holobionts. Together, these results provide insight into how coral-algal-microbial interactions structure the holobiont and the overall ecosystem.

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Disentangling inter-kingdom and functional traits associated with pulse thermal resistance in corals

E. Santoro Peçanha[1], H. Villela[1], G. Duarte[1,2], C. Vilela[1], A. Michelato[1], C. Rachid[1], R. Toscan[3], F. Côrrea[3], J. P. Saraiva[3], R. Kallies[3], A. Macrae[1], U. Rocha[3], R. Peixoto[1,2,4]

[1]*Federal University of Rio de Janeiro, Microbiology, Rio de Janeiro, Brazil*

[2]*Instituto Museu Aquario Marinho do Rio de Janeiro-AquaRio, Rio de Janeiro, Brazil*

[3]*Helmholtz Centre for Environmental Research, Department of Environmental Microbiology, Leipzig, Germany*

[4]*University of California, Genome Center, Davis, CA, United States*

Thermal-resistant corals have been reported in the Brazilian coast over the last few years. We hypothesized that Beneficial Microorganism for Corals (BMCs) play a role on conferring thermal resistance to their host. Fragments of the coral *Mussismilia hispida* were sampled from Maraú, Brazil after recovering from a massive coral bleaching event in 2016. Both affected (sensitive) and unaffected (resistant) colonies of the same coral species were collected. Coral fragments were exposed to high temperatures in two microcosm experiments, the first with resistant and the second with sensitive organisms, which started at 26ºC, moving on to high temperatures (32ºC/30,5°C, respectively) and going back to 26ºC. Coral fragments from each treatment were sampled before, during the peak of temperature and after the recovery period; and the DNA was extracted and sequencing to disentangling microbiome roles in thermal resistance. Among all physicochemical factors measured, thermal stress was the main factor in NMDS analysis promoting significant changes in the bacterial structure of both resistant and sensitive communities. No significant difference on the maximum quantum yield of the algae-associated Photosystem II (*Fv/Fm*) values and no visible signs of tissue damage were observed for resistant corals after the recovery period, while all sensitive corals bleached at 30,5 ºC. Amplicon sequencing analysis of the 16S rRNA gene showed that, even though both resistant and sensitive coral types are classified as being from the same species, the microbial community was distinct since the beginning of experiment and some bacterial taxa were identified as putative bioindicators of resistance. Alphaproteobacteria was the most abundant class among resistant indicators, sheltering ASVs from Rhizobiales, Parvularculales and Rhodospirillales orders. Metagenomic data from the beginning of the experiment and the peak of temperature, suggested proteins that are associated with thermal-resistant corals, such as cytochrome c and b complexes, zinc transport and Photosystem I and II, which may indicate the harboring of resistant species of *Symbiodinium* and functional traits related with resistance for oxidative stress. Also, 932 viral contigs were found in the metagenome, such as Caudovirales, virus order known to harbor Vibrio phages, which were more related with resistant corals and may play an important role in controlling the pathobiome.

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Uncovering virus-bacterial host interactions across ecological gradients in soil

L. Sungeun[1], H. Christina[1], N. Graeme W.[1]

[1]*Ecole centrale de Lyon, Ecully, France*

Soil viruses have potential to influence microbial community structure and subsequent ecosystem functioning by directly affecting the abundance of host cells by lysis and through their ability to transfer genes between hosts. However, in contrast to other environments, an understanding of the extent to which virus-bacterial host interactions regulate soil bacterial populations is lacking. While viruses will have the ability to infect a range of hosts in highly diverse bacterial soil communities, coevolutionary processes may still tightly control the susceptibility of hosts through virus-bacterial interactions and local adaptation within distinct ecological niches. This work tested the hypothesis that host bacteria are more susceptible to infection from co-localised virus populations in soil.

Virus-bacterial host interactions were investigated across a continuous soil pH gradient that has been maintained for over 50 years, and which have different prokaryotic communities at pH 4.5 and 7.5. Bacterial strains were isolated from soils at the extremes of the pH gradient, and virus enrichments obtained from pH 4.5, 5.5, 6.5 and 7.5 soil were applied to the host bacteria and infectivity was quantified using a plaque assay approach.

The plaque assay approach was first optimized and validated to ensure that there was no direct impact of soil pH on the isolated strains and virus enrichments. The results demonstrated that infectivity (plaque-forming units) was greater when viruses and host bacterium were isolated from the same pH soil compared to when isolated from different pH soils, indicating a stronger infectivity when virus and host co-occur in the same soil niche.

Findings provide evidence for local adaptation in natural populations, and that virus-bacterial host interactions play an integral part in the regulation of soil bacterial populations. Our plaque assay approach allowed enrichment for the viruses capable of infecting specific host bacterium in order to address specific hypotheses regarding virus-bacterial host interactions in soil. This method can be adapted for use with various nutrient media, allowing for the characterization of phages with a range of ecologically important bacteria.

P 120

Identifying key components of microbial communities driving major changes in the microbiome

M. Bulling[1], A. Burian[1], M. Sweet[1]
[1]*University of Derby, Environmental Sciences , Derby, United Kingdom*

The recognition that the microbiome can affect the physiology of host organisms has led to extensive interest in characterising microbial communities associated with hosts. However, these communities are usually species rich and complex, showing considerable spatiotemporal variation both within and between hosts of the same species. The current challenge is to understand the dynamics of these communities and the functional roles any given microbe provides to their host and within the microbiome. Given the inherent background variation in community structure, it is challenging to identify key and meaningful changes driving significant overall shifts in community composition. In this work we develop several metrics aimed at identifying individuals and/or clusters of microbes likely to drive the observable shifts in the microbiome. We illustrate their use and compare the results with established metrics on microbial community shifts associated with the progression of diseases in corals.

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Bacterial community composition change in temperate octocoral, *Scleronephthya gracillima* exposed to heat stress

S. Woo[1]
[1]*KIOST, Marine Biotech Research, Busan, South Korea*

Coral diversity is affected by climate change. Environmental impacts can induce the change of relationship between coral and its symbiotic microbial community. Furthermore the microbial community change could lead the coral diseases considered one of the reasons of coral death and the opportunistic infections in corals exposed to the increased temperature. In this study we collected a temperate octocoral, *Scleronephthya gracillima* in Jeju,Korea and exposed to various seawater temperature (26, 28 and 30ºC) to compare the composition of bacterial community using the Next Generation Sequencing technique. The results showed total 88 species of bacteria were found in *S.gracillima* in wild condition and they were classified to 72 genus, 41 family, 32 order, 16 class and 13 phylum. Soft coral, *S.gracillima* were enriched in OTUs from the families Hahellaceae, Mycoplasmataceae, Alteromonadaceae, Anaplasmataceae, and Rhodobacteraceae. The number of bacteria species belong to following 8 families, Flavobacteriaceae, Bacillaceae, Comamonadaceae, Alteromonadaceae, Pseudoalteromonadaceae, Hahellaceae, Pseudomonadaceae and Vibrionaceae robustly increased in responses to the heat stress in 26, 28 and 30ºC groups and the species *Mesoflavibacter sabulilitoris, Vibrio tubiashii, Pseudomonas azotoformans, Oceanospirillum beijerinckii, Neptuniibacter Caesariensis*, and *Amphritea spongicola* showed proportionally increase by temperature. The number of *Endozoicomonas elysicola* showed an increase up to 26ºC then decrease in 28 and 30ºC groups.

Fig. 1

Poster Presentations

Session 2

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Harnessing the performance of the soil microbiome in agriculture

F. Andreote[1]
[1]*University of Sao Paulo, ESALQ, Soil Science, Piracicaba, Brazil*

The soil is the most diverse and complex system for microbiome assembly. The theoretical knowledge on this subject has grown in the last decades, but it remains scarcely explored in the agriculture, where it can support innovative practices and applications, leading agriculture to new levels of yield and sustainability. The limited use of microbes in agriculture has shown its great capacity to fix nitrogen in leguminous plants or to control specific soil-borne pathogens. However, it is only the first examples of how microbes can harness food production worldwide. Our research group has targeted the use of theories of microbial ecology to provide solid support for innovations in agriculture. We targeted microbiome composition modulation and learned that shifts in the microbiome composition and manipulation of its diversity and richness may collapse important soil functions, such as the connection of plants to beneficial mycorrhizae, or the availability of soluble phosphorus to plants. Based on that, we listed agricultural management that can improve soil diversity and richness, ultimately supporting a better functioning of the soil microbiome. We also advanced in the development of important indicators for better biological qualities of soil, such as roots and plants phenotypes connected to microbial players. Also, we have worked in the development of laboratory analyses, like microbial activity in soils, or friendly visualization of high throughput sequencing methodologies, that can be used by farmers to better monitor soil quality and microbiome performance in agricultural soils.

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The use of root endophytic bacteria to boost lettuce growth at low temperature conditions

P. Antoine[1], S. Goormachtig[1]
[1]*VIB-UGent, Plant Systems Biology, Ghent, Belgium*

In flanders, Belgium, lettuce farmers create a yearly turnover of 37 million euro, making it the regions second most grown vegetable crop. Lettuce growth is optimal at temperatures ranging between 20°C and 25°C, making it possible for farmers to grow it up to a consumable size in six weeks during spring and summer. During the winter time on the other hand, this process takes up to four months. A promising discipline to promote plant growth under stress conditions is the use of plant growth promoting rhizobacteria (PGPR). Through 16S amplicon sequencing, we aim at comparing the rhizo and endomicrobiome of different lettuce cultivars grown at low temperature conditions compared to control conditions to detect the enriched rhizosphere and endophytic bacterial genera in the cold. In addition, we will isolate these PGPR out of the lettuce root and evaluate them for their plant growth promoting potential by adding an overdose of them to the plants. Subsequently, we want to know which plant associated molecular pathways are triggered by these bacteria to promote the plant"s growth. The above mentioned experiments will provide us with insights into which bacteria live inside lettuce roots, which of these bacteria can promote lettuce growth and how they influence the plant"s molecular pathways to do so. This project will significantly aid lettuce farmers living in temperature climate areas by increasing the crop"s turnover rate during the cold season.

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Long-term agricultural management practices affect rhizosphere microbiota and plant health

D. Babin[1], S. P. Chowdhury[2], L. Sommermann[3], S. Windisch[4], G. Neumann[4], M. Rothballer[2], J. Geistlinger[3], K. Smalla[1], R. Grosch[5]
[1]*Julius Kühn-Institut, Institute for Epidemiology and Pathogen Diagnostics, Braunschweig, Germany*
[2]*Helmholtz Zentrum München, Institute of Network Biology, Neuherberg, Germany*
[3]*Anhalt University of Applied Sciences, Institute of Bioanalytical Sciences, Bernburg, Germany*
[4]*Universität Hohenheim, Institut für Kulturpflanzenwissenschaften, Stuttgart, Germany*
[5]*Leibniz Institute of Vegetable and Ornamental Crops (IGZ), Plant-Microbe System, Großbeeren, Germany*

Enhancing productivity and output of agriculture, while avoiding negative long-term consequences for the quality of soils and their microbiota is essential to cope with world-wide growing demands for food and energy. Maintenance or rise of crop yields in the long term requires the sustainable use of fertile soils and productive lands. The soil microbiota plays an integral role in virtually all soil processes and has a critical role as determinant of soil quality in maintaining soil functions at the system level. We hypothesized that agricultural management affects microbial communities in the soil as well as in the rhizosphere of crop plants with significant impact on plant growth and health. We used soils from three long-term experimental sites (LTEs) established in 1978 (DOK-LTE, Switzerland), 1992 (LTE-1, Germany), and 2006 (HUB-LTE, Germany) to grow the model plant lettuce (*Lactuca sativa*, L. cv. Tizian) for 10 weeks under controlled climate chamber conditions. This allows to assess the impact of various management strategies (tillage practice, fertilization regime, crop rotation) on soil and rhizosphere microbiota under consideration of plant growth and plant health. High-throughput sequencing of bacterial 16S rRNA genes or fungal ITS fragments, respectively, PCR- amplified from total community DNA of rhizosphere and soil samples showed significant differences in microbial community compositions depending on the field site that soils originated from and the long-term farming practices. Moreover, differences depending on long-term agricultural management in plant growth and health as measured by RT-qPCR of stress-related plant genes were observed. Quantity and quality of lettuce root exudates differed among the soil variants and indicated different stress potentials of farming practices on plants. Under controlled growth chamber conditions, we could show that the lettuce rhizosphere microbiota assemblage as well as plant growth and health are significantly influenced by farming practices. We suggest a farming-dependent legacy on the performance of the subsequent crop generation via belowground plant-microbiota interactions.

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Does the agricultural management affect the priming capacity of barley rhizomicrobiota against powdery mildew?

N. Bziuk[1], D. Lauterbach[1], S. J. Sørensen[2], A. Schikora[1], K. Smalla[1]
[1]*Julius Kühn-Institute , Epidemiology and Pathogen Diagnostics, Braunschweig, Germany*
[2]*University of Copenhagen, Department of Biology, Copenhagen, Denmark*

The plant's immune response towards pathogens is assumed to be enhanced by interactions with its rhizomicrobiota in a process called priming. We could show that the rhizosphere microbial community composition of barley grown in agricultural soil is influenced by the agricultural management. Our hypothesis is that the treatment-shaped microbial communities of agricultural soils differ in their capacity to enhance the resistance of barley cultivar "Golden Promise" towards infection by the fungal leaf pathogen *Blumeria graminis* f. sp. *hordei*. A greenhouse experiment was set up with soils from a long term field experiment with four different agricultural managements (mouldboard plough (MP) and conservation cultivator tillage (CT) with standard N-fertilization with pesticide application (I) or reduced N-fertilization without pesticides (E)). Barley plants were grown in these four differently managed soils until growth stage 13 and subsequently infected with *B. graminis*. Gene expression pattern of the defense related genes *PR1b* and *PR17b* were identified before and 24 hours after infection. The relative gene expression was higher in all infected samples with a significant difference between uninfected and infected plants grown in MP-I soil. Furthermore, the amount of fungal hyphae was determined in a detached leaf assay and the dry weight of shoot and root were assessed. The prokaryotic community composition was analyzed by 16S rRNA gene amplicon sequencing amplified from total community-DNA directly extracted from rhizosphere and bulk soil samples taken 10 days after infection. Our results indicated that the rhizomicrobiota were shaped by the agricultural management and that they slightly changed after aboveground infection. The plant's response to the pathogen seemed to depend on the microbiota. This study therefore opens up new insights into agricultural managements to enhance crop resistance.

P 126

Recovering soil quality in vegetable farming systems by reduced tillage and organic fertilization and the effect on soil microbiome

M. V. Cerecetto[1,2], D. Babin[1], J. C. Gilsanz[2], C. Leoni[2], K. Smalla[1]
[1]*Julius Kühn-Institut, Institute for Epidemiology and Pathogen Diagnostics, Braunschweig, Germany*
[2]*INIA Uruguay, Las Brujas, Uruguay*

Soil being a non-renewable resource makes maintenance of soil quality mandatory for ecosystem functioning. Soil microorganisms as key players of many soil processes mediate e.g.nutrient cycling,soil structure, plant growth and health. We are still lacking a clear understanding how the intensification in agriculture changes the soil microbiome and soil properties. Long-term field experiments (LTEs) become increasingly important to study effects of agricultural management. Here, we selected a LTE in Uruguay which was established in 2012 to compare the potential of conventional vs. reduced tillage treatments combined with different fertilization variants to improve soil quality in vegetable farming systems. Bulk soil and rhizosphere soil from table beet (*Beta vulgaris*) cultivated in these soils under field conditions were collected in November 2017. Physico-chemical parameters of bulk soil were determined. Microbial communities from bulk soil were evaluated by cultivation-independent techniques. Total community DNA directly extracted from soil after a harsh lyses was used for 16S rRNA gene and ITS fragment-based analysis of the microbial community by denaturing gradient gel electrophoresis (DGGE) and Illumina MiSeq sequencing. First results showed that conventional tillage with mineral fertilization had different soil aggregate size distribution with less aggregates >2mm and labile carbon than undisturbed soil sampled nearby the agricultural site. DGGE fingerprinting indicated differences between these soils in the total Bacteria and Actinobacteria community composition. In contrast, the treatment with reduced tillage, green manure, chicken manure and compost fertilization showed similarities with undisturbed soil regarding soil bacterial community composition. Our present data suggest thatreduced tillage combined with organic fertilization presents a sustainable farming alternative with the potential to restore or improve soil quality.

P 127

The bacterial community of the food-waste reducing insect *Hermetia illucens* is shaped by diet and developmental stage

E. Crotti[1], M. Callegari[2], R. Marasco[2], M. Fusi[2], C. Jucker[1], F. Mapelli[1], S. Borin[1], S. Savoldelli[1], D. Daffonchio[2]
[1]*University of Milan, Milan, Italy*
[2]*King Abdullah University of Science and Technology, Thuwal, Saudi Arabia*

With the growing world population, the worldwide consumption of proteins for human and animal nutrition is foreseen to increase by over 30% by 2050 compared with 2000. In this perspective, insects have been proposed as potential source of animal proteins. The black soldier fly (BSF), *Hermetia illucens* (L.) (Diptera: Stratiomyidae), is an efficient bioconverter of organic waste and an interesting alternative protein source as livestock feed in the circular economy framework. Since the primary role exerted by microbial symbionts in many different aspects of the host biology and physiology, a comprehensive knowledge of the microbial dynamics in the insect gut is therefore auspicable.
The aim of our work was to investigate the influence of three different diets (i.e. standard, fruit waste and vegetable waste) on the growth performance and gut bacterial community structure of BSF considering different developmental stages (larval, pupal and adult ones). Diet effect was evaluated on i) insect growth, development and survival, ii) gut physicochemical conditions, and iii) gut bacterial community structure and networking. Diet significantly influenced BSF growth performance, as well as the physicochemical conditions (oxygen partial pressure, redox potential and pH) occurring in the insect gut compartments. Significant differences were found among the bacterial communities both considering the diet sources and the developmental stages. Diet-specific bacterial components and key players involved in the host sustenance were revealed. In conclusion, our results indicate that diet and developmental stage affect BSF bacterial community and suggest that the selection of diet-specific microbial members could influence host growth and development.

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Integrating an evolutionary perspective to reveal the core microbiome of plant roots

F. Dini-Andreote[1], S. Abera[1], D. Etalo[1], J. M. Raaijmakers[1]
[1]Netherlands Institute of Ecology, Microbial Ecology, Wageningen, Netherlands

1. In natural ecosystems, plants have co-evolved with their microbiome to help them overcome the adverse effects of (a)biotic stressors. Modern agricultural practices and plant breeding have short-circuited this co-evolution by making crop plants less dependent on their microbiome for nutrient acquisition and stress protection.
2. The objective of this study was to define the core microbiome of Sorghum taking into account distinct plant genotypes and soils from the centre of origin.
3. We start by profiling the microbiome of a total of 43 soils collected at the centre of origin of Sorghum in Ethiopia, in an area extending over 2500 km. We experimentally grew 12 distinct Sorghum genotypes including wild varieties, landraces, and modern cultivars, in a subset of these soils. Sorghum rhizosphere samples were collected using standard methods and subject to microbiome profiling and analysis. Last, we used a probabilistic model to define the core microbiome of Sorghum.
4. Microbiome analysis of the 43 Ethiopian soils revealed a clear biogeographical pattern, in which most of the variation was explained by region, followed by soil pH and organic carbon. Sorghum root microbiome analysis revealed a significant effect of soil type and genotype on the community assembly. Disregarding the soil type, we found signatures in taxa consistently associated with particular Sorghum genotypes. The use of a probabilistic model based on Poison distribution resulted in the well-defined core microbiome of Sorghum. The advantage of such method is the absence of arbitrary thresholds and inclusion of a greater number of rare taxa in the core.
5. This study provides valuable information on the core microbiome of Sorghum by integrating an evolutionary perspective on the host and soil type. Information gathered here will help to guide microbial isolation and culturing and, potentially, provide the basis to harness the power of the microbiome to improve plant production and health.

P 129

Seasonal and spatial bacterial variability of the Lake Balaton

M. Farkas[1], E. Kaszab[1], J. Radó[1], J. Háhn[2], G. Tóth[1], G. Bordós[3], î Bokor[1], A. Táncsics[2], B. Kriszt[1], S. Szoboszlay[1]
[1]Szent István University, Department of Environmental Safety and Ecotoxicology, Gödöllő, Hungary
[2]Szent István University, Regional University Center of Excellence in Environmental Industry, Gödöllő, Hungary
[3]Wessling Hungary Kft, Budapest, Hungary

Introduction: The Lake Balaton with surface area of 594 km2 is the largest central European shallow lake. As a result of high external nutrient load the lake was hypertrophic in the '70s, therefore strict regulations were introduced. In the last decade the water quality stayed between meso- and eutrophic values.
Objectives: Due to the changing trophic level and the touristic significance of the lake numerous ecological research were carried out, but none of them was focused on the seasonal and spatial variability of the microbial community. Nowadays establishment of sustainable fish farming for angling purposes come more to the fore in Lake Balaton. To achieve this aim the examination of food web including planktonic and benthic bacterial diversity is necessary.
Materials & methods: To reveal the spatial heterogeneity of planktonic bacterial community 16 sampling areas were marked out on Balaton and the inflow Zala River. To track the seasonal dynamics 5 time points were set. In addition, at the first time point the benthonic community was also sampled. Bacterial diversity was investigated by 16S rDNA T-RFLP fingerprinting at first and due to the results 4-4 samples were chosen for 16S rDNA amplicon sequencing. The amplicon sequencing of last time point samples is in progress by the time of writing.
Results: The bacterial composition of the Lake Balaton markedly different from main inflow Zala River, regarding both sediment and water samples. Mostly well-known freshwater bacteria such as actinobacterial "*Candidatus* Nanopelagicus", cyanobacterial Synecoccales, Nostocales or proteobacterial LD12 group were abundant in the planktonic samples. The benthonic bacterial diversity was much higher than planktonic and according to this result we cannot mention any dominant benthic genus.
Conclusion: While some bacterial lineages showed seasonal dynamics (e.g. members of Verrucomicrobia, Cyanobacteria) some genera (e.g. actinobacterial "*Candidatus* Nanopelagicus", unknown Ilumatobacteraceae) were constantly dominant members of the community. The riverine nutrient input has only detectable microbial effect (higher cyanobacterial abundance) on the western basin of the lake which is close to the estuarine area.
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P 130

The impact of microbial diversity loss on phosphorus availability to maize

Y. Florentino[1], A. C.F. Dias[1], A. M. Miranda[1], R. G. Taketani[1], F. D. Andreote[1], S. Raposo Cotta[2,1]
[1]*ESALQ, Piracicaba, Brazil*
[2]*Cena, Piracicaba, Brazil*

Nowadays, agriculture represents a complex paradox. It is world"s single largest driver of global environmental change and, at the same time, it is most affected by these changes. Here, we testes effects of microbial biodiversity on the functioning of phosphorus availability. Our hypothesis is that a more diversity microbial community promotes a greater availability of phosphorus to the plants. To test this hypothesis, we developed a gradient of soil microbial diversity and then inoculated into microcosms containing organic (Calcium Phytate) and inorganic (Araxá Rock Phosphate and Supertriple Phosphate) phosphorus source with and without corn seed. The experiment was conducted in the greenhouse for 30 days and after that period, analyses were performed regarding plant features (dry weight, root, shoot measurements and leaf and root phosphorus content) and the microbial community and their activity. In the most available sources of phosphorus (CP and STP), the effect of reducing microbial diversity was more prevalent, where as the diversity is reduced, extractable phosphorus decreases. In relation to the microbial community and its functions, both acid phosphatase activity and abundance of phoD gene were reduced in less diverse communities. More studies are being carried out, however, it is possible to observe the importance of the conservation of high levels of microbial diversity for the full functioning of ecosystems, thus reflecting the health of the plant.

P 131

Metagenomic insights into bacterial succession during vermicomposting of the white grape marc *Vitis vinifera* v. Albariño

M. Gómez Brandón[1], M. Aira[1], M. Ferneda[2], D. Pimentel[3], D. do Nascimento[4], J. Dominguez[1]
[1]*University of Vigo, Ecology and Animal Biology Department, Vigo, Spain*
[2]*Universidade Federal de Santa Maria, Departamento de Ciencia do Solo, Rio Grande do Sul, Brazil*
[3]*Universidade Federal de Pelotas, Facultade de Agronomia Eliseu Maciel, Rio Grande do Sul, Brazil*
[4]*Universidade Federal de Santa Maria, Departamento de Defesa Fitossanitaria, Rio Grande do Sul, Brazil*

Introduction: Winemaking produces millions of tons of grape marc, a byproduct of grape pressing, each year. It is nutrient rich but also characterized by a low pH and high polyphenol content, which are hazardous to the environment in agricultural applications. Previous work has shown that grape marc can be successfully stabilized via vermicomposting, which simultaneously reduces the polyphenol content dramatically. Furthermore, the application of grape marc vermicompost has been shown to positively improve vineyard soil through enhanced availability of nitrogen and carbon mineralization. During vermicomposting microbial communities experience significant changes in composition associated to the decomposition of dead organic matter, which may affect the process in terms of reproducibility, performance and quality of the end product.
Objectives: To evaluate the changes in bacterial communities during vermicomposting of white grape marc derived from *Vitis vinifera* v. Albariño, which represents 95% of the annual white grape harvest in the Northwest of the Iberian Peninsula.
Materials & methods: Bacterial succession was characterized in a pilot-scale vermireactor at day 0 and after 7, 14, 28, 42 and 91 days of vermicomposting by using next-generation sequencing (Illumina MiSeq) of 16S rRNA–V4 amplicons, sequence variant analysis (dada2 pipeline) and functional prediction (PICRUSt).
Results: Significant changes in the bacterial community composition of grape marc vermicompost were observed by day 7 and throughout the duration of the experiment unitl day 91. Likewise, taxonomic and phylogenetic α-diversity increased throughout the experiment, and estimates of β-diversity differed significantly between time points. There was a large increase in gene abundances classified as "metabolism" in the KEGG functional hierarchy, as well as increases in specific metabolic processes including cellulose metabolism, plant hormone synthesis, and antibiotic synthesis.
Conclusion: These findings indicate potential mechanisms for enhanced plant performance in soil amended with vermicompost, and support the use of grape marc vermicompost for sustainable agricultural practices in the wine industry.

P 132

Metabolic potential of acidobacteria for carbon degradation in soils cultivated with sugarcane fertilized with nitrogen and vinasse

M. Gonçalves de Chaves[1], R. Rossetto[2], G. Gueiros, Silva[3], R. Alan Edwards[3], T. Siu Mui [1], A. A. Navarrete[4]
[1]*UNIVERSIDADE DE SÃO PAULO, CENTRO DE ENERGIA NUCLEAR NA AGRICULTURA, Piracicaba, Brazil*
[2] *Agency for Agribusiness Technology, Piracicaba, Brazil*
[3]*San Diego State University, 2Computational Science Research Center , San Diego, CA, United States*
[4] *Federal University of São Carlos, Sorocaba, SP, Brazil., Department of Environmental Sciences, Sorocaba, Brazil*

Acidobacteria is a predominant bacterial phylum in tropical agricultural soils. Previous studies have revealed Acidobacteria subgroups as potential early-warming indicators of agricultural soil management effects in different global soil regions. However, few cultured representatives are available, thereby restricting the knowledge and evaluations of such changes based on the functional role of different Acidobacteria subgroups. A culture-independent approach based on high-throughput DNA sequencing and microarray technology (GeoChip) was used to perform taxonomic and functional profiling of the Acidobacteria community in a tropical soil under sugarcane (*Saccharum* spp.) that was supplemented with nitrogen (N) and nitrogen in combination with vinasse residue (NV). These analyses were conducted to identify the subgroup-level responses to chemical changes and the carbon (C) degradation potential of the different subgroups. The analysis revealed that gene subcategories "starch" and "chitin" had the highest hybridization signal intensity in the these treatments (56% of the total). Subgroups Gp13 and Gp18 presented the highest number of positive correlations with gene families (70%), especially those involved in "hemicellulose" degradation. However, both presented low abundance in the treatment NV (0.9%) in relation to the treatment N (1,2%), which indicates those chemical conditions imposed by the addition of this organic residue to the soil has a negative effect in these subgroups. On the other hand, the Gp4 subgroup was the most abundant in NV (15%) when compared to treatment N (7%), but did not present positive correlations with the gene families analyzed in this study. In general, the results showed that the metabolic potential of C degradation of the different subgroups of Acidobacteria can be modulated by increasing nutrient availability, reducing the abundance of potentially involved subgroups with C degradation in this agricultural soil.
Keywords: soil metagenome, high-throughput DNA microarray, mineral and organic fertilizers, carbon cycling

P 133

Biochar amendments affected composition and activity of the soil microbial community, and the severity of common scab, a soil-borne disease of potatoes

J. Kopecky[1], P. Madrova[1], V. Kristufek[2], J. Divis[3], M. Sagova-Mareckova[1]
[1]*Crop Research Institute, Epidemiology and Ecology of Microorganisms, Prague, Czech Republic*
[2]*Biology Centre of the Czech Academy of Sciences, České Budějovice, Czech Republic*
[3]*University of South Bohemia, České Budějovice, Czech Republic*

The study aimed at assessing the effects of biochar additions on soil microbiota and on severity of the potato common scab. The common scab is a soil-borne disease known to be affected by nutrient availability, as well as by abundance of the pathogen and also by overall composition of soil bacterial community. Two agricultural field soils with high incidence of the disease were used in a pot experiment. Biochar was added in five replicates to the fresh soil in the spring season of three consecutive years, resulting in a set of soils differing in the time passed from the biochar application. A common scab susceptible potato variety Agria was grown in the pots during vegetation season of the third year, and five replicates of the untreated soils were used as a control. Respiration activity of the soil community was measured using OxiTop-C system, and composition of the soil bacterial communities was assessed by Illumina MiSeq 16S rRNA gene amplicon sequencing. Common scab severity responded to freshly added biochar differently in the two soils, resulting in a slight increase and significant decrease, respectively. On the contrary, in the second and third year after the treatment, the disease severity was significantly elevated in the both soils. Significant increase of soil respiration activity was observed after biochar addition, while during the following two years it gradually decreased becoming comparable with respiration activity of the untreated control soil. Bacterial community composition differed between the two soils. Within the samples of each of them, no shift of community composition was observed in the first season, while in the following two years the communities significantly differed from the untreated control, but not from each other. The responding groups of bacteria belonged mainly to the predominating phyla *Proteobacteria* and *Actinobacteria*.
In conclusion, the observed effects of biochar addition were time dependent. The immediate increase of overall microbial activity was followed by change in composition of the soil bacterial community and increase of potato common scab severity in the two years following the treatment.

P 134

Effect of glycerol feed-supplementation on seabass (*Dicentrarchus labrax*) gut microbiota

A. Louvado[1], F. JRC Coelho[1], M. Palma[2], R. Osório[3], L. Magnoni[3], I. Viegas[2], N. Gomes[1]
[1]*Aveiro University, CESAM & UNIVERSIDADE DE AVEIRO, Aveiro, Portugal*
[2]*Centre for Functional Ecology, Department of Life Sciences , Coimbra, Portugal*
[3]*Interdisciplinary Centre of Marine and Environmental Research (CIIMAR), Porto, Portugal*

Glycerol is an increasingly abundant byproduct of biodiesel production, for which innovative uses are needed. As an inexpensive and energetic ingredient, its supplementation in animal feed formulation has been shown to improve rentability and quality of terrestrial livestock production when added at an optimal dosage. However, its potential for aquaculture feed has been ignored. In this study we investigated the effects of glycerol feed-supplementation, at 2.5% and 5% (w/w), on European sea bass (*Dicentrarchus labrax*) growth, metabolism and gut bacterial community composition. Three fish groups were reared in an experimental recirculating aquaculture systems (RAS) for 60 days and subjected to isonitrogenous diets supplemented with glycerol and/or an excipient (cellulose) at different dosages: 0%, 2.5% and 5 % (w/w). Our results showed that glycerol feed-supplementation had no significant effect on the overall structure and diversity of the fish gut bacterial communities, nor did it alter growth performance parameters and nitrogen excretion of reared fish. However, in a dataset clearly dominated by OTUs related to Lactobacillales, a 2.5% glycerol diet did induce significant alterations among some specific and relevant bacterial groups, namely, an increase of the phyla Bacteroidetes and Proteobacteria and the order Bacillales. Curiously, 5% glycerol supplementation lead to a significant increase of the order Vibrionales. Overall, glycerol supplementation did not show adverse effects on fish performance but lead to group specific alteration of fish gut bacterial communities.

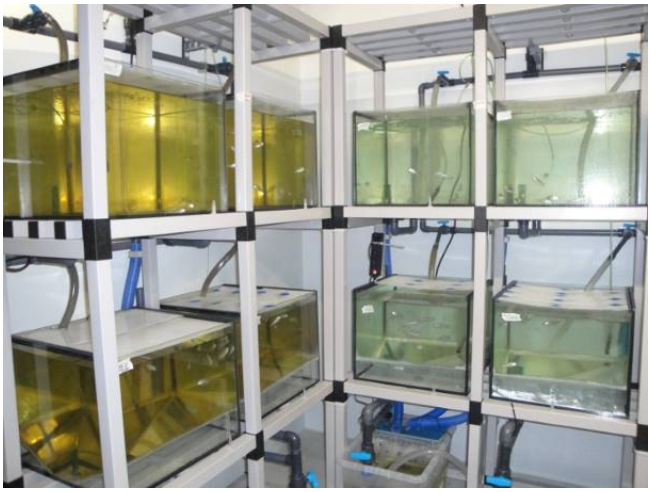
P 135

Humic acid modulates bacterioplankton and fish microbiome in a recirculating aquaculture system (RAS)

A. Louvado[1], L. Pereira[2], F. Coelho[1], P. Pousão-Ferreira[3], R. Ozório[2], N. Gomes[1]
[1]*University of Aveiro, Department of Biology - CESAM, Aveiro, Portugal*
[2]*CIIMAR, University of Porto, Porto, Portugal*
[3]*Portuguese Institute for the Sea and Atmosphere, Olhão, Portugal*

The modulation of host-associated bacterial communities through prebiotics and probiotics can be considered an alternative to current antimicrobial strategies in the aquaculture sector. A key, but overlooked, route for this modulation may be through the chemical manipulation of rearing water. In this study we used humic acid (HA) as a chemical modulator of microbial communities in an experimental recirculating aquaculture system (RAS) (Fig. 1) for production of juvenile European seabass (*Dicentrarchus labrax*) (28-days experiment). High-throughput sequencing of 16S rDNA gene of the bacterioplankton and host associated (fish gut and skin mucus) bacterial communities were analyzed alongside fish growth performance, enzymatic and oxidative stress and water physicochemical parameters. Our results revealed that HA significantly increased the diversity and altered the composition of bacterial communities in water, skin mucus and, to a *lesser extent*, fish gut. The composition analysis at higher taxonomic levels showed that water and skin mucus bacterial communities responded similarly to HA treatment, yet responsive OTUs were different. Therefore, suggesting a niche-specific response to HA modulation in the aquaculture system. In addition, HA exposure significantly increased the relative abundance of members of the Roseobacter clade in water and skin mucus. Interestingly, HA treatment also significantly reduced the relative abundance of an OTU closely related to *Acinetobacter johnsonii*, an emerging fish pathogen. Overall, HA did not affect fish growth, significantly increased the activity of chymotrypsin, glutathione-S-transferase and catalase and reduced the concentrations of nitrite and nitrate in water. Here, we showed for the first time, that HA, a common substance found in soil, sediment and water, has a strong effect on bacterioplankton and may play a critical role in the modulation of fish skin mucus bacterial communities in aquaculture systems.

Fig. 1



P 136

Influence of pea genotype on root associated fluorescent pseudomonads, consequences for plant iron nutrition

T. Lurthy[1], L. Avoscan[1], J. P. Lemaître[1], C. Barraud[1], P. Lemanceau[1], S. Mazurier[1]
[1]INRA, UMR1347 Agroecology, Dijon, France

Pea has a high potential in agroecology because of its ability to fix atmospheric nitrogen and in Human nutrition because of the high amino-acid content of its grains. However, pea may suffer from a susceptibility to iron deficiency in calcareous soils. This susceptibility varies depending on cultivars. Pseudomonad siderophores, pyoverdines (pvd), were shown to promote iron nutrition in Arabidopsis and tobacco. Thus, we hypothesize that variations in iron susceptibility between pea cultivars could be related to differences in their ability to recruit fluorescent pseudomonad (fp) contributing to siderophore-mediated iron nutrition.
Our aims were to compare i) the impact of a susceptible (S) and a tolerant (T) cultivar of pea on fp biodiversity with a focus on their pvd, and in return ii) the impact of the corresponding bacteria and pvd on pea iron nutrition.
The strategy consisted in cultivating S & T pea cultivars in a calcareous soil (low iron bioavailability). fp associated with roots of S & T and corresponding pvd were compared. In return, representative pvd from fp associated with S and T were tested for i) their effect on the transcription of genes involved in plant iron uptake and homeostasis (rt-qPCR) and ii) their impact on plant growth, iron content and ionome (HRICP-MS).
Differences recorded between fp associated with T and S roots were related to i) their low susceptibility to Fe deficiency and ii) their pvd types as characterized by isoelectrofocalisation. Representatives of major pvd types impacted differently S & T for i) iron content, plant ionome and growth of pea and ii) the transcription of genes involved in plant iron metabolism.
The results obtained support the principle of an extended phenotype in which associated bacteria contribute to plant nutrition, showing the importance to consider root microbiota to develop strategies aiming at overcoming iron susceptibility in pea. Further studies are in progress to unravel the mechanisms implied.

P 137

Microbial disruption of the signalling between *Sorghum* and the parasitic weed *Striga*

R. Masteling[1,2], F. Dini-Andreote[1], W. de Boer[1,3], J. M. Raaijmakers[1,2]
[1]NIOO-KNAW, Microbial Ecology, Wageningen, Netherlands
[2]Leiden University, Institute of Biology, Leiden, Netherlands
[3]Wageningen University & Research, Soil Quality, Wageningen, Netherlands

Recently, a paradigm shift in the plant sciences emerged in which microbiomes are seen as a functional driver of plant growth and health. Microbiomes can substantially expand the genomic and metabolic capabilities of plants, providing essential functions including nutrient acquisition, modulation of immune responses and enhanced (a)biotic stress tolerance. However, there is still scarcity in the literature with respect to the potential of soil and plant-associated microbiomes in disrupting the lifecycle of plant parasitic weeds. Particularly the root parasitic weed *Striga*, which is the major biotic constraint to food production in Africa, severely affecting smallholder farmers and food security.
We aim to characterize the mechanisms by which microbes can suppress *Striga* and modulate its interaction with the host *Sorghum bicolor*.
We screened a collection of microbes, comprising 146 strains of various bacterial genera, for their ability to disrupt the molecular signalling between *Sorghum* and *Striga* in pre-defined phenological stages of the parasite"s lifecycle. These constitute the initial phases of the infection, that is, the germination of *Striga* seeds and the formation of the haustorium (parasitic structure).
We identified several bacteria from different phyla that significantly suppress *Striga* seed germination via microbial volatile organic compounds (mVOCs) profiled using GC/Q-TOF. We generated the complete genome sequence of all isolates and are focused on identifying genomic regions responsible for the suppressive phenotype. Ultimately, we aim to integrate the genomics data with the metabolomics data to identify putative biosynthetic gene clusters for the volatiles resulting in a suppressive phenotype.
Our work provides new insight into the yet unknown metabolic and genomic potential of soil and plant-associated microbes and makes progress in identifying the mechanisms and microbial metabolites involved in the suppression of root parasitic weeds.

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Nitrogen fixation of iron reducing bacteria in rice paddy soils – potent agents for sustainable crop production with low nitrogen input

Y. Masuda[1], H. Yamanaka[1], H. Itoh[2], Y. Shiratori[3], S. Amachi[4], T. Aono[1,5], K. Senoo[1,5]
[1]*The University of Tokyo, Department of Applied Biological Chemistry, Tokyo, Japan*
[2]*AIST, Hokkaido centre, Sapporo, Japan*
[3]*Niigata agricultural research institute, Nagaoka, Japan*
[4]*Chiba University, Graduate School of Horticulture, Matsudo, Japan*
[5]*The University of Tokyo, Collaborative Research Institute for Innovative Microbiology, Tokyo, Japan*

Japanese agronomists say, "Wheat is harvested from fertilizer, while rice is harvested from soil fertility". This means crop growth in upland field depends on nitrogen fertilizer application, while rice growth in rice paddy fields (wetland rice fields) depends on soil nitrogen fertility. In fact, good rice yields in non-nitrogen fertilization plots are observed in long-term experimental paddy fields in Japan. Microbial nitrogen fixation greatly contributes to the sustainable nitrogen fertility of rice paddy soils. As the major nitrogen fixers (diazotrophs) in paddy soils, phototrophic *Cyanobacteria* and rhizospheric *Alpha*-, *Beta*-, and *Gammaproteobacteria* have long been studied.
Here, we report predominant but previously-overlooked diazotrophs found in rice paddy soils. We performed metatranscriptomic analysis of rice paddy field soils to investigate the active diazotrophic microbiome there. Most of nitrogen fixation gene (*nif*) transcripts were, surprisingly, derived from *Deltaproteobacteria*, particularly the genera *Anaeromyxobacter* and *Geobacter,* known as iron-reducing bacteria predominated in paddy soils. In addition, our *in silico* analysis of soil metagenomic data revealed the ubiquitous presence of *nif* genes derived from *Anaeromyxobacter* and *Geobacter* in various soil environments. These results suggest that they could be important drivers of nitrogen fixation not only in paddy soils but also other soil ecosystems.
Nitrogen fixing ability of *Anaeromyxobacter* spp. has not been confirmed yet although that of *nif* harboring *Geobacter* strains have been reported. We examined nitrogen fixing ability of *nif* harboring *Anaeromyxobacter* sp. PSR-1 and Red267 isolated from soil under cultivation condition. These strains showed nitrogen fixing activity by utilizing Fe(III)-NTA and ferrihydrite as electron acceptors at even lower concentration than in paddy soils. High activity of nitrogen fixation was detected in the presence of ammonium at the similar concentration level as paddy soils. Their nitrogen fixing activity was confirmed in the paddy soil microcosm as well.
Our study provides novel insights into the pivotal role of *Anaeromyxobacter* and *Geobacter* as diazotrophs which support soil nitrogen fertility of rice paddy fields. These bacteria could be potent agent for sustainable crop production with low nitrogen input.

P 139

Deliberate introduction of invisible invaders – impact of microbial inoculant to soil microbial communities

P. C. Mawarda[1,2], X. Le Roux[3], J. D. van Elsas[1], J. F. Salles[1]
[1]*University of Groningen, Microbial Ecology, Groningen, Netherlands*
[2]*Indonesian Institute of Sciences, Research Unit for Clean Technology, Bandung, Indonesia*
[3]*Université Lyon 1, Laboratoire d'Ecologie Microbienne, Lyon, France*

Non-target effects of deliberately releasing organisms into a new environment are of great concern due to their potential impact on the biodiversity and functioning of the ecosystem. While these studies often focus on macro-organisms, the impact of microbial inoculants on resident microbial communities is often expected to be negligible. In contrast, we posit that such introductions often impact native microbial communities leading to cascading effects on higher trophic levels, which may have major ecological and societal implications. Taking soil communities as an example and using a literature search, we examine the impact of microbial inoculation (often the release of beneficial microorganisms in agricultural systems) on the resident microbial communities. Through the knowledge gathered from each study, we proposed a mechanistic framework explaining how inoculants might alter resident microbial communities. We advocate that resource competition, antagonism and synergism represent the main mechanisms through which inoculation can affect soil microbial community. We further discuss the longer term consequences of microbial inoculants by placing these invasions in the context of the resilience of the resident community, and identify challenges as well as future approaches to shed more insight on this unseen reality.
Keywords: microbial inoculation, resilience, microbial communities

P 140

Breeding for resistance in common bean drives distinct response to pathogen infection in the root system

L. W. Mendes[1], V. C. Bravo[2], M. Hollander[2], A. F. Chiorato[3], R. Mendes[4], J. M. Raaijmakers[2], S. M. Tsai[1]

[1]*Center for Nuclear Energy in Agriculture, University of Sao Paulo, Piracicaba, Brazil*

[2]*Netherlands Institute of Ecology, Microbial Ecology, Wageningen, Netherlands*

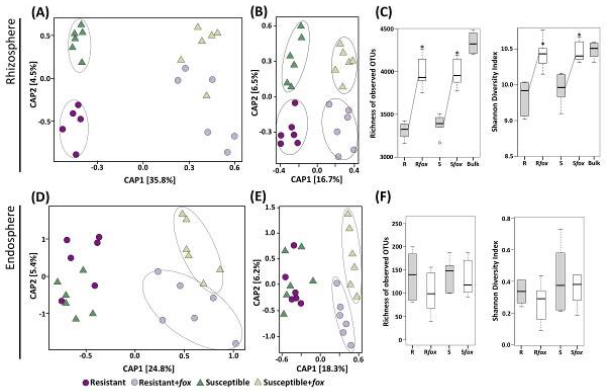
[3]*Agronomomic Institute of Campinas, Campinas, Brazil*

[4]*Embrapa Environment, Laboratory of Environmental Microbiology, Jaguariúna, Brazil*

Plants harbor a wide diversity of microorganisms both inside and outside their tissues, and these associated microbiomes play key roles in plant growth and health, including the defense against root infections by soil-borne pathogens. Here, we investigated the response of rhizosphere and endosphere microbiome of common bean cultivars (*Phaseolusvulgaris*) with contrasting levels of resistance to the root pathogen *Fusariumoxysporum*(*Fox*), i.e. one *Fox*-resistant and one susceptible. The analysis of 16S rRNA sequencing and metagenome revealed that each cultivar recruitsa distinct rhizosphere microbiome and presentsa different response to the *Fox*invasion. On the other hand, the endophytic community is similar for both cultivar, but becomes distinct after *Fox*infection. There was also an increased diversity after infection in rhizosphere samples (Figure 1). To further investigate the response of the microbiomes toward pathogen infection, we conducted co-occurrence network analysis and showed an increased complexity after *Fox*infection. Considering the groups that responded to the pathogen infection in both rhizosphere and endosphere we can highlight the bacterial families Flavobacteriaceae, Cytophagaceae, Comamonadaceae, Pseudomonadaceae and Oxalobacteriaceae. Taken together, these results indicate a prompt response of the microbial communities in the presence of the pathogen, with distinct community assembly between rhizosphere and endosphere niches and clear differences between the resistant and susceptible cultivar. Further analysis will explore in detail the differences between cultivars in the search for specific microbial groups and functions that could help the plant to fend-off the pathogen.

Figure 1. Structure and diversity measurements of rhizosphere and endosphere microbiomes of two common bean cultivars with contrasting level of resistance to the pathogen Fusarium oxysporum. Constrained Analysis of Principal Coordinates (CAP) performed on taxonomic profile based on (A) 16S rRNA at OTU level and (B) metagenome data for rhizosphere community; (C) richness and diversity measurements of microbial communities from rhizosphere. CAP analysis based on (D) 16S rRNA and (E) metagenome data for endophytic community; (F) richness and diversity measurements for endophytic community.

Fig. 1



P 141

Comparative genomics reveals high genomic diversity in the drought-tolerant diazotroph

S. D. Nishu[1], H. R. Hyun[1], T. K. Lee[1]
[1]*Yonsei University, Environmental Engineering, Wonju, South Korea*

The role of water availability is important in plant-microbe interaction, and drought-adapted microbial communities have been shown to be beneficial for plant fitness under drought stress. Despite of important role of microbe under the drought stress, studies comparing the genomes of various drought-tolerant symbiotic microbe with plants in drought conditions are very scarce. Here we compared the genomes of drought-tolerant diazotrophs which were isolated from the nodules and rhizospheric soil of the legume exposed to temporary and periodic drought in Korea (37 sampling sites). The nitrogen fixing capability of isolates were evaluated under the drought stress condition (-0.735 Mpa).The 10 isolates were taxonomically identified as *Arthobactor, Pseudomonas, Bacillus*, *Stenotrophomonas* and *Sphingobacterium* and featured large genome size variations (4.4 to 7.3 Mb) and two distinct groups in GC contents (closed to 40% or 65%). Whole genome sequence of these strains confirmed the presence of osmotic-stress resistant genes such as trehalose synthesis genes (*TreS*, *SugB*) and Na+/H+ antiporter (*Nha*) in all genomes. Further comparisons helped to identify functional genes which is essential to the plant-bacteria symbiosis including IAA synthesis (*Tropl*, *SugB*), siderophore production (*SbnA*), phosphate solubilization (*Pqq*), and gene clusters encoding secretion system Type I, II and IV. A core gene cluster resulting from comparative genomics is uncovered as potentially important for plant-bacteria symbiosis. A comprehensive insight is also provided of how this gene cluster involved in plant-microbe interaction under the drought stress.

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Microbial activity and community in long-term organic and conventional farming systems

K. Peltoniemi[1], H. Fritze[1], T. Pennanen[1], S. Velmala[1]
[1]*Natural Resources Institute Finland (Luke), Natural Resources, Helsinki, Finland*

Several studies have shown enhanced soil fertility and higher microbial biodiversity in organically grown plots compared to conventionally grown. Organic systems have been reported to have 32% to 84% greater microbial biomass, carbon and nitrogen content, total phospholipid fatty acids and enzyme activities than conventional systems. Higher microbial diversity in organically farmed fields is expected to impact positively, e.g. to carbon sequestration, GHG mitigation, nutrient and water uptake and pathogen control. Thus, our aim was to detect changes in the activity, biomass, abundance and community composition of microbiota between organic and conventionally farmed systems in boreal region.

Our study sites locate in Toholampi (63°49'N, 24°09'E), in Central Finland, and have been a long-term experimental site for cereal and dairy crops (100 m x 16 m) under organic and conventional farming since 2001 and 1997, respectively, with 4 replicate plots each. The first sampling was conducted from the first 10 cm soil layer in May 2018 before sowing and the second after the harvest in August. Microbial activity was measured as basal respiration, e.g. CO2 production determined by gas chromatography. Microbial biomass was determined by the fumigation extraction method (FE), the community composition by PLFA analysis, and gene copy amounts by qPCR with group specific primers. Microbial diversity for fungi (ITS region for all fungi and 18S for arbuscular mycorrhizas) and bacteria (16S rRNA gene) were conducted with Illumina amplicon sequencing.

The microbial activity did not differ between samplings. There was, however, significantly higher microbial activity in the field of organic cereal crop production compared to the corresponding conventionally farmed in August samples. On the contrary, the microbial activity of dairy crop production fields did not differ between systems. There were more fungal ITS copies in the field of organic cereal crop production compared to that of conventional one in August samples. There were no distinctive differences in community composition of arbuscular mycorrhiza between farming systems. Genera *Glomus*, *Archaeospora*, *Paraglomus*, *Claroideoglomus* and *Ambispora* were the most dominating AMF taxa. Other analyses of microbial biomass and microbial community composition are still in progress. Preliminary results indicate that organic farming system has increasing impact on the microbial activity and fungal abundance after the harvest.

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Bioinoculants as modulators of rhizospheric microbiome

S. Sharma[1], G. Anand[1], R. Sharma[1], V. S. Bisaria[1]
[1]*IIT Delhi, Department of Biochemical Engineering and Biotechnology, New Delhi, India*

The changing climate along with the indiscriminate usage of chemicals in fertilizers has imposed immense pressure on the agricultural system to adopt an eco-friendly, long term and sustainable alternative. This is in view of increasing crop productivity so as to face the glaring global food crisis. Bioinoculants provide a potential effective eco-friendly alternative. However, the bottleneck of this technology is their reduced efficacy and survivability of the in field. There is limited understanding of the non-target impacts of these bioinoculants on the rhizospheric microbiome, and ultimately soil health. Majority of the studies have focussed on the target impacts of these bioinoculants i.e. on the plant growth and productivity. The prime focus of our study revolves around the potential impacts of these bioinoculants as single inoculants and as consortia on the rhizospheric microbiome that eventually leads to enhanced plant growth and productivity. The study holds significant importance in the light of newer concept of "holobiont" represented by the plant with its associated microorganism that has been evolving together for continual survival of the metaorganism. The model system of our study is the legume *Cajanus cajan* that has high significance in terms of economic and dietary perspective in India. The structural and functional diversity of the bacterial community was targeted utilizing a metagenomic approach. The bioinoculants led to enhancement of established and potential plant growth promoting bacteria involved in various biogeochemical cycles. The cumulative impact of these bioinoculants had a beneficial influence on the rhizospheric microbiome which led to enhanced plant growth and productivity. The study is yet another step in deciphering the mechanism of action, and to further the development of newer and improved bioinoculants with enhanced efficacy and survivability.

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Microbial ecology of Czech spa thermal springs

T. Šmrhová[1], G. Nováková[1], M. Strejcek[1], J. Šuman[1], O. Uhlík[1]
[1]*University of Chemistry and Technology Prague, Biochemistry and Microbiology, Prague, Czech Republic*

Thermal water springs represent an ideal model of ecosystem to investigate microbial ecology of thermal aquatic habitats with low nutrient content, due to their isolation from human activity and broad geochemical gradients across geographic distances. Besides that, these springs represent habitats of potentially biotechnologically attractive microorganisms. Such microorganisms and their secondary metabolites could serve as a source of biologically active compounds and enzymes with technologically interesting characteristics such as thermal, proteolytic, detergent or salt tolerance. Therefore, there is an effort to obtain pure cultures of microorganisms isolated from such habitats. Despite a big effort it is not an easy task to obtain pure cultures of microorganisms indigenous in thermal springs. Therefore, it is necessary to design appropriate cultivation conditions simulating the natural environment. Aim of this study is to obtain pure cultures of bacteria inhabiting spa thermal springs and perform biochemical, physiological and phylogenetic characterization of these cultures, leading to description of the microbial ecology of selected springs. Four spa thermal springs, differing in temperature and chemical composition, located in the Czech Republic were examined in this study. The cultivation was performed with six different nutritionally poor cultivation media, using corresponding filtered spring water instead of distilled water in the formula. Obtained cultures were subjected to MALDI TOF mass spectrometry analysis and the obtained spectra were used for reduction of number of identical isolates. The phylogenetic identity of cultures will be assessed by sequencing of 16S rRNA genes. To the present day we possess a collection of pure cultures of bacteria originating in the examined thermal springs, which will be further characterized. In this work we aim to extend the knowledge on microbial ecology of such extreme habitats.

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Microbial inoculants as engineers of crop and soil microbiomes – an ecosystem service approach

A. Soares[1,2], L. Carvalho[1,2], P. Correia[1,2], C. Cruz[1,2]
[1]*Soilvitae, Teclabs, Campus Fcul, Lisbon, Portugal*
[2]*cE3c, University of Lisbon, Lisbon, Portugal*

Microbial communities shape planetary ecosystems and influence the health of humans, plants, and animals. Direct and indirect benefits of improving soil biological management in agricultural systems can be assessed in terms of economic, environmental and food security benefits. Many pre-competitive works support the use of microbial inoculants as promoters of productive, societal, and natural capital. In this work we tested whether applying microbial inoculants in crop fields could simultaneously promoted both plant productivity and soil quality.
Plant growth promoting actinobacterial inoculant was applied in a tomato (*Solanum lycopersicum*) greenhouse farm under a regular fertilizer crop programme. Plant growth and production were determined. Soil community structure assessment was based on phospholipids fatty acid (PLFA) profiles for total microorganisms, bacteria, fungi and arbuscular mycorrhizal fungi (AMF). Soil microbial activity and function were evaluated by measuring the activity of extracellular enzymes.
Microbial inoculant application promoted crop productivity compared with the control treatment and induced higher soil abundance of bacterial and fungal communities. Furthermore, it stimulated important soil microbial functional groups as the AMF community. Enzymatic activity results indicated a promotion of soil microbial function with the application of microbial inoculant.
All together the results show the potential of microbial inoculants as a tool to engineer plant and soil microbiomes increasing productivity, diversity and soil quality, and therefore contributing to a neutral land degradation world.

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Biological control of green mould disease of *Agaricus bisporus* by antagonistic *Bacillus* spp. strains

O. Stanojević[1], T. Berić[1], I. Potočnik[2], S. Stanković[1], S. Milijašević-Marčić[2]
[1]*University of Belgrade, Faculty of Biology, Microbiology, Belgrade, Serbia*
[2]*Institute of Pesticides and Environmental Protection, Laboratory of Applied Phytopathology, Belgrade, Serbia*

One of the most devastating diseases of *Agaricus bisporus* is green mould caused by several *Trichoderma* species. Disease control is usually achieved by application of fungicides during mushroom cultivation. Given the developing resistance in pathogens and the negative environmental impact of such chemicals, biological control emerged as a promising alternative. The potential of *Bacillus* species for biocontrol is extensively examined due to their competitiveness and ability to produce various secondary metabolites.
The objective of this study was to evaluate the efficacy of three *Bacillus* spp. strains for the control of *Trichoderma aggressivum* f. *europaeum* T77 *in vivo*, as well as to determine their impact on the white button mushroom yield.
The *in vivo* experiment consisted of two groups, uninoculated and inoculated with the pathogen, and six treatments within the groups (*B. subtilis* B-233, *B. amyloliquefaciens* B-241, *B. pumilus* B-138, commercial biofungicide *Bacillus subtilis* QST 713, fungicide prochloraz-Mn and water). Mushrooms were hand-picked and the healthy and diseased fruiting bodies were counted and weighed. Two values were calculated for each treatment: the efficacy for pathogen control (E) and the impact of treatments on crop yield (biological efficiency – BE).
The most efficient control of the pathogen was achieved by prochloraz-Mn (80.10%), followed by *B. amyloliquefaciens* B-241 (67.61%), *B. subtilis* QST 713 (57.27%), *B. pumilus* B-138 (56.33%) and *B. subtilis* B-233 (45.52%). When it comes to impact on mushroom yield, BE values ranged from 58.65% to 81.42%, with no significant differences amongst the treatments.
When compared to *B. subtilis* QST 713 and prochloraz-Mn, *Bacillus* spp. strains, especially *B. amyloliquefaciens* B-241, showed good efficacy for the control of *T. aggressivum* f. *europaeum* T77 and had no negative effect on the crop yield. Strain *B. amyloliquefaciens* B-241 showed potential for biocontrol of green mould during mushroom production.

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Valorization of solid wastes – improvement of soil properties

H. Stiborova[1], M. Kracmarova[1], P. Tlustos[2], J. Szakova[2], O. Uhlík[1], M. Strejcek[1], K. Demnerova[1]
[1]*University of Chemistry and Technology, Biochemistry and Microbiology, Prague, Czech Republic*
[2]*Czech University of Life Sciences Prague, Department of Agro-Environmental Chemistry and Plant Nutrition, Prague, Czech Republic*

Biochar is a carbon rich material prepared by thermochemical decomposition of organic matter. Its production and utilization serve as an environmentally friendly strategy for valorization of various types of organic wastes. Nowadays, biochar belongs to the most extensively studied soil amendment, which can improve the soil properties. The other approaches are the regular application of organic or inorganic fertilizers.
Biochar from two different feedstock (plant biomass and waste from poultry slaughterhouse) were prepared at 350°C and 500°C and characterized using advanced spectroscopic methods to obtained their surface and structure characteristics. Biochars were applied at two ratios of 2% and 5% (w/w) in two different soils, Cambisol and Luvisol, and their impact on soil microbiota (both prokaryotic and fungal) and physicochemical parameters of treated soil will be evaluated at different time intervals within one year.
The impact of different type of fertilization on bacterial and fungal community structures using 16S rDNA and ITS region sequencing with Illumina Miseq method on these two type of soils was already analyzed. The fertilization significantly affected both fungal and prokaryotic communities in all soil types. Furthermore, significant relationships between genera occurrence and applied fertilizers or crop rotation were analyzed using an indicator species analysis. Amendments were significantly associated with an abundance of a few taxa belonging to Firmicutes, Proteobacteria (alpha- and gamma-) and phylum Actinobacteria and fungi of phyla Ascomycota and Basidiomycota.

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Isolation of lactic acid bacteria with plant growth promotion activities from the rhizosphere of wheat at different phenological phases

S. Straffella[1], P. Murgese[1], F. Minervini[1], C. Crecchio[1]
[1]*University of Bari Aldo Moro, Dept of Soil, Plant and Food Sciences, Bari, Italy*

Studies about plant-microbe interactions showed the importance of some microbial communities to promote plants" health and growth because of their bioprotective and biostimulant actions. This heterogeneous group of microrganisms, known as Plant Growth Promoting Bacteria, include the Lactic Acid Bacteria (LAB). In order to realize biofertilizers to be used for a sustainable agriculture, the aim of this study was to isolate lactic acid bacteria from wheat rhizospheric soil, to study their Plant Growth Promoting activities and, through in vitro and in vivo tests, to evaluate improvements on physiological and productive plant parameters.
Rhizospheric soil samples were randomly collected from two wheat crops located in Southern Italy during the following phenological phases: tillering, elongation, earing and physiological maturity. For each field and each phase ten samples were randomly collected and pooled to form two sub-samples, considered as random replicas.
Metagenomics analysis of 16S rDNA was performed to characterize the structure of microbial communities and to investigate the presence of lactic acid bacteria in rhizospheric soil samples. The root-associated microbial community is primary composed of Proteobacteria, Actinobacteria and Bacteroidetes. In both soils, Firmicutes, phylum to which lactic bacteria belong, represents about 3% of the total microbial community.
Culture-dependent methods were performed to isolation lactic acid bacteria from soil samples. GYP (*Glucose, Yeast, Peptone*) and MRS (*De Man, Rogosa and Sharpe*) were used as growth medium to isolate the presumptive LAB. An enrichment in liquid medium before plating out on agarized selective growth media allowed to isolate a relevant number of microbial species that, by 16S rDNA sequencing, were identified as belonging to the genus *Lactococcus*, *Enterococcus* and *Sporolactobacillus,* an endospore-forming lactic acid bacterium. The characterization of their plant growth promoting traits is in progress.

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The microbial ecology of disease suppression in alternative soils for mushroom cropping systems

T. Taparia[1], E. Hendrix[1], M. Hendriks[1], J. Baars[1], J. van der Wolf[1]

[1]Wageningen University and Research, Wageningen Plant Research, Wageningen, Netherlands

Introduction: Mushroom cropping systems are shaped by and rely heavily on the dynamic interactions between *Agaricus bisporus* and beneficial microbes in the casing soil. The inherent microbiota of these peat-based soils initiate pinhead formation of the vegetative mycelium into fruiting bodies. However, the peat also introduces several pathogens into mushroom farms. The rapid decline of peat reserves world-wide, and the associated ecological costs of mining peatlands, also add to its disadvantages.

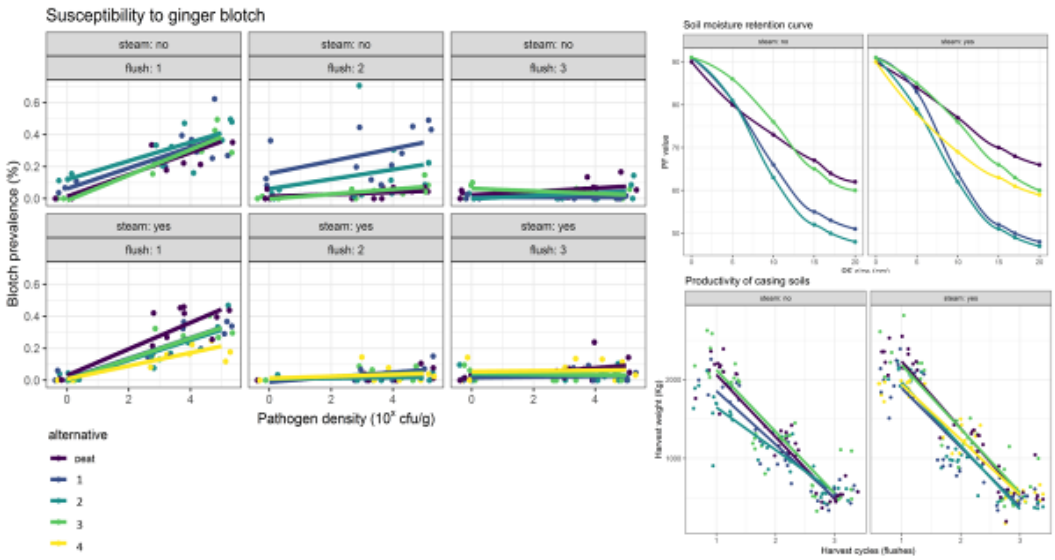
Objectives: This research aims to reduce the disease pressure in mushroom farms while making the cropping system more sustainable, by the use of alternative materials to proportionally replace peat in casing soils. This study explores the susceptibility to bacterial blotch diseases in these alternative soils, the microbial ecology of the pathogens in the mushroom beds, and the mechanisms of disease suppression.

Methods: Bacterial blotch prevalence in alternative soils was studied via pathogen-inoculated semi-field experiments, mimicking the conditions of conventional mushroom farms. Soil microbiome and physico-chemical characteristics were analyzed for effect on productivity and disease prevalence.

Results: The type and proportion of alternatives in the soil, produce differing mushroom yields, and are comparable to that of peat-soils. Alternative soils also differ vastly in their ability to suppress blotch development. Steaming of the alternatives, prior to mixing in the soil, allows a regulated restoration of the microbiome, and confers increased disease suppressive characteristics to some alternative soils. However, entirely steamed peat-soil loses its ability to suppress blotch with the loss of its native microbiome. After peak outbreaks in the first harvest, the consequent cultivation cycles show a steep decline in blotch, despite increased pathogen populations, across most soil types. For some soil types, this generic blotch suppression is enhanced with steaming.

Conclusions: Despite a promising performance, strong trade-offs exists between the harvest yield, the disease suppressiveness and the economic costs for mushroom cultivation in alternative soils. Several microbiological and physical soil properties vary between suppressive and conducive soils, and have the potential to be explored as disease indicators.

Fig. 1



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Microbiota of white button mushroom (*Agaricus bisporus*) compost production with special emphasis on potential mycotoxin degrading bacteria

F. Tóth[1], B. Vajna[1], M. Cserháti[2], A. Risa[2], K. Márialigeti[1]
[1]*Eötvös Loránd University, Department of Microbiology, Budapest, Hungary*
[2]*Szent István University, Department of Environmental Safety and Ecotoxicology, Gödöllő, Hungary*

There is an increasing risk of potential mycotoxin contamination of the cultivation raw materials of the popular white button mushroom, also influenced by the global climate change. Although scant details about mycotoxin effect on *Agaricus bisporus* are currently available, their importance in the food chain is evident. The members of the microbial community, having a substantial role in mushroom compost production, are potentially able to degrade or neutralize mycotoxins present in the substrate.
The aim of the current work was to (i) identify the microbial community members of white button mushroom compost production, (ii) isolate potentially mycotoxin degrading bacteria from the compost and (iii) test the isolated strains for successful mycotoxin degradation.
The compost production chain of a local large-scale mushroom producer was sampled extensively. Microbial community members were identified via amplicon sequencing of the 16S rRNA encoding gene. Potential mycotoxin degrader bacteria were enriched in BYE broth supplemented with trichotecene analogues. After isolation strains were identified and subjected to mycotoxin degradation experiments.
The raw materials of the compost harboured a diverse bacterial community with members from Actinobacteria, Bacteriodetes, Firmicutes and Proteobacteria. During Phase I composting *Thermus* spp., Bacilli and Clostridia became dominant, whereas members of Phase II compost derived mainly from Actinobacteria (e.g. *Microbispora*, *Thermobispora*, *Thermomonospora* spp.) and Proteobacteria (e.g. *Pseudoxanthomonas* spp.). The mature Phase III compost was built up by several minor members of Proteobacteria, Actinobacteria, Firmicutes and Bacteriodetes including many uncultivated bacteria. Most of the isolated bacteria from the trichotecene analogues containing enrichments belonged to the genus *Pseudomonas*, but genera *Comamonas*, *Bacillus*, *Glutamicibacter*, *Lysinibacillus* and *Lactococcus* were also represented by a few strains. Some of them exhibited trichotecene degradation.
The described bacterial succession of *Agaricus* compost production resembled to previous literature results and some bacterial strains with promising trichotecene degradation were isolated. To reveal, what kind of effect mycotoxins in higher concentration have, artificially contaminated composts will be used in small-scale *Agaricus* production in the near future.
This research was supported by the National Competitiveness and Excellence Programme (NVKP_16-1-2016-0035).

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Effects of tillage or no-till on soil aggregates, enzymes, respiration and prokaryotic community structure

L. Gabbarini[1], E. Figuerola[2], J. Frene[1], B. Robledo[1], F. Ibarbalz[2], D. Babin[3], C. Kleine[4], K. Smalla[3], L. Erijman[2], L. Wall[1]
[1]*University of Quilmes, CONICET, Bernal, Argentina*
[2]*INGEBI, CONICET, Buenos Aires, Argentina*
[3]*Julius Kühn-Institut, Braunschweig, Germany*
[4]*Fundación Funke, Tornquist, Argentina*

Soil tillage in the semiarid south-western Argentine pampas is causing the exhaustion of agricultural lands, driving ecosystems towards desertification processes. In this experimental study we used along-term field experimenton side-by-side plots, aimed at comparing the effects of conventional tillage (CT) vs. no-till (NT) agriculture, excluding plant and crop effects. One half of the plot under CT was switched to NT and vice versa. Soil enzymes, soil respiration, soil aggregates distribution and the bacterial community structure were analyzed in three consecutive samplings along 30 months after the switch in tillage practices. Soil functional profiles responded to the changes in till management much earlier than the overall bacterial community structure. Thirty months after switching from CT to NT, the levels stratification observed in long-term NT soils were already recovered in the new NT soils, as revealed by significant differences in beta diversity between soil layers (measured by Bray-Curtis dissimilarities). We found many possible bacterial OTUs that responded to NT, related to *Bacillus nealsonii*, *Candidatus nitrosocosmicus*, *Candidatus udaeobactercopiosus*, and OTUs that responded to CT, related to *Arthrobacter*sp., *Nitrososphaera viennensis*and *Chloroflexi*. The response of these bacterial groups occurred at different rates for NT to CT and CT to NT, suggesting that CT induced a longer-term soil disruption than NT. A Spearman correlation analysis of functional and microbial structures, corroborated by random forest tests, revealed coherent structure-functional elements for soils. These findings contribute to better understanding fundamental mechanisms underlying the microbiology of conservative soil management strategies.

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Improvement of a polymeric carrier for efficient cowpea rhizobial formulations

G. Xavier[1], J. Fernandes Carvalho[2], V. Oliosi Favero[3], G. Cavalcanti Alves[3], J. Ribeiro Costa Rouws[1], P. Jansen de Oliveira[4], N. Gouvêa Rumjanek[1]

[1]Embrapa, Seropédica, Brazil

[2]Federal Rural University of Rio de Janeiro, Crop Science, Seropédica, Brazil

[3]Federal Rural University of Rio de Janeiro, Soil, Seropédica, Brazil

[4]Federal Rural University of Rio de Janeiro, Chemical engineering, Seropédica, Brazil

Cowpea beans are a legume that benefits from association with nitrogen-fixing bacteria. Seed inoculation with efficient and adapted strains is a technique that contributes to the process. Until 1996, researches directed to the selection of efficient cowpea strains were neglected in Brazil, due to the broad range symbiosis among cowpea and its microsymbiont. Since 2004, inoculation of cowpea has become a reality in the country, and today it reached a market of around 400,000 doses, with yield gains ranging from 30 to 100%, based on peat formulations. Restrictions to peat extraction have motivated the development of alternative carriers capable of guaranteeing bacterial cell viability. A carrier based on carboxymethylcellulose (CMC) and starch was developed and showed results similar to peat formulations regarding biological nitrogen fixation. However, cell viability were dependent on the reagents used. To extend the use of the CMC / starch carrier, this study aimed to improve a formulation with desirable characteristics for cell viability maintenance of strain BR 3262 of *Bradyrhizobium pachyrhizi* up to 180 days at room temperature. Four types of starch and two of CMC were tested with MgO as the compatibilizing agent ranging from 0.1 to 1% on a dry basis. Two sterilization conditions (121ºC for 30 and 60 min at 1 atm) were also evaluated. Higher cell survival was obtained after 38 days of storage in a polymer blend compatibilized with 0.2 to 0.5% MgO. Two starches out of four were capable of holding pH between 7.5 and 8.0 after 180 days of blend preparation. Nine formulations are capable to maintain approximately 1.7 x 10^7 CFU g^{-1} until 168 days after inoculation. The CMC / starch polymer mixture represents an alternative to peat, but it is important to ensure its production according to standard procedures considering mainly the type of starch and the content of MgO as a compatibilizing agent.

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Influence of plant residues as a convergence factor on the rhizosphere core microbiome of sugarcane and inoculated maize

C. A. Yoshiura[1,2], A. M. Venturini[2], A. G. França[2], M. C. C. Lyra[3], F. S. Cannavan[2], J. L. M. Rodrigues[4], C. L. Aguiar[1], S. M. Tsai[2]

[1]University of São Paulo - Luiz de Queiroz College of Agriculture, Agroindustry, Foods, and Nutrition, Piracicaba, Brazil

[2]University of São Paulo - Center for Nuclear Energy in Agriculture, Division of Agroindustrial Productivity and Foods, Piracicaba, Brazil

[3]Agronomic Institute of Pernambuco, Recife, Brazil

[4]University of California - Davis, Department of Land, Air, and Water Resources, Davis, CA, United States

Recently, the core microbiome of several crops has been characterized for different plants parts, soils, and influence of environmental factors. Roots in association with plant residues (PR) can select and shape microbial communities resulting in crop yield and production increments. The determination of the rhizosphere core microbiome of several types of crops may provide directions for the development of microbial inoculants that can provide faster response for plant growth. The purpose of this study is to compare the rhizosphere microbiome of several crop experiments (sugarcane, maize, and others from public databases), and the microbiome of PR decomposition obtained by 16S sequencing in order to find the core microbiome for potential microbial inoculants. For an initial comparison, the following experiments were considered: (1) sugarcane planted in soils from burnt- and green-harvesting systems (i.e. different levels of PR) and collected after 180 days – stage with intense organic matter accumulation; (2) maize under two inputs (*Azospirillum brasilense* as microbial inoculant on seeds and maize stover coverage as PR) collected after 50 days at the 15th mature leaf – stage with intense biomass gains for reproductive stage; (3) and the PR from a maize decomposition experiment in maize and Amazonian Dark Earth (ADE) soils carried out for 80 days. Following DNA extraction and 16S sequencing, the datasets were analyzed using Qiime2 platform and STAMP v2.1.3 software for similarities analysis. As results, sugarcane and maize soils have great similarities with its PR samples (R2 = 0.943 to 0.987). Furthermore, the combination of inoculant and maize stover samples presented more similarities to PR samples of sugarcane and decomposition in maize soil (R2 = 0.875) in comparison to the other treatments of maize experiment (R2 = 0.448). ADE soil with PR trended in convergence to PR samples of other experiments (R2 = 650) than ADE control group (R2 = 642). In consequence, rhizosphere and PR core microbiomes were determined from samples (22 and 11, respectively, from 319 orders in 11,888 ASVs), in which presented in common, respectively: Bacillales (12% and 12%), Micrococcales (9% and 7%), Rhizobiales (4% and 2%), Sphingomonadales (3% and 2%), and other ≤ 1%; indicating a prevalence for these orders that can be the candidates to looking for microbial inoculants. Financial Support: FAPESP (13/05087-7, 15/13546-7, 15/08564-6, 16/11268-2), CNPq, and CAPES.

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Aerobic and oxygen-limited enrichment of BTEX-degrading biofilm bacteria: dominance of *Malikia* versus *Acidovorax* species

T. Benedek[1], F. Szentgyörgyi[1], F. Révész[1], B. Kriszt[2], A. Táncsics[1]
[1]*Szent István University, Regional University Centre of Excellence in Environmental Industry, Gödöllő, Hungary*
[2]*Szent István University, Department of Environmental Safety and Ecotoxicology, Gödöllő, Hungary*

Bacterial biofilms due to their increased resistance against environmental perturbations are ubiquitous and most of the time are associated with unwanted phenomena in industry or even in hospital settings. However, as a result of high taxonomic and functional diversity bacterial biofilms by using the appropriate sampling site can be isolation sources of biotechnologically important bacteria e.g. involved in bioremediation.
Our previous study revealed the high phylogenetic and metabolic diversity of a biofilm developed in a BTEX-contaminated groundwater. Within the biofilm representatives of more than 600 hundred genera of 40 classes of bacteria could be identified. Functional genes involved in aerobic, microaerobic and anaerobic BTEX-degradation could also be detected. Despite the fact that from taxonomic and metabolic viewpoints the community composition of the investigated biofilm is well known the clear identity of aerobic and/or hypoxic (oxygen-limited) BTEX-degrading and/or biofilm forming community members has not been uncovered. The present study aimed at filling this research gap.
By using a biofilm sample BTEX-amended aerobic and oxygen-limited enrichments were set up for five weeks. Temporal dynamics from taxonomic and functional points of view throughout of the enrichment period were followed by conventional molecular microbiological techniques (16S rRNA and functional gene based T-RFLP and molecular cloning), as well as next generation sequencing (Illumina 16S rDNA amplicon sequencing).
The obtained results indicated that the concentration of dissolved oxygen may be the strongest selective force in the evolution and final structure of microbial communities developed in hydrocarbon-contaminated environments. Representatives of the genus *Malikia* proved to be the most dominant bacterial community members of the aerobic BTEX-degrading enrichments. *Acidovorax* and *Pseudomonas* species dominated the oxygen-limited setup as evidenced by both phylogenetic and functional marker genes based analyses.

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Microbial bioremediation of aquifer affected from chloroethenes and petroleum hydrocarbon contamination

M. Bertolini[1], S. Zecchin[1], L. Cavalca[1]
[1]*Università degli Studi of Milan, DeFENS, Milan, Italy*

In 2014, 20% of contaminated site in Europe were treated by bioremediation techniques, prevalent contaminants being: petroleum hydrocarbons, chlorinated compounds and heavy metals.
Mixed contaminations can be lowered by anaerobic (reductive dechlorination) and aerobic (oxidation) microbial metabolisms. Successive anaerobic-aerobic systems can be used for biodegradation of by-products, such as vinyl chloride, and petroleum hydrocarbons.
In order to assess feasible treatment to improve reductive dechlorination at a contaminated site (Dogaletto, VE, Italy), anaerobic microcosms were set up by using groundwater samples added or not with molasses. GC-MS analysis demonstrated that the addition of the substrate improved reductive dechlorination reactions, leading to higher concentrations of vinyl chloride and ethenes. The addition of molasses was then applied at the contaminated aquifer in the frame of an anaerobic active permeable barrier treatment. Two year field monitoring evidenced that molasses addition improved dehalogenation of high chlorinated ethenes (i.e. perchloroethene from 900 to 450 µg/l) with concomitant formation of vinyl chloride that increased from 33"00 to 69"000. The treatment affected the microbial community structure: *Bacteroidetes*, glucose fermenting bacteria, relatively increased in relation to dechlorinating bacteria of the *Dehalococcoides*genus. Despite this, *Dehalococcodes*gene copy number increased, as determined by q-PCR. In the aerobic active permeable barrier, vinyl chloride and petroleum hydrocarbons decreased, respectively, from 13"550 to 2"600 µg/l and from 3"171 to 1"368 µg/l. A low number of BTEX-degrading bacteria were isolated and characterized and vinyl chloride degrading populations are under study.
The obtained data demonstrated that bioattenuation processes were active at the contaminated aquifer and specialized anaerobic and aerobic bacterial populations can be further exploited for a complete bioremediation.

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Community shift of a mixed culture during benzene degradation under nitrate reduction conditions

F. Borim Corrêa[1], S. Eziuzor[2], S. Peng[3], J. Schultz[1], S. Kleinsteuber[1], C. Vogt[2], U. Nunes da Rocha[1]
[1]*Helmholtz-Zentrum für Umweltforschung UFZ, Department of Environmental Microbiology, Leipzig, Germany*
[2]*Helmholtz-Zentrum für Umweltforschung UFZ, Department of Isotope Biogeochemistry, Leipzig, Germany*
[3]*Chongqing University, Department of Environmental Science, Chongqing, China*

A microbial community mineralizing benzene under nitrate-reducing conditions was enriched from a benzene-contaminated aquifer and has been maintained in the lab for several years. A previous study revealed that the community was dominated by Gammaproteobacteria, Ignavibacteria and Anaerolineae. However, a controlled succession experiment is yet to be described and could reveal microbial interactions and bioindicators for mineralization.
Here, we monitored the succession of species in our model community during the degradation of benzene and reduction of nitrate. The experiment was set in 24 bottles inoculated with the previously proved degrading community plus 3 bottles without the inoculum. In total, 15 bottles were fed with ^{13}C-benzene while 9 bottles served as controls. We measured indicators of activity as $^{13}CO_2$, nitrate and nitrite and sampled the solid (sand) and liquid phases at five time points. DNA was extracted, 16S rRNA genes were PCR-amplified and the amplicons were sequenced using Illumina MiSeq. Amplicon Sequencing Variants (ASVs) were generated using DADA2 and classified using SILVA version 132.
From an average of 108,000 sequences per sample, a total of 58 distinct ASVs were identified. Confirming our previous study, Proteobacteria were detected and increased in abundance at the last two time points when the mineralization was at its highest. Notably, the superphylum Patescibacteria was considerably abundant and present in all samples and phases. The identified Patescibacteria, which have not been detected in our previous study, belong to the candidate phylum WWE3. This phylum was recently detected in groundwater and has a very small genome (<0.9Mb).
In conclusion, community shifts were seen along the degradation of benzene. Currently, we are preparing metagenomics and SIP-proteomics data from all samples and time points. These will be used to understand the mechanism underlying anaerobic benzene mineralization under nitrate reduction conditions.

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Compositions of enriched bacterial communities involved in the aerobic degradation of BTEX and vinyl chloride from chlorinated solvent-contaminated groundwater

A. Chebbi[1], T. Stella[1], I. Pietrini[2], G. CARPANI[2], A. Franzetti[1]
[1]*University of Milano - Bicocca, Dept. of Earth and Environmental Sciences (DISAT), Milan, Italy*
[2]*Eni S.p.A., Decarbonization and Environmental R&D, Milan, Italy*

Introduction: Groundwater contamination caused by BTEX and chloroethenes is remaining a worldwide challenge for leading industrial countries, including Italy. Among chlorinated ethenes, vinyl chloride (VC) is the most cancerogenic compound, which is widely threatening both human and animal health.
Objectives: The aim of this study is to enrich microbial consortia capable of degrading BTEX and VC under aerobic conditions.
Materials & methods: Cultures were set up using contaminated groundwater as inoculum, spiked with BTEX and VC for the enrichment. The biodegradation capacities and the microbial community structure were determined using GC-FID and MiSeq-Illumina sequencing, respectively.
Results: Based on GC-FID results, the autochthonous microbial communities in a contaminated piezometer were able to degrade toxic volatile organic compounds efficiently, as they constitute major pollution problems in groundwater. After microbial enrichment during 99 days, the VC condition (1-2 ppm) revealed predominantly the presence of *Xanthobacteraceae* (29.4%), *Hyphomicrobiaceae* (27.8%), *Peptococcaceae* I (12.5%), and *Rhizobiaceae* (4.7%), while in the presence of BTEX (50 ppm), *Xanthobacteraceae* (35.7%), *Peptococcaceae* I (26.8%), *Rhizobiaceae* (10.1%), and *Caulobacteraceae* (6.2%) were the most abundant families. Finally, the mixture (VC+BTEX) condition (3) showed the family dominance of *Peptococcaceae* I (36.4%), *Xanthobacteraceae* (26.2%), *Rhizobiaceae* (9.0%), and *Hyphomicrobiaceae* (7.2%). In comparison to the initial bacterial structure of the groundwater, the results clearly indicate the appearance of predominant novel bacterial communities, which would be correlated to the significant biodegradation capacities of these three consortia on VC and BTEX, under biostimulation conditions.
Conclusion: This study adds further knowledge for the bioremediation of groundwater aquifers impacted by both BTEX and VC.
Keywords: Biodegradation, Vinyl chloride, BTEX, GC-FID, MiSeq Illumina, Groundwater.

P 158
Cancelled

P 159
The impact of bacteriophages in bacteria removal associated with soba stabilisation station efficiency
A. Elshayeb[1]
[1]*Elbarkal Multi Activities, Dept. Research & Consultancy, Microbiology, Khartoum, Sudan*

Introduction: Bacteriophages are viruses that infect and lyse bacteria, the applications of phage" techniques in wastewater treatment systems improve effluent and sludge emissions into the environment.
Methods: The existence of bacteriophages in wastewater of Soba Stabilisation Station was determined by isolating and identifying methods for their activities against *Escherichia coli* and *Staphylococcus aureus* isolated from the anaerobic, facultative and maturation ponds.
Results: The general viable count of the bacteria showed an average of 2.0x 106 cfu/ml. In broth media the affection of the bacteriophage interactions with bacteria showed increasing of bacteriophages with concomitant decrease in bacteria due to culture clearance, where the readings of the turbidity for the first and second infection showed statistical significant of light transmission among *E. coli* phages" samples due to place of sample collections as follows: from the anaerobic and facultative ponds $P>0.05$, facultative and maturation $P<0.05$ and anaerobic and maturation $P>0.05$. Whilst, the *S. aureus* phages samples" light transmission from the anaerobic and facultative $P<0.05$, facultative and maturation $P<0.05$ and anaerobic and maturation $P>0.05$. On solid media the affection of the bacteriophage was recognised by the phage plaque formation on bacterial cultures. The linear equations of phages" densities and distributions according to their wavelength were $y = 0.0008x + 0.0303$ for *E. coli* phage and $y = -0.0102x + 0.2438$ for *S. aureus* phage.
Conclusion: This study concluded that phages naturally present where their hosts present and naturally destroyed bacteria which aided to recover from polluted environment.

P 160
Characterization of phyllospheric bacterial communities hosted by *Platanus x acerifolia*in European urban areas
I. Gandolfi[1], W. Smets[2], L. M. Spada[1], A. Franzetti[1], S. Lebeer[2]
[1]*University of Milano-Bicocca, Dept. of Earth and Environmental Sciences, Milan, Italy*
[2]*University of Antwerp, Dept. of Bioengineering, Antwerp, Belgium*

Introduction: air pollution is a global problem because it is increasing worldwide due to rapid economic growth and increased energy consumption, and it affects both human health and ecosystem functions. Phyllospheric bacteria, hosted by plant leaves, have been reported to be able to biodegrade or transform pollutants, but their potential for air remediation is still largely unexplored.
Objectives: phyllospheric bacterial communities of London Plane trees (*Platanus x acerifolia)* were characterized in relation to different air pollution levels in Antwerp and Milan. The colonization of young leaves by bacteria hosted by other parts of the tree was also assessed.
Materials & Methods: a total of 180 samples (buds, leaves, branches and trunks) were analysed in order to describe the bacterial communities through the Illumina sequencing of the V4 hypervariable region of 16S rRNA gene. Pollution levels on leaves were estimated by SIRM (Saturation Isothermal Remanent Magnetization) analysis, used as a proxy for PM10 concentrations.
Results: bacterial communities were generally dominated by orders *Sphingomonadales, Cytophagales* and *Burkholderiales,* which are already known to be part of the phyllosphere. However, Milan samples were dominated by *Cytophagales*, while Antwerp samples by *Sphingomonadales*. Alpha-diversity was also higher in Antwerp samples. Therefore, geography and climate had a clear impact on the biodiversity of bacterial communities. On the contrary, it was not possible to observe wide differences between the bacterial communities of samples collected near high traffic roads and the samples collected in parks, in both cities. Nevertheless, this lack of significant differences might be also due to pollution levels similar for high traffic roads and urban parks, as indicated by SIRM analysis. Great differences could be observed in the bacterial community structure between leaf samples and all the other samples. This may suggest that the bacteria harboured by trunk and branches can colonize the buds, but the bacteria present on leaf surface in its early life can derive mainly from other sources, such as the atmosphere.
Conclusions: a deeper knowledge on phyllospheric bacterial communities is needed to develop a sustainable technology for contaminated air clean-up. More plant species should be examined to elucidate the relationship between phyllosphere bacteria, their host plants, and various air pollutants.

P 161

Use of next-generation sequencing to characterize changes in water microbiota across an urban watershed in San Juan Puerto Rico

E. Lemuel-Tosado[1], F. Vazquez-Sanchez[1], A. Marquez-Trejo[1], E. Torres-Rivera [2], F. Godoy-Vitorino[1]
[1]University of Puerto Rico, School of Medicine, Microbiology and Medical Zoology, San Juan, Puerto Rico
[2]Inter American University of Puerto Rico, Metropolitan Campus, Natural Sciences, San Juan, Puerto Rico

Introduction: The Rio Piedras river is the most important urban watershed in Puerto Rico (San Juan area). Over the years, this watershed has been impacted by urban development and contaminants as result of anthropogenic activities. Currently only standard culture-methods are used to evaluate water diversity and quality. We hypothesized that the structure and diversity of the water bacterial communities change according to the anthropogenic impact levels.
Objectives: We aimed at characterizing the microbiota of this body of water using 16S rRNA sequencing.
Materials & methods: Water samples were collected on the river in four sites over an urbanization gradient. Water was filtered, gDNA was extracted from the filters and 16S rDNA genes were amplified and sequenced by Illumina MiSeq. Data analyses were done using QIIME1 with the SILVA database as reference.
Results: A total of 29 phyla and 213 genera were found, in varying complexity along river sites. The less impacted site showed only 5 phyla with a dominance of Proteobacteria and Actinobacteria the medium impact sites had 15 phyla and a dominance of Chlorobi, Actinobacteria and Bacteroidetes while the most impacted site had a higher diversity and number of phyla (26) with a a dominance of Proteobacteria, Firmicutes (*Faecalibacterium*) and Bacteroidetes (*Bacteroides*) – fecal matter derived taxa. Richness was significantly higher in the most impacted site. We found 4 genera of Enterobacteriaceae including Enterobacter and Yersinia, in the high impact sites.
Conclusion: With a 16S rDNA approach, we were able to define the varying and dynamic microbiota of this urban watershed to a much more detailed depth revealing a relation to anthropogenic impact. We found an unprecedented diversity of microbial communities in water samples directly linked to an increase in human impact of overall environmental and enteric bacteria.

P 162

Effect of mycorrhizal inoculation on the phytomanagement of polluted soil

J. Hidalgo [1], M. ANZA[1], L. Epelde[1], J. Urra[1], J. M. BECERRIL[2], C. GARBISU[1]
[1]neiker-Tecnalia, Conservation Of Natural Resources, Derio, Spain
[2]university Of Basque Country, Plant Biology And Ecology, Leioa, Spain

Introduction: Soil pollution with organic compounds is an environmental problem of great concern. Our study area, i.e. an industrialized site located in the Basque Country (Spain), is characterized by the presence of high concentrations of polycyclic aromatic hydrocarbons (PAHs) and total petroleum hydrocarbons (TPHs). Among the different strategies currently available for the remediation of polluted sites, phytomanagement appears as a cost-effective, sustainable option for soils degraded by the presence of organic pollutants. Within phytomanagement initiatives, plants are often inoculated with mycorrhizal fungi to facilitate plant survival and growth, and simultaneously to collaborate in the biodegradation of organic pollutants in the rhizosphere. *This work was funded by the NANORRIZORREM-2 project (AGL2016-76592-R).*
Objectives: The aim of this study was to assess the effectiveness of mycorrhizal inoculation on a phytomanaged soil heavily polluted with a mixture of TPHs and PAHs.
Methods: We carried out a mycorrhizal-assisted phytomanagement study using an organic amendment, poplar trees and alfalfa. A factorial design was used to assess the expected beneficial effects of treatments on the (i) degradation of organic pollutants, (ii) improvement of soil health and (iii) restoration of ecosystem services. To quantify the effect of treatments on soil health, microbial activity, biomass and diversity were measured, as well as soil physicochemical parameters.
Results: Microbial parameter values increased in inoculated treatments, especially for alfalfa. Microbial activity was enhanced by the application of the amendment.
Conclusion: We observed a phytomanagement-induced recovery of soil health. In fact, soil microbial communities and plant growth were stimulated by the applied treatments.

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Alien pesticide-degrading bacteria challenged to remove micropollutants from drinking water: Physiological and ecological consequences of starvation

B. Horemans[1], D. Springael[1]
[1]*KULeuven, Department of Earth and Environmental Sciences, Leuven, Belgium*

In recent years, bioaugmentation was explored again as a technique to remove micropollutants such as pesticides from drinking water by applying pesticide-degrading bacteria in existing sand filters which are part of the drinking water production process. However, pesticide-degrading bacteria from agricultural soils treated with high loads of pesticides repeatedly over the years, suddenly experience a new and harsher world in sand filters where pesticide loads are continuous but extremely low. Suddenly, being able to grow on pesticides isn"t a real advantage anymore.
Challenges for pesticide-degrading bacteria to invade the sand filter environment were explored by proteomics and genomics revealing signs of starvation stress and loss of catabolic enzymes needed for pesticide degradation. Starvation clearly puts pesticide-degrading bacteria in a bad position to invade the resident sand filter biofilm community well-adapted to oligotrophic conditions in sand filters. A synthetic ecological approach using sand filter bacterial isolates revealed, however, that an "alien" pesticide-degrading bacterium relies on associations with resident bacteria in the sand filter environment to assure its survival.

P 164

Bioremediation of metal-contaminated Doce River Hydrographic Basin based on microbial biofilms

R. da S. Pereira[1], L. Seldin[1], C. Neumann Keim[1], D. Jurelevicius[1]
[1]*Universidade Federal do Rio de Janeiro, Instituto de Microbiologia Paulo de Goes, Rio de Janeiro, Brazil*

In 2015, the breakdown of a mining tailings dam (Mariana, Minas Gerais, Brazil) released approximately 50 million/m^3 of sludge containing fine particulate, iron oxides and quartz in Doce River Hydrographic Basin (DRHB). Microbial biofilms are well known to retain metals and, then, represent a potential tool for the bioremediation of metal-contaminated environments. The objective of this study was to evaluate the formation of microbial biofilms and their capacity to capture metals from contaminated water. For microbial biofilms development, sterile glass slides associated with floats were placed on the riverbed of the Gualaxo do Norte River (GNR) and Doce River (DR) for a period of 14 days. Water samples from the two rivers were also collected at the beginning and at the end of the experiment. Subsequently, the iron quantification present in water and in biofilms samples was determined by the ferrozine method in flame atomic absorption spectrophotometry (FAAS). The biofilms and the presence of the metals associated with the biofilms were analyzed by Optical Microscopy and by Scanning Electron Microscopy (SEM) associated with the X-ray Spectroscopy by Energy Dispersion (EDX). In addition, the bacterial community present in each sample was characterized by high-throughput sequencing of the gene coding for 16S rRNA. The results obtained by FAAS showed the presence of 0.245 mg/L of total iron and 0.041 mg/L of total manganese in the DR water. In the GNR, the iron concentration was below the detection limit of the ferrozine method (<0.28 mg/L of total iron). After 14 days complexes biofilms structures were formed on the glass slides placed on both rivers. SEM/EDX analysis showed the association of iron and manganese with microbial biofilms. On average, 64 mg/m^2 of total iron was quantified in the DR biofilms. 16S rRNA sequencing analysis showed the structure of the bacterial community of the GNR biofilms was different from that observed in the DR biofilms. While *Advenella*, *Pseudomonas*, *Enterococcus*, *Delftia*, and *Pantoea* were the main genera observed in biofilms from GNR, in biofilms from DR the main genera were *Corynebacterium*, *Methylobacterium*, and *Cloacibacterium.* Furthermore, the results and knowledge obtained in this research can be used to design bioremediation methods to clean-up metal contaminated waters.

P 165

Microbial community structure and transformation of xenobiotics in Moscow Free Water Surface Treatment Wetland

S. Kharitonov[1], M. Semenov[2], K. Ribka[1], S. Nataliya[1]
[1]*Moscow State University, Soil Science, Moscow, Russian Federation*
[2]*V.V. Dokuchaev Soil Science Institute, Moscow, Russian Federation*

Treatment Wetlands (TW) are very effective in the wastewater purification. Their biocenoses are capable to degrade various organic pollutants. However, not much is known about the selection of bacteriocenosis takes place in TW, and how the structure of such complex community reflects its functions, useful for water treatment.
The aim of the study is to analyze composition of bacterial community in different zones of TW, detect all functional groups of bacteria and compare this data with results of chemical analysis.
The object of study is Free Water Surface Constructed Wetland in Moscow, it receives stormwater and some industrial effluents. It is a flow reactor divided into 5 zones. The structure of bacteriocenosis in sludge and water, content of organic pollutants (polycyclic aromatic hydrocarbons, phthalates, surfactants) in different zones of TW were investigated. Sequencing of 16S rRNA was carried out on Illumina MiSeq. The pair-end reads were filtered and merged using MeFiT (https://github.com/nisheth/MeFiT) (Parikh et al., 2016). Taxonomy assignment of the OTU sequences up to genera level with adjustment for the gene copy number was performed using the RDP database (http://rdp.cme.msu.edu/) with the RDP classifier (Cole et al., 2014). Microbial diversity was estimated using the Shannon, Chao1, and abundance-based coverage indixes. Bray-Curtis dissimilarity was used to explore the variation in prokarytic community structures among all the samples. GC-MS (Agilent GC 6890 MSD 5970), HPLC (Agilent 1200) were used for chemical analysis.
We have detected 17 genera of methanogens. The methanotrophic community was represented only by 4 genera. A total of 21 genus of methylotrophic microorganisms were detected. Phototrophic microorganisms were among the dominant in TW, especially in anaerobic sites. The most representative phototrophs were cyanobacteria, reached 20–25% of the total microbiome. Content of the sulfate-reducing microorganisms ranged from 0.5 to 3%.
The patterns of distribution of the main phylogenetic groups of microorganisms, which are biologically active components of TW were identified. As a result, relationships between community structure and water treatment efficiency in each zone were determined. Zones with plants proved to be most effective in removing xenobiotics.

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cancelled

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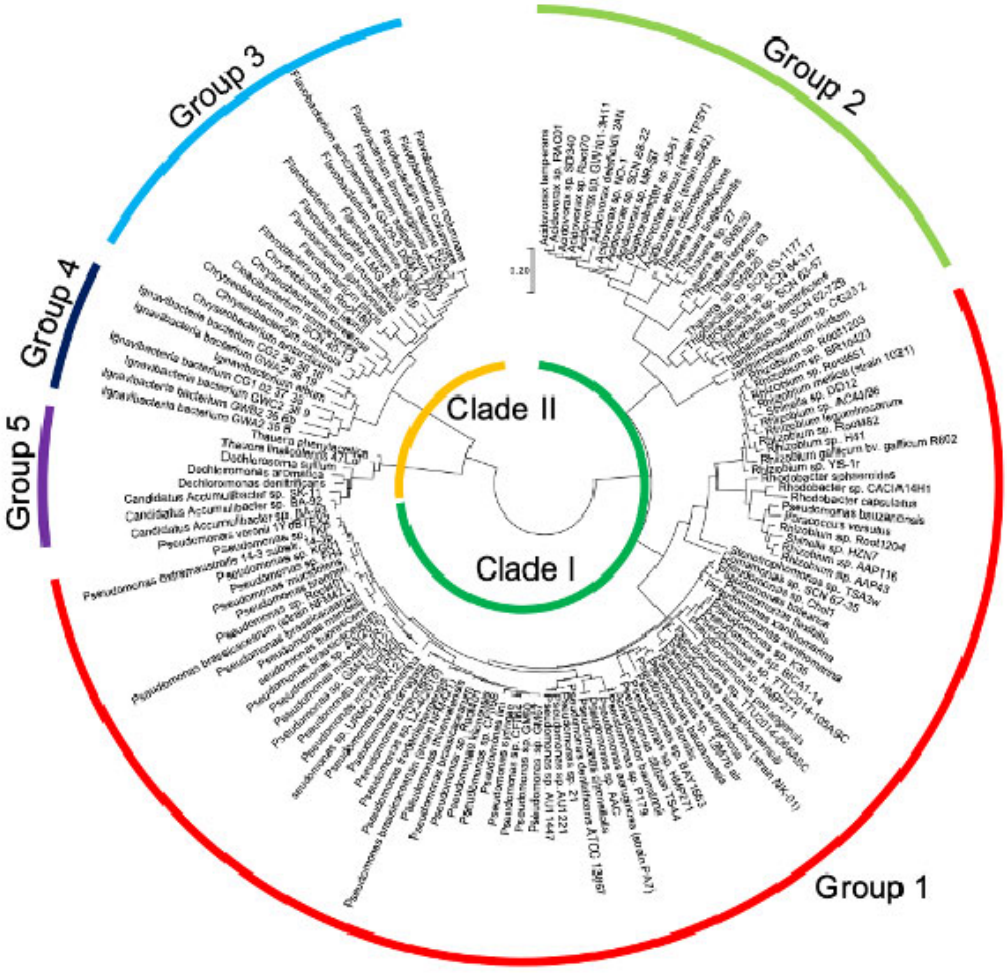
Development of group-specific *nosZ* primers for quantification of active high-affinity nitrous oxide reducing organisms in complex environmental samples

D. D. Kim[1], H. Yoon[1], T. Yun[1], D. Park[1], S. Yoon[1]

[1]KAIST, Civil and Environmental engineering, Daejeon, South Korea

Despite the recent interest in *nosZ*-possessing organisms as the sink of the N2O, their quantification has relied on antiquated qPCR techniques using degenerate primers developed before the broad diversity of *nosZ* genes was revealed. Due to the divergence of their sequences, *nosZ* genes, especially of the recently discovered clade II, have evaded attempts for development of molecular tools for group-specific quantification. Here, we have combined culture-based technique with computational methods to develop Taqman-based qPCR reactions for quantification of microorganism actively consuming N2O with high affinity. An activated sludge sample was enriched in a fed-batch reactors with continuous stream of N2 gas containing 20 ppmv and 200 ppmv N2O. The enriched N2O-reducers were identified with 16S rRNA amplicon sequencing. The amino acid sequences of NosZ affiliated to these taxa were collected from UniProt and grouped into five subdivisions. A TaqMan primer/probe set exclusively and comprehensively targeting each subdivision was then designed and validated against unspecific amplification with extensive *in-silico* PCR analyses. Unexpectedly, the enriched high-affinity N2O reducers included *Pseudomonas* and *Acidovorax* (clade I) as well as *Dechloromonas* (clade II) as the most abundant genera, according to amplicon sequencing data. The group-specific qPCR assays were able to corroborate the abundance of *nosZ* affiliated to these organisms. These observations suggested that subgroups of both clade I and clade II were responsible for high-affinity N2O reduction and that simple clade I / clade II dichotomy cannot solely explain the important sink of the potent greenhouse gas

Fig. 1



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cancelled

P 169
Purification of anaerobically digested livestock slurry with aim to reclaim nutrients and water resource from digestates by bioleaching approaches
L. Zhou[1], J. Liang[1], H. Zhang[1]
[1]Nanjing Agricultural University, College of Resources and Environmental Sciences, Nanjing, China

At present, the annual production of anaerbiocally digested slurry from livestock reaches 1 billion tons in China. The improperly or directly discharged digested swine slurry, characterized by high pollution load (high concentration of COD, NH3-N, and SS), threat greatly human health through damaging the quality of surface and underground water, soil, and atmosphere. In recent years, conventional industrialized biological treatment such as A/O and A2O processes for purifying the digested slurry often exhibits low efficiency due to high concentration of SS in the slurry. Here bioleaching approach was used to remove drastically or recovery SS prior to the slurry entering subsequent biological treatment system. The results showed that the digestion time affected drastically the properties of digested slurry and also the slurry bioleaching effectiveness. After digested slurry bioleaching, the specific filtration to resistance of the bioleached digested slurry could fall to below 5×1011 m·kg-1 and its dewaterability improved greatly. The leaching percentages of heavy metals of the digested slurry were 72.5% for Cu and 82.7% for Zn. SS could be removed or recovered up to nearly 100% through bioleaching pretreatment followed by diagram press filter. SS, NH3-N, COD, and total phosphorus of filtered water were reduced to about 0 mg/L, 650 mg/L, 395~483 mg/L, and 0.6~1 mg/L from initial 56000 mg/L, 1894 mg/L, 51650 mg/L, and 1086 mg/L, respectively. These filtrate easily was purified by subsequent biological treatment system such as A2O to meet the related discharge standard. As a result, about 90% of organic matter and nutrients in digestate can be reclaimed through solid and liquid separation after bioleaching. Purified water resource after biological treatment can be reutilized in livestock farms.

P 170
Can secondary plant metabolites stimulate bacterial degradation of dioxins and furans?
E. Lopez-Echartea[1], J. Šuman[1], O. Uhlík[1]
[1] University of Chemistry and Technology, Prague, Department of Biochemistry and Microbiology, Prague, Czech Republic

Massive production, use and release of persistent organic pollutants (POPs) in the last century represents now an environmental burden. Several technologies have been used to remove POPs from the environment. However, bacterial degradation may be a more cost-effective and eco-friendly methodology to remove these contaminants. Microbes have the ability of degrading POPs; therefore, they can be used in bioremediation applications. Xenobiotics like polychlorinated dioxins and dibenzofurans are more often cometabolized by microorganisms. Cometabolism is based on a broad specificity of degradative enzymes, in this case oxygenases, which transform recalcitrant compounds in the presence of a primary substrate. Secondary plant metabolites (SPMEs) appear as a suitable alternative to primary substrates such as biphenyl and dibenzofuran due to their structural similarities to the contaminants, natural origin and structural diversity. SPMEs can also serve as carbon and/or energy sources and inducers of degradative enzymes. The knowledge of the relation of SPMEs to biodegradative enzymes is insufficient, requiring further research.
The aim of the presented project was to test the potential of SPMEs to induce dioxygenase genes in a culture of *Pseudomonas veronii* capable of using different POPs as growth substrates. The bacterial culture was grown on sodium acetate until mid-exponential phase and then exposed to selected SPMEs or dibenzofuran in a resting-cell-like assay. Total RNA was extracted and reverse transcription was performed, followed by the quantification of the transcripts using primers targeting hydroxylating dioxygenase and 16S rRNA genes. Our results show that specific SPMEs can act as inductors of the transcription of dioxygenase genes. In the future, natural materials rich in these metabolites could be used to enhance biodegradation in remediation applications.
Support of the Ministry of Industry and Trade of the Czech Republic is acknowledged (grant no. FV10471)

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Application of nzvi and cheese whey to aquifer contaminated by chlorinated ethenes – effect on autochthonous bacteria and remediation

H. Macková[1], D. Vlková[1], P. Lacina[2], A. Ševců[1], I. Dolinová[1]
[1]*Technical University of Liberec, Institute for Nanomaterials, Advanced Technologies and Innovations, Liberec, Czech Republic*
[2]*GEOtest, Brno, Czech Republic*

Chlorinated ethenes (CEs) are common environmental contaminants due to past improper handling and storage. Owing their adverse effect on the environment and living organisms including humans, cleanup of aquifers contaminated by CEs has been a major task for decades. Current remediation strategies include treatment by nanoscale zero-valent iron (nZVI) followed by application of biological substrates, such as cheese whey. Particularly this combination seems to be very promising tool, because it increases efficiency of entire remediation process and simultaneously induces optimal conditions for subsequent natural attenuation.
The main goal of this study was to compare treatment using nZVI and cheese whey when applied in different order and evaluate the best approach. Field experiments were focused on saturated zone of the CE-contaminated area in an industrial factory in the Czech Republic. Following treatments were studied: a) nZVI followed by cheese whey, b) cheese whey followed by nZVI, c) nZVI alone, d) cheese whey alone. The interval between applications was six months and one non-treated well was monitored as a reference. Effect of different treatments on dechlorination process as well as on autochthonous microorganisms was evaluated. Molecular genetic analyses based on quantitative polymerase chain reaction (qPCR) were focused on detection of genes encoding enzymes vinylchloride reductases (bvcA and vcrA) and bacteria capable to degrade CEs: *Dehalococcoides* spp. (DHC-RT), *Dehalobacter* spp. (Dre) a *Desulfitobacterium* spp. (Dsb).
nZVI application caused only short-term negative effect on autochthonous microorganisms. In longer perspective, nZVI effectively reduced CEs and enabled higher efficiency of biodegradation processes. Cheese whey application showed positive effect to autochthonous microorganisms in all cases. CEs concentrations decreased to almost zero in all monitored wells. The best remediation strategy is application of nZVI and after three months application of cheese whey.
This study was supported through the "Environmental Friendly Nanotechnologies and Biotechnologies in Water and Soil Treatment Project" (NanoBioWat, Project TE01020218) by Technology Agency of the Czech Republic

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Detection of functional genes involved in petroleum biodegradation

A. Menezes Neto[1], E. Oliveira[1,2], D. Oliveira[1], A. Queiroz[1], D. Lima[1]
[1]*Federal University of Bahia, Institute of Geosciences, Salvador, Brazil*
[2]*State University of Feira de Santana, Department of Biology, Feira de Santana, Brazil*

The autochthonous microbial community is responsible for the bioremediation process, due to the metabolic capacities of these organisms to remove pollutants. The use of microbial consortia in the remediation of impacted areas makes it possible to better understand the metabolic functions of each microorganism as well as how they act together. Hydrocarbons can be degraded in two distinct forms, aerobic and anaerobic, since oxygen is indispensable for the activation of degradation by aerobic bacteria. The objective of this study was to identify the presence of oil degrading genes in a bacterial consortium composed of isolates from mangrove sediments in Brazil, as well as to evaluate the diversity of these genes. To reach the objective, genomic DNA was extracted from 10 isolates, the quantification of the samples performed in NanoDrop One, amplification of alkB and bssA genes performed in thermocycler and quantification in 2% agarose gel, sequencing was performed in ABI3500XL Genetic Analyzer. The alkB gene was successfully amplified in 90% of the isolates, while the bssA gene was amplified in the 10 isolates that make up the microbial consortium. The alkB gene is often reported as a biomarker for characterization studies of microbial populations containing medium and long chain n-alkane degrading aerobic bacteria in various environmental samples, as well as in bioremediation processes. The bssA gene has its action in the anaerobic degradation of monoaromatic and polycyclic aromatic hydrocarbons, and can be treated as a universal marker for these organisms. Thus, the microbial consortium presents functional aerobic and anaerobic oil degrading genes for different oil fractions and point out the potential of these isolates to remedy areas impacted by oil and its derivatives.

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Evolution of bacterial populations in *m*-cresol-containing environment

I. Metsik[1], T. Ilmjärv[1], M. Kivisaar[1]
[1]*Institute of Molecular and Cell Biology, University of Tartu, Depratment of Genetics, Tartu, Estonia*

Due to human activities there are many toxic aromatic compounds called xenobiotics in the environment. Xenobiotics are persistent in the environment for a very long time because of their chemical structure and stability. Since most of these aromatic compounds contain foreign structures there are missing necessary catabolic pathways in microbes for the degradation of these compounds. Despite of that there has been isolated many bacterial strains that are capable to degrade such compounds. Microbial ability to evolve to new environmental conditions and their metabolic versatility contributes to the evolution of new catabolic pathways which enables to use new aromatic compounds as an energy and carbon source.

In this work we carried out laboratory evolution experiment with constructed *Pseudomonas putida* strains that degrade toxic phenolic compound called *m*-cresol (Cre^+ phenotype). The aim of this experiment was to examine what kinds of changes could occur in bacterial populations throughout the laboratory evolution experiment which enabled bacteria to degrade or tolerate *m*-cresol more efficiently. Bacteria were cultivated in glucose-containing medium in the presence of 5 mM *m*-cresol (335 to 427 generations). *P. putida* wild-type strain which did not carry genes necessary for the degradation of *m*-cresol was used as a control population. In order to evaluate impact of increased mutation frequency on the evolution of bacterial populations in the presence of *m*-cresol, *P. putida* Cre^+ lineages deficient in DNA mismatch repair (lack of functional *mutS* gene) were also included in the evolution experiment. We wanted to investigate whether *mutS*-deficient bacterial population could evolve and adapt more efficiently to these environmental conditions compared with bacterial population which had lower mutation frequency. Initial screenings of the evolved lineages implied that despite the frequency of mutations, in each lineage there are subpopulations evolved via different evolutionary trajectories either leading to increased efficiency of *m*-cresol catabolism or higher tolerance to this substrate with concomitant loss of corresponding catabolic genes. These results indicate that a "bet-hedging" strategy could be prevailing in populations of *P. putida* when exposed to toxic phenolic pollutants.

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Soil loss prevention by rainfall through Microbially Induced Calcite Precipitation (MICP) and its application on heavy metals-contaminated soil

K. Nam[1]
[1]*Seoul National University, Civil and Environmental Engineering, Seoul, South Korea*

Microbially Induced Calcite Precipitation (MICP) was applied to prevent soil loss by rainfall through its soil-cohesion effect, especially in heavy metals-contaminated. *Sporosarcina pasteurii* KCTC 3558 was used as a urease-producing bacterium of which kinetic parameters of urea hydrolysis were 6.95 mM/min-OD of Vmax, 252.65 mM of KM. Optimum composition of MICP-inducing solution in soil was determined based on the degree of cohesion of the MICP-induced soil. Presence of nutrient (tryptic soy broth), different calcium sources (i.e., calcium chloride, calcium nitrate, and calcium acetate), and different urea and calcium concentrations were tested, and the final composition was determined to be 4 g/L tryptic soy broth, 450 mM each of calcium chloride and urea, and *S.pasteurii*. As MICP was conducted in sand, the concentration of $CaCO_3$ precipitates increased linearly (9.8 mg $CaCO_3$/g-soil/application). After seven-repeated applications, 72.9±3.7 mg-$CaCO_3$/g-soil was precipitated, but penetration resistance, as an indicator of soil cohesion, reached a plateau of 25.1±2.4 N/mm. In artificial rainfall test with sand and sandy loam, up to 84% of reduction in loss rate was accomplished in sand at the hardest condition (rainfall intensity of 75 mm/hr, slope of 15°) while only 58% of reduction rate was obtained after five-repeated applications in sandy loam with 2.6% organic carbon, indicating that particle size and organic carbon content seemed to influence the effect of MICP. In an experiment to investigate the applicability of MICP on heavy metals-contaminated soil, the inhibitory effect of Cu on urea hydrolysis was observed, showing about 50% reduction of urea hydrolysis at 250 mg-Cu/kg-soil. However, the inhibition was highly correlated to Cu concentration in soil solution rather than the total concentration in soil. Interestingly, calcium ion, which is an essential element for MICP, increased the concentration of Cu in soil solution probably because it exchanged soil-sorbed Cu and released it more in soil solution, causing higher inhibitory effect.

Fig. 1. Effect of MICP on (a) calcium carbonate concentration and (b) penetration resistance in sand

Fig. 2. Effect of (a) soil Cu and (b) calcium on the inhibition of urea hydrolysis. As calcium concentration increased Cu in soil solution increased, generating decreased ammoia production.

Fig. 1

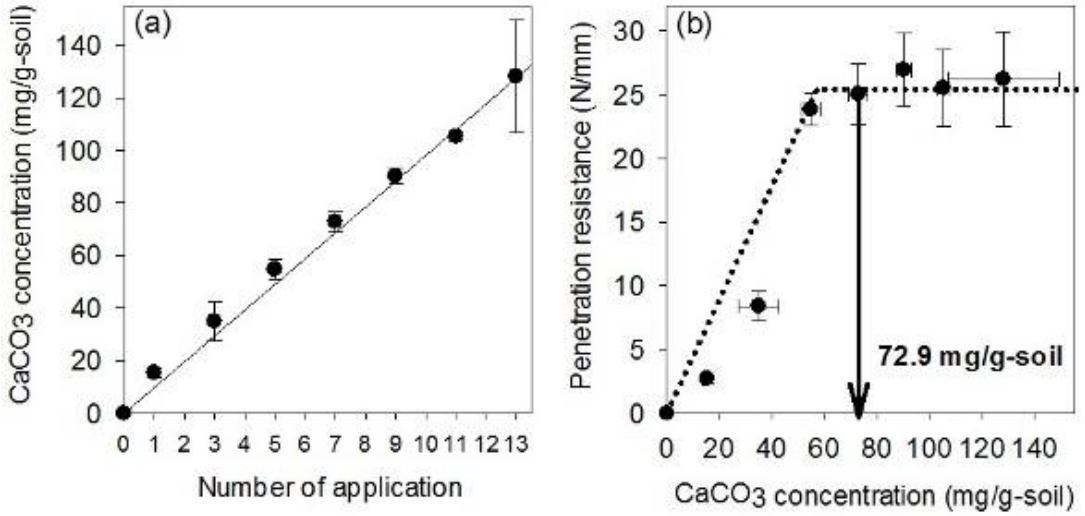
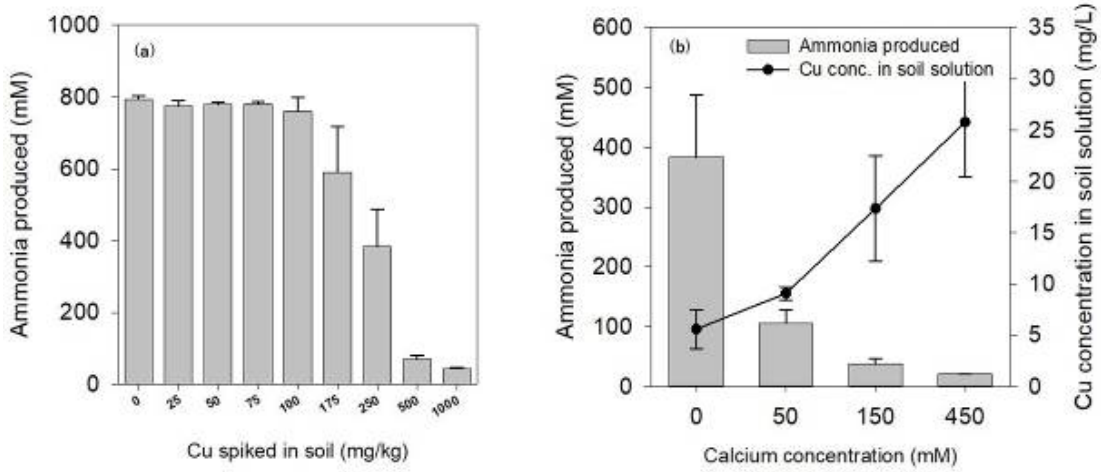


Fig. 2



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Desulfurization of crude oil from bacteria isolated from palaeoenvironmental niche of Greece

G. Ntroumpogianni[1], A. Savvides[1], D. Filioti[1], E. Katsifas[1], A. Karagouni[1]
[1]*National and Kapodistrian University of Athens, Biology, Athens, Greece*

The process of oil combustion causes the emission of certain hazardous pollutants into the atmosphere. Among these pollutants, the sulfur dioxide (SO2), causes severe environmental impacts, such as air pollution and acid rain. Currently, deep desulfurization of oil and its fractions is performed by hydration at high temperature and pressure, which makes the process rather expensive. An alternative low cost process and also complementary to this technology is the microbial desulfurization of crude oil and its various derivatives. Relevant studies have designated a number, of mostly aerobic, microbial strains that can efficiently perform desulfurization provided sufficient oxygen supply to the atmosphere. So far they have been focused in isolating sulfur assimilating strains from sites with oil-contamination history of only a few decades. Our aim was first to isolate desulfurizing bacteria from the palaeoenvironmental setting of *Keri Lake* on Zakynthos Island. The site is non-commercially exploitable and remains intact for all recorded historical period, at least 2000 years. A total of 38 bacterial isolates were recovered from soil samples. All the isolates were grown in sulfur-free medium (MSSF) using the crude oil as sulfur source. Only 6 out of 38 were able to grow on MSSF plates and were characterized according to their 16S rRNA sequence. The isolates were then examined for their biodesulfurization activity in batch cultures using high-sulfur fuel oil as a sole sulfur source. Biomass production, sulfur concentration, total hydrocarbon concentration and hydrocarbon profile were estimated during a 60 days period. In conclusion, the data of desulphurization and degradation are promising for microbial treatment especially if they combined with the process in refineries.

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Biochemical characterization of two nitrilotiracetate monooxygenases from *Gordonia* NB4-1Y thought to mediate the transformation of 6:2 fluorotelomer sulfonate to 6:2 fluorotelomer aldehyde

G. O'Connell[1], C. Milton-Wood[1], E. Bottos[1], K. Haegedorn[1], B. McAmmond[1], M. Norman[1], C. Vickers[1], D. Wang[2], J. Liu[2], J. Van Hamme[1]
[1]*Thompson Rivers University, Biological Sciences, Kamloops, BC, Canada*
[2]*McGill University, Civil Engineering, Montreal, QC, Canada*

The way we understand bacterial transformation of sulfonated hydrocarbons traditionally focuses on three groups of enzymes: dibenzothiophene monooxygenases (DszA/B), taurine dioxygenases (TauD) and alkanesulfonate monooxygenases (SsuD). Bacteria may use these enzymes to break carbon-sulfur bonds, releasing sulfite and an aldehyde. In recent years, fluorinated surfactants, such as 6:2 fluorotelomer sulfonamide alkyl betaine (6:2 FTAB) in aqueous film-forming foams (AFFF) for firefighting, have emerged as contaminants of concern due to their resistant chemical properties. A 6:2 FTSA breakdown product, 6:2 fluorotelomer sulfonate (6:2 FTSA), is a partially fluorinated version of octane sulfonate often observed in the environment following AFFF deployment. The bacterium *Gordonia* NB4-1Y was found to be able to partially degrade 6:2 FTSA to short chain aldehydes and carboxylic acids, presumably via the production of the yet to be detected 6:2 fluorotelomer aldehyde (6:2 FTUA). Proteomic analysis of NB4-1Y revealed that two flavin-dependent nitrilotriacetate monooxygenases (NtaA) were produced under sulfur-limiting conditions with 6:2 FTSA as the sole added source of sulfur. In order to know if NB4-1Y is able to use the carbon-nitrogen bond cleavage activity of NtaAs to remove sulfur from 6:2 FTSA, two NB4-1Y NtaAs (ISGA 1218 and 1222) were cloned into pMAL-c2 and pET23d vectors for heterologous expression in *Escherichia coli* BL21. Maltose binding protein-tagged ISGA 1218 and 1222 have been expressed and purified as shown by proteins of 96 and 91 kDa on SDS-PAGE gels. Expression of His-tagged ISGA 1218 and 1222 is currently underway. Purified SsuD from *E. coli* BL21 and NB4-1Y are being used to develop kinetic assays against octanesulfonate as a positive control. Simultaneously, 6:2 FTSA transformation by NB4-1Y NtaAs is being tested in the presence of *E. coli* flavin reductase and anoxic photoreduction of flavin. The generation of ISGA 1218 and 1222 protein crystals will shed light on how NtaAs may accommodate 6:2 FTSA into active sites.

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Effect of silicon on the biosorption and accumulation of chromium by arthrobacter globiformis 151b type bacteria

M. Osepashvili[1], A. Rcheulishvili[1], L. Tugushi[1], E. Ginturi[1], M. Gurielidze[2], H. Y. Holman[3]
[1]*Ivane Javakhishvili Tbilisi State University E. Andronikasvili Institute of Physics, Department of physics of biological systems, Tbilisi, Georgia*
[2]*S. Durmishidze Institute of Biochemistry and Biotechnology of the Agricultural University, Laboratory of the Nitrogen Fixation and Assimilation, Tbilisi, Georgia*
[3]*University of California, Lawrence Berkeley National Laboratory (LBNL), Oakland, CA, United States*

Heavy metal pollution in soil is a critical global environmental problem. Heavy metals are considered systemic toxicants that are known to induce multiple organ damage, even at lower levels of exposure. Chromium, which is widely spread in environment, is highly toxic, mutagenic, and carcinogenic. Therefore, environmental protection from Cr(VI), including by microbial remediation, is extremely important. However, the heavy metal uptake by bacteria can be interfered by different metals/chemical elements, spread in environment. Silicon is the second-most abundant element on the planet, after oxygen. Si is able to alleviate heavy metal toxicity in plants but less is known about the joint action of chromium and silicon and their influence on metalloresistant bacteria.

Our studies addresses the effect of Si on the biosorption and accumulation of chromium in the metal resistant Arthrobacter globiformis 151B, isolated from the most polluted region of Georgia (Kazreti). Arthrobacter species that are capable of microbial remediation can induce intracellular reduction of chromium. The studies were conducted on A. globiformis 151B bacterial culture at the different growth phase in growth media (Triptic Soy Broth (TSB) containing 7.1 µg/ml chrome) enriched with various concentration of Silicon (50 µg/ml, 200 µg/ml, 800 µg/ml), incubated at 26°C. The concentration changes of intracellular and membrane-associated total chromium over a time period was detected in bacteria at different growth phases (17, 24, 48, 96 and 144 hours) by atom-absorption spectrometer - Analyst 800 (air-acetylene flame).

The obtained data from Arthrobacter globiformis 151B showed that chrome uptake is highly correlated to the presence of Silicon. Low concentration of Silicon (50 µg/ml) hinders intracellular accumulation of chrome at each growth phase of A. globiformis 151B during 17-144 h. Additionally, the increased concentration of Silicon (especially at 200 µg/ml) facilitates the chrome uptake for first 24h of bacterial growth. Afterwards, the chrome intracellular concentration significantly decreases.

Our studies suggest Arthrobacter globiformis 151B can activate efflux systems that induce the intracellular chrome concentration changes due to Silicon. Silicon may play role in the reduction of chromium toxicity in Arthrobacter through the complex molecular cellular mechanisms to avoid chromium biosorption and intracellular accumulation.

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Molecular and metagenomics investigations in oxidative vinyl chloride biodegradation

I. Pietrini[1], T. Stella[1], G. Carpani[1], A. Franzetti[1], L. Zaninetta[1]
[1]*Eni, San Donato Milanese, Italy*

Introduction: Chloroethenes have been widely used for industrial purposes. As a consequence of improper disposal and landfill leaching, chloroethenes have led to a significant groundwater contamination worldwide. The occurrence of the lesser chlorinated ethenes, such as vinyl chloride (VC) in groundwater is primarily due to the incomplete anaerobic reductive dechlorination of the more highly chlorinated ethenes. VC is toxic and it is a known human carcinogen. Thus, its presence in groundwater is a public health threat. The clean-up of VC-contaminated groundwater *via* oxidation by aerobic microorganisms is potentially the most suitable bioremediation strategy.
Objectives: The aim of this work was to assess the effectiveness of VC oxidation under both natural (NA) and enhanced biological (ENA) processes. Moreover, site-specific molecular markers of VC-aerobic biodegradation were identified to monitor this process *in situ*.
Materials & methods: Contaminated groundwater was collected from 2 contaminated sites (Site A and F) and aerobic microcosms with indigenous cultures in NA and enhanced ENA (addition of inorganic nutrients) conditions were set up to evaluate VC degradation rates (GC-FID analyses). Moreover, microbial community structure by next generation sequencing (NGS) and the presence of the catabolic gene *etn*C encoding for the alkene monooxygenase (AkMo), which is potentially involved in VC biodegradation process, were assessed.
Results: Concerning the site A, VC was degraded up to 46.9% of the original content within 20 days of incubation in ENA microcosms, while no degradation occurred in NA microcosms. The copy number of *etn*C gene did not change over time, whereas NGS results revealed the enrichment in *Xanthobacter* ssp. genus, which represented up to 52.6% of the total sequences. As for site F, VC was completely removed in ENA microcosms in 20 days. *etn*C gene copy number increased from 107 up to 109 copies per liter of groundwater and the microbial community was enriched in *Ancylobacter* and *Mycobacterium* genera.
Conclusion: The catabolic gene *etn*C encoding for AkMo can be use as functional biomarker for the aerobic VC degradation at site F. However, a more comprehensive analysis of the functional profile of the enriched cultures able to degrade VC is needed. Therefore, metagenomics analysis are in progress.

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Soil moisture-induced enhancement of soil N2O emission linked to distinct response of nitrite-reducing and nitrous oxide-reducing microbial communities in acid paddy soil

H. Qin[1]
[1]*Institute of Subtropical Agriculture, Chinese Academy of Sciences, Changsha, China*

Much greater cumulative emissions of N2O was found in winter fallow season than that in rice growing period, however, the mechanisms of N2O emissions affected in fallow paddy field without fertilizer inputs have barely been investigated and still unclear. An incubation experiment was designed including five treatments: WFPS levels 25%, 50%, 75%, 100% and 125%, aiming to figure out the soil moisture threshold influencing N2O emission and key communities of *nirS*-, *nirK*-, and *nosZ*- containing denitifiers regulating N2O emission in acid fallow paddy field. Real-time PCR (qPCR) and Illumina MiSeq sequencing approaches were applied to investigate the size, composition, and diversity of the *nirS*-, *nirK*-, and *nosZ*- containing denitrifying bacterial communities. The results showed that the fallow paddy field under WFPS75% and WFPS100% treatments had higher N2O fluxes, *nirS* and *nosZ* gene copy numbers, but lower Shannon index for *nirS*-, *nirK*-, and *nosZ*- containing bacterial communities and lower phylogenetic diversity for *nosZ*-denitrifying bacterial communities during the 48h higher flux period. At 48h incubation time, there were differential responding patterns in structure of the *nirK*-, *nirS*-, and *nosZ*-denitrifying bacterial communities to soil moisture, which the *nirK*- and *nosZ*-denitrifying bacterial communities were mutant-varied, but gradually-varied for *nirS*- gene with the increasing soil water. N2O flux positively related with the decrease of nitrate-N and the increase of ammonium-N, positively related with the abundance and phylogenetic diversity of PD of *nosZ* gene, but not for nirK or nirS gene. Of the top thirty phyla at the OTU level, there were five OTU belong to *Ralstonia*, *Bradyrhizobium*, *Paraburkholderia* and *Pseudomonas* for *nirK*- denitrifier, four OTU belong to *Herbaspirillum*, *Arthrobacter* and *Ideonella* for *nirS*- denitrifier, fourteen OTU belong to *Ralstonia*, *Herbaspirillum* and *Massilia* for *nosZ*- denitrifier enriched by increasing soil moisture and also closely related with N2O flux. In conclusion, denitrifying bacteria harboring *nirK, nirS*, and *nosZ* genes differently respond to soil moisture, and the enriched special species might be play key role by denitrifricaiton to control N2O flux from fallow paddy soil, and nitrous oxide-reducing bacteria may played a key role in N2O emissions rather than nitrite-reducing bacteria.

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Microbial community analysis of crude oil/gasoline mixture amended aerobic and hypoxic enrichment cultures

F. Révész[1], T. Benedek[1], B. Kriszt[2], S. Banerjee[1], A. Táncsics[1]
[1]*Regional University Center of Excellence in Environmental Industry, Szent István University, Gödöllő, Hungary*
[2]*Department of Environmental Safety and Ecotoxicology, Faculty of Agriculture and Environmental Science, Szent István University, Gödöllő, Hungary*

Introduction: Saturated hydrocarbons (alkanes) are quantitatively the most abundant fraction among all petroleum hydrocarbons. The most rapid biodegradation of these contaminants can be observed under aerobic conditions. Nevertheless, in subsurface ecosystems the availability of oxygen is often restricted. However, only a handful of studies have investigated alkane degradation under oxygen-limited conditions or alkane degrading microbial communities in oxygen-limited environments.
Objectives: The hypothesis of the present study was that different microbial communities can be observed in aliphatic hydrocarbon contaminated environments under aerobic and hypoxic conditions.
Methods: To investigate this hypothesis a pilot experiment was performed by establishing aerobic and hypoxic bacterial enrichments amended with a crude oil – gasoline mixture.
Results: Results have shown that aerobic enrichments had much greater microbial diversity than the hypoxic enrichments. The aerobic microbial communities were dominated by Betaproteobacteria (36.5%) followed by Gammaproteobacteria (28%), Alphaproteobacteria (8.7%) and Actinobacteria (5.6%). The most dominant genus was *Polaromonas* (14%), followed by *Pseudomonas* (10.5%), *Acidovorax* (6.7%) and *Rhodococcus* (5.6%). Contrarily, the hypoxic communities showed highly reduced diversity and were overwhelmingly dominated by Gammaproteobacteria (79%) followed by Betaproteobacteria (20%). The most dominant genus was *Acinetobacter* (66.3%), followed by *Acidovorax* (11%), *Pseudomonas* (11%) and *Variovorax* (1.8%).
Conclusion: Consequently, results shed light on the fact that the excellent aliphatic hydrocarbon degrading *Rhodococcus* species favor clear aerobic conditions, while oxygen-limited conditions can facilitate the high abundance of *Acinetobacter* species in aliphatic hydrocarbon contaminated subsurface environments (e.g. oil reservoirs).

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Optimization of the technology to improve the efficiency of microbial degradation of chlorinated aliphatic hydrocarbons in contaminated groundwater

C. Romsics[1], Z. Nagymáté[1], L. Jurecska[1], V. Bódai[2], P. Sátorhelyi[2], B. Erdélyi[2], K. Márialigeti[1]
[1]*Eötvös Loránd University, Department of Microbiology, Budapest, Hungary*
[2]*Fermentia Ltd., Budapest, Hungary*

Chlorinated aliphatic hydrocarbons (CAHs) are frequent contaminants in soil and groundwater. Several groups of bacteria are able to degrade CAHs partially. *Dehalococcoides* sp. is the only bacterial group which is capable of the complete dehalogenation of chlorinated ethenes to non-toxic ethene. *In situ* biological remediation could be a useful technique to degrade CAHs by biostimulation and bioaugmentation.
The goal of this study is to optimize bioremediation technology to improve the efficiency of the microbial degradation of CAHs in contaminated groundwater. Our team has developed a contaminated site-specific *bioaugmentation culture,* a large anaerobic fermentation process and an initial bioremediation technology previously.
Pilot-tests were performed in order to optimize *in situ* bioaugmentation technology. Two plots with comparable contamination were selected for our project. Anaerobic solution was added for one week in order to maintain anaerobic conditions. Different amounts of bioaugmentation inoculum cultures were dosed (1 unit & 4 unit) three months long along with application of the same quantity of biostimulation agent during the pilot period.
Pilot tests were monitored by chemical and molecular biological techniques of polyphasic approach. Dynamics of the biodegradation were monitored by gas chromatography. Catabolic gene tests were applied to assess the existing dechlorination potential, and to determine the presence and activity of dehalorespiring bacteria. Changes of microbial community structure were assessed by T- RFLP techniques.
Community fingerprint analyses revealed that the microbial community had been significantly altered by the bioaugmentation and biostimulation treatments. *Dehalococcoides* spp. were identified from samples taken from the injection wells with several methods. The total CAHs contamination has been decreased significantly in both plots tested during the pilot tests.
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Soil and pharmaceuticals – what shapes microbial community composition in farming lands?

P. Sardar[1], A. Chroňáková[1], Z. Frková[1], Y. Vystavna[1], R. Grabic[2], R. Kodešová[3]
[1]*Biology Center CAS, Institute of Soil Biology, České Budějovice, Czech Republic*
[2]*University of South Bohemia, Faculty of Fisheries and Protection of Waters, Vodňany, Czech Republic*
[3]*Czech University of Life Sciences, Faculty of Agrobiology, Prague, Czech Republic*

Many pharmaceuticals and personal care products (PPCPs) including antibiotic drugs has been introduced into agricultural soils via irrigation system with treated wastewater and biosolids application. Such contamination is alarming because the risks to the soil microbial community and the ecosystem causing by most of these chemicals are unknown. The current study examines effects of six pharmaceuticals: angiotensin II receptor blocker Irbesartan, antihistamine Fexofenadine, antibiotics Clindamycin and Sulfamethoxazole, antidepressants Carbamazepine and Citalopram on microbial community composition in five agricultural soils (Greyic Phaeozem, Haplic Chernozem, Haplic Cambisols, Haplic Luvisol and Stagnic Chernozem Siltic). Experimental design involved single application of six pharmaceuticals and a cocktail of six pharmaceuticals to the soils at known concentration and analyze soil microbial community at 0, 1, 13 and 61 days after treatment. The response of soil microbial communities (prokaryotic and eukaryotic) has been investigated using high throughput sequencing and bioinformatics. Soil types exhibits the most significant effect on microbial community composition, followed by incubation time, but this effect varies in different soil types. In depth statistical tests were also performed to check for the relatedness of the factors: Soil, Treatment and Time. In pairwise comparison it was found that except the pair Haplic Cambisols and Stagnic Chernozem Siltic, all other soil pairs are significantly different from each other in terms of diversity in microbial community composition. Observed effects of different pharmaceutical amendments were rather weak in shaping microbial community and mostly varies across soils. We observed decreased richness in Greyic Phaeozem at 13 and 61 days after application of Sulfamethoxazole and pharmaceutical cocktail. Contrastingly, treated Haplic Chernozem soil sampled at 61 days showed higher richness as compared to untreated soil for all the pharmaceuticals that were used. However, both trends cannot be generalized. These data suggest that microbial community in the agricultural soils are mostly distinguished according to the soil properties and that their responses to pharmaceutical application are rather soil and chemical compound dependent.

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Mycotoxin degradation and antifungal activity against mycotoxigenic fungi by *Streptomyces*

J. S. Hwang[1], H. G. Song[1]
[1]*Kangwon National University, Biological Sciences, Chuncheon, South Korea*

Question: Mycotoxins produced by several fungi including *Aspergillus* spp. and *Fusarium* spp. can be found from contaminated cereals. They are extremely toxic and carcinogenic, and pose a severe threat to animal health and brings about huge economic losses. Therefore, their production has to be inhibited and mycotoxins already produced should be removed to prevent economic losses from contamination of crops. This study aims to explore degradation of aflatoxin B1 (AFB1) and fumonisin B1 (FB1) and antifungal activity against mycotoxigenic fungi by isolated bacteria.
Methods: Mycotoxin degrading bacteria were isolated from various sources including animal feces using coumarin medium, and their antifungal activity (inhibition of mycelia growth, sporulation, spore germination) was examined, and degrading capability of AFB1 and FB1 were measured by HPLC.
Results: *Streptomyces sporoverrucosus* JS383 and *S. lavendulae* JS669 degraded AFB1 (0.1 mg/L) by 93.7 and 96.8%, respectively in nutrient broth (72 h, 30°C). They also degraded FB1 (0.1 mg/L) by 91.2 and 95.9%, respectively on same conditions. JS383 and JS669 showed excellent thermostability in AFB1 degradation (up to 121°C), and JS383 also displayed broad temperature range (4~75°C) for FB1 degradation. The antifungal activity of JS383 and JS669 was evaluated by co-culture with 3 strains of aflatoxigenic *A. flavus* (KACC44986, 45068 and 45146) and 4 strains of fumonisigenic *Fusarium* spp. (*F. fugikuroi* KACC46888 and 48352, *F. verticillioides* KACC48354 and *F. proliferatum* KACC48356). JS383 and JS669 effectively inhibited mycelial growth of target mycotoxigenic fungi (68.4~90.2%) and suppressed sporulation of target organisms up to 97.3 and 97.2%, respectively. They also displayed inhibition of spore germination by 99.0 and 97.3%, respectively. Ethyl acetate extracts of bacterial cultures showed low minimum inhibitory concentrations (1.25~5 mg/ml) on target mycotoxigenic fungi.
Conclusion: JS383 and JS669 can be used for mycotoxin biodegradation and control of mycotoxigenic fungi in agriculture and also in food and feed industry.

Fig. 1

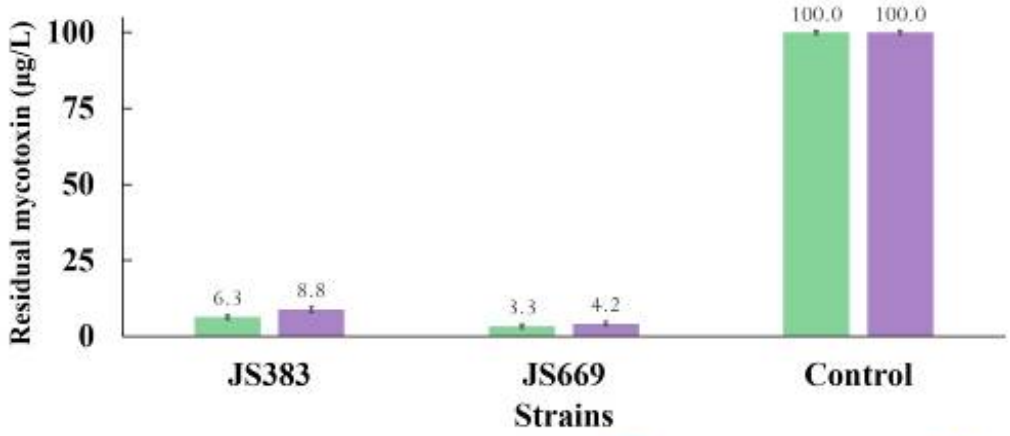

Fig. 1. Degradation of 100 μg/L of aflatoxin B_1 (■) and fumonisin B_1 (■) by strains JS383 and JS669 in nutrient broth (30°C, 150 rpm, 72 h).

Fig. 2

Table 4. Minimum inhibitory concentration (MIC) of antimicrobial substances from JS383 and JS669 strains against toxigenic fungi (unit: mg/ml)

Target organism	Strains	
	JS383	JS669
A. flavus KACC44986	1.7	2.5
A. flavus KACC45068	3.3	5.0
A. flavus KACC45146	3.3	5.0
F. fugikuroi KACC46888	1.3	2.5
F. fugikuroi KACC48352	1.3	2.5
F. verticillioides KACC48356	2.5	2.5
F. proliferatum KACC48354	2.5	2.5

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Catabolism of the common groundwater micropollutant 2,6-dicholorobenzamide (BAM) in *Aminobacter* sp. MSH1 involves two plasmids and an unusual metabolic pathway

D. Springael[1], B. Raes[1], J. T'Syen[1], B. Horemans[1], H. P. Köhler[2]
[1]*KU Leuven, Division of Soil and Water Management, Heverlee, Belgium*
[2]*EAWAG, Dübendorf, Switzerland*

Aminobacter sp. MSH1 uses the common groundwater micro-pollutant 2,6-dichlorobenzamide (BAM) as sole source of carbon and energy and is considered a prime biocatalyst for BAM contaminated groundwater bioremediation in Drinking Water Production Plants (DWPPs). In a first step, MSH1 converts BAM to 2,6-dichlorobenzoic acid (2,6-DCBA) by means of the BbdA amidase. encoded by the *bbdA* gene residing on an unusual IncP-1 plasmid designated as pBAM1. Information about the genes and degradation steps involved in 2,6-DCBA metabolism in MSH1 or any other organism is currently lacking. We found that the genes for 2,6-DCBA degradation in strain MSH1 reside on a second catabolic plasmid of 53.9 kb belonging to the *repABC* family. The plasmid was designated pBAM2 and is the first *repABC* family plasmid with demonstrated organic xenobiotic degradation functions. This leaves the BAM catabolic genes of MSH1 dispersed over two catabolic plasmids and hence MSH1 needs both plasmids to use BAM as a carbon and energy source. Evidence was obtained that the DCBA catabolic pathway encoded by pBAM2 follows an unusual route involving two mono-oxygenation steps leading to a non-vicinal dihydroxylated compound, followed by a reductive dehalogenation step to remove a first chlorine, an hydrolysis step to replace the second chlorine with a hydroxyl group leading to two vicinal hydroxyl groups and *meta*-cleavage of the ring structure. The hydrolysis step involves a dehalogenase related to alkane dehalogenases and is the first of this family to act on chlorinated aromatics. We further show that MSH1 is prone to BAM-catabolic instability and that especially pBAM2 is easily lost under non-selective conditions including those encountered in the oligotrophic environment of DWPPs .

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***Hedera helix* phyllobiome structure and (pan)genomic survey of bacterial isolates with regard to phytoremediation of airborne pollutants**

V. Stevens[1], S. Thijs[1], N. Weyens[1], J. Vangronsveld[1]
[1]*Hasselt University, Diepenbeek, Belgium*

Question: Air pollution, containing fossil fuel-related volatile organic compounds (VOCs), constitutes a major problem in most urban areas. Worldwide, more people die from exposure to air pollution than from malaria and HIV infection together [1]. Many years of research show that plant–microbe interactions can be exploited to significantly enhance phytoremediation of contaminated environments [2]. *Hedera helix* (common ivy), an evergreen plant known for its hardiness and climbing ability, proves to be an excellent host species in the context of phytoremediation of air pollution [3]. Could microorganisms living on ivy leaves constitute promising candidates to detoxify harmful VOCs and promote plant growth?

Methods: We employed 16S rRNA gene amplicon-based metagenomics to characterize the taxonomic structure of phyllospheric microbial communities associated with *H. helix*. Further, we investigated 33 bacterial isolates from 8 different genera using culture-dependent approaches and whole genome sequencing.

Results: Our results indicate that the relative composition of the *H. helix* phyllobiome (**Fig.1**) is similar to that of the model plant *Arabidopsis thaliana* [4]. The presence of pathways related to detoxification of fossil fuel-related VOCs could be confirmed by genome-level analyses of bacterial isolates showing a high *in vitro* capacity to metabolize selected VOCs and promote plant growth [5, 6].

Fig. 1 Taxonomic diversity of the *Hedera helix* bacterial phyllobiome. ProtB, *Proteobacteria*; ActB, *Actinobacteria*; Bact, *Bacteroidetes*; Firm, *Firmicutes*; AciB, *Acidobacteria*; Uncl., Unclassified.

Conclusion: We provide an integrated description of the *H. helix* phyllobiome, including the identification of interesting bacteria with significant potential as inoculants in phytoremediation approaches.

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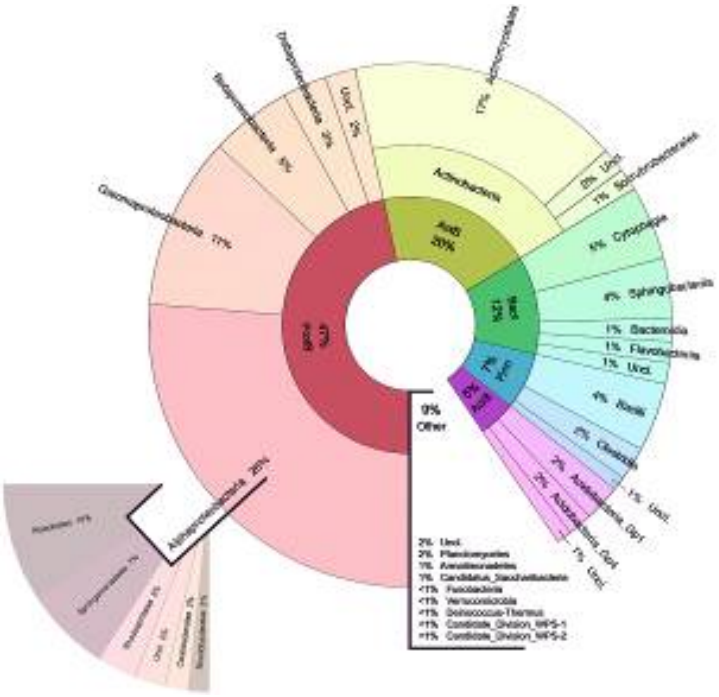
Fig. 1



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Involvement of bacterial biphenyl dioxygenases in *ortho*-phenylphenol degradation

J. Šuman[1], S. Fraraccio[1], T. Veselá[1], T. Cajthaml[2,3], T. Uhlik[1]

[1]*Institute of Chemistry and Technology, Prague, Dpt. of Biochemistry and Microbiology, Prague, Czech Republic*

[2]*Czech Academy of Sciences, v.v.i., Institute of Microbiology, Prague, Czech Republic*

[3]*Faculty of Science, Charles University, Institute for Environmental Studies, Prague, Czech Republic*

O-phenylphenol (OPP) is a widely used agricultural fungicide mostly applied for waxing citrus fruits. Its bacterial degradation has been known to be initiated by monooxygenase-mediated hydroxylation yielding 2,3-dihydroxybiphenyl that is subjected to further degradation. In this study, we aimed to investigate whether aromatic-ring-hydroxylating dioxygenases, namely biphenyl dioxygenases, are involved in bacterial degradation of OPP.

As a source of OPP-utilizing bacteria, soil samples collected from long-term sludge-fertilized fields were used. OPP- and biphenyl (BP)-enrichment cultures were prepared and subjected to (i) a cultivation-based procedure for the isolation of OPP- and biphenyl-degrading bacteria, and (ii) determination of the structure and phylogeny of the enriched microbial populations through 16S rRNA gene amplicon sequencing by Illumina MiSeq platform. In total 30 and 23 isolates were retrieved from BP- and OPP-enrichment cultures, respectively. 16S rRNA gene amplicon analysis revealed that far more diverse taxa were enriched on BP versus OPP, including *Flavobacteriaceae*, *Alcaligenaceae*, *Pseudomonas*, *Acinetobacter*, *Sphingobacterium*, *Rhodococcus*, *Chitinophagaceae*, etc. OPP-enrichment cultures were strongly dominated by one sequence affiliated to *Pseudomonas fluorescens* group. Its 16S rRNA gene was identical to that of one of the isolates from OPP-enrichment culture named *Pseudomonas* sp. P1B16. The complete sequence of *bphA* gene encoding for biphenyl dioxygenase large subunit was retrieved from the P1B16 genome using inverse PCR. The sequence of corresponding protein BphA showed 98% identity with those from the soil polychlorinated biphenyl-degrading strains both *Pseudomonas pseudoalcaligenes* KF707 and *Pseudomonas alcaliphila* JAB1. In order to investigate the involvement of biphenyl dioxygenases in OPP degradation, the *bphA* gene was deleted from the P1B16 genome of the P1B16 strain. Moreover, P1B16-borne *bphA* gene was used for the replacement of that in the *P. alcaliphila* JAB1. Based on OPP- and BP-utilization/degradation tests performed with the P1B16- and JAB1-derived recombinant strains and wild-type controls, the involvement of biphenyl dioxygenase in OPP-degradation in the P1B16 strain was proven. Based on the analysis of reaction intermediates, the presumed mechanism of a biphenyl dioxygenase-mediated OPP-degradation was inferred.

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Effects of long-term phytoremediation on rhizosphere and endophytic microbial communities at high latitude

J. Papik[1], M. Strejcek[1], L. Musilova[1], M. C. Leewis[2], M. B. Leigh[3], O. Uhlík[1]
[1]*University of Chemistry and Technology, Prague, Dpt. of Biochemistry and Microbiology, Prague, Czech Republic*
[2]*United States Geological Survey, Menlo Park, CA, United States*
[3]*University of Alaska Fairbanks, Fairbanks, AK, United States*

Petroleum hydrocarbons (PHCs) are common soil contaminants at high latitudes due to their widespread and improper usage, storage and disposal. A long-term assessment of PHC phytoremediation was performed which capitalized on a study established in Fairbanks, Alaska in 1995. The original study sought to determine how the introduction of plants (*Festuca rubra*, *Lolium multiflorum*), nutrients (fertilizer), or their combination would affect degradation of PHC-contaminated soils (crude oil or diesel) over time. The site was re-examined in 2011, after 15 years with no active site management. Native and non-native vegetation had extensively colonized the site, with more abundant vegetation found on the diesel-contaminated soils than the more nutrient-poor, more coarse, and acidic crude oil-contaminated soils. Microbial communities in bulk soils also reflected both the original treatment and contaminant type (Leewis et al. 2013; Cold Regions Science and Technology 96: 129–137). We re-examined the site in 2014, by which point PHC concentrations were below detection limits (< 0.5 ppm; EPA Method 8015M) in all treated soils, and analyzed structure of microbial communities in the rhizosphere and endosphere of the plants that had colonized the site. We sought to understand whether structure of plant-associated microbial communities is influenced by the original treatment type or changes in PHC concentrations associated with treatments, and to quantify the interactions between rhizosphere and endosphere communities across plant species. Together, this study suggests that initial treatment with native tree species in combination with grasses could be an effective means for phytoremediating petroleum contaminated-soils and promoting ecological recovery in cold regions.

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Effects of the endocrine disruptor 17α-ethinylestradiol on bacterial community associated with the scleractinean coral *Mussismilia harttii*

C. Vilela[1,2], H. Villela[1,2], C. Rachid[1], G. Duarte[1,2], E. Santoro Peçanha[1,2], R. Peixoto[1,2]
[1]*Federal University of Rio de Janeiro, General Microbiology, Rio de Janeiro, Brazil*
[2]*Marine Aquarium of Rio de Janeiro - AquaRio, Rio de Janeiro, Brazil*

Increasing concentrations of an estrogenic endocrine disrupting compound (EDC) commonly associated with changes in the reproductive cycles of several animals in aquatic environments, known as 17α-ethinylestradiol (EE2), is becoming a major concern. While several studies have focused on revealing EE2 impacts on physiology, behavior and morphology of different marine organisms, no data has been published about the changes in their associated microbial communities. Indeed, effects of EE2 on corals, especially regarding gene expression profiles under exposition, are also poorly understood. Therefore, the aim of this work was to investigate potential EE2 effects on *Mussismilia hartti* health, gene expression and associated bacteria. Thus, a closed mesocosm system with circulating water was built, where 3 different conditions were tested in quadruplicates (4 controls without EE2, 4 aquaria containing 100ng/L of EE2 and 4 with 100µg/L). The experiment was carried out for 17 days and sample collections were performed at days 0, 3, 9 and 17. Water parameters (such as pH, temperature and nutrients) were constantly monitored. Indeed, water samples were also taken for DNA and RNA analyses in sampling days, as well as coral samples. Maximum photosynthetic efficiency (Fv/Fm) of corals" zooxanthellae was measured throughout the experiment as a proxy for coral health. No difference on the values was detected and the average ranged around 550 of Fv/Fm. Ordination test showed that the collection time is statistically significant on the microbiome structure, what is not true for EE2 presence. Taxonomic investigation demonstrated that there were no significant microbial community changes among treatments. However, the bioindicator analysis showed significantly divergent bacterial groups in different treatments, suggesting that the presence of EE2 could affect specific OTUs associated with corals. The preliminary results seem to be positive regarding coral physiology and homeostasis, once its health and associated community were not impacted by the endocrine disruptor using the tested concentrations, although deeper analyses should be performed to corroborate these findings. RNA was extracted in order to assess changes in the relative abundance of stress-related and estrogen-metabolism genes (17β-HSD type 1, P-gp, Hsp70, Hsp 90 and GnRH). These results will help us to better elucidate the effects of EE2 contamination on corals.

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Synergism in lignocellulose degrading microbial consortia

Y. Wang[1], L. Cortés Tolalpa[1], D. Jiménez[2], J. D. van Elsas[1]
[1]*University of Groningen, Science and engineering, Groningen, Netherlands*
[2]*Universidad de los Andes, Bogotá, Colombia*

Lignocellulose biomass (LCB) are mainly composed of cellulose, hemicellulose, lignin, and pectin, with largely inaccessible structures. Given the difficulty of working with high diversities of required enzymes, LCB biodegradation by microbial consortia has received more attention recently. In previous work, different LCB-degrader communities have become available, as derived from various inoculum on diverse substrates. Even though the composition of the microbial communities varied, some core microorganisms could be discerned. A focus was placed on two bacteria, i.e. *Citrobacter freundii* so4 and *Sphingobacterium multivorum* w15, next to the fungus *Coniochaeta* species (strain 2T2.1). Synergistic interactions were found in both the bacterial-bacterial and bacterial-bacterial-fungal consortia when growing together on wheat straw. However a full understanding of the exact mechanisms underlying the synthetic consortia is at yet lacking, which is of great value for a fundamental understanding of the biodegradation process. Our objectives are 1) to foster our understanding of the mechanisms underlying the synergism behavior of the three strains. 2) to determine the relevance of conditions for the roles of the three synergistic partners. 3) to elucidate the genome sequences of the three synthetic organisms. Draft genome sequences of these two bacteria were analyzed, and the relevance of conditions were studied in aerobic cultures using wheat straw as the sole carbon and energy source. The analysis of the draft genome sequences of the two bacteria shows they have parallel and potentially complementary catabolism. *S. multivorum* w15 acts as a secretor of hydrolytic enzymes that attack hemicellulose while *C. freundii* so4 works on cellulose. *C. freundii* so4 could produce and excrete secondary metabolites that *S. multivorum* w15 can consume, and detoxify the system by reduction of accumulated by-products. The positive interactions between these strains can be defined as cooperative cross-feeding. And experimental work shows that the pH is a major factor for the synthetic consortia, and also the fungus plays an important role during the degradation process. The bacteria grow better and had higher enzymatic activities. Unfortunately, genome data of the fungus is still working on. and transcriptome and proteomics analysis will done in future.

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Application of endophytic bacteria to reducing PAH contamination of plants

X. Zhu[1]
[1]*Nanjing Agricultural University, College of Resources and Environmental Sciences, Nanjing, China*

Greatly increasing accumulations of polycyclic aromatic hydrocarbons (PAHs) have been found in multiple environments. Due to their high hydrophobicity and affinity for fatty tissues, the PAH contents in plants increased. Although microbial degradation has been thought as one of the main applications for PAH remediation in the environment, most bacteria could not effectively degrade PAHs in environment. Remarkably, plant-endophytic bacteria symbioses have the nutrients and a niche provided by plants for the bacteria, protecting them from competition with other native bacteria.

Our research was focused on isolation high PAH-degrading endophytic bacteria and investigation on their potential in protecting plants against PAHs contamination. Pyrene-degrading endophytic bacteria were isolated from plants grown in PAH-contaminated soil. The capacities of endophytic strains to degrade pyrene were tested in vitro, and colonization efficiency was tested in vivo by inoculation of endophytic strains on seedlings.

8 strains of endophytic bacteria (2 strains *of Stenotrophomonas* sp., 2 strains *Serratia* sp. *Pseudomonas* sp., *Paenibacillus* sp., *Pantoea* sp., *Bacillus* sp.) were isolated from plants grown in PAH-contaminated soil. *In vitro* test, these strains could degrade more than 95% phenanthrene from media within 7 days. Among them, *Serratia* sp. and *Paenibacillus* sp. could degrade more than 50% of pyrene from media within 7 days. *In inoculation test*, *Serratia* sp. successfully colonized into roots and leaves of inoculated plants, and reduced the PAH contamination interior plants. In the roots and shoots of the inoculation plants, increasing activities of polyphenoloxidase, peroxidase, and catechol 2, 3 dioxygenase were observed, suggesting that high PAH-degrading endophytic bacteria could enhance the biodegradation of PAHs interior plant tissues.

PAH-degrading endophytic bacteria have the potential for protecting plants against PAH contamination and mitigating the threat of pyrene to human health through food consumption.

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Bioactive secondary metabolites from octocoral-associated microbes — new chances for blue growth

I. Raimundo[1], S. G. Silva[1], T. Keller-Costa[1], R. Costa[1]

[1]*Universidade de Lisboa, Institute for Bioengineering and Biosciences (iBB), Department of Bioengineering, Instituto Superior Técnico, Lisbon, Portugal*

Octocorals (Cnidaria, Anthozoa Octocorallia) are magnificent repositories of natural products with fascinating and unusual chemical structures and bioactivities of interest to medicine and biotechnology. However, mechanistic understanding of the contribution of microbial symbionts to the chemical diversity of octocorals is yet to be achieved. This work inventories the natural products so-far described for octocoral-derived bacteria and fungi, uncovering a true chemical arsenal of terpenes, steroids, alkaloids, and polyketides with antibacterial, antifungal, antiviral, antifouling, anticancer, anti-inflammatory, and antimalarial activities of enormous potential for blue growth. Genome mining of 15 bacterial associates (spanning 12 genera) cultivated from *Eunicella* spp. resulted in the identification of 440 putative and classifiable secondary metabolite biosynthetic gene clusters (BGCs), encompassing varied terpene-, polyketide-, bacteriocin-, and nonribosomal peptide-synthase BGCs. This points towards a widespread yet uncharted capacity of octocoral-associated bacteria to synthesize a broad range of natural products. However, to extend our knowledge and foster the near-future laboratory production of bioactive compounds from (cultivatable and currently uncultivatable) octocoral symbionts, optimal blending between targeted metagenomics, DNA recombinant technologies, improved symbiont cultivation, functional genomics, and analytical chemistry are required. Such a multidisciplinary undertaking is key to achieving a sustainable response to the urgent industrial demand for novel drugs and enzyme varieties.

Fig. 1

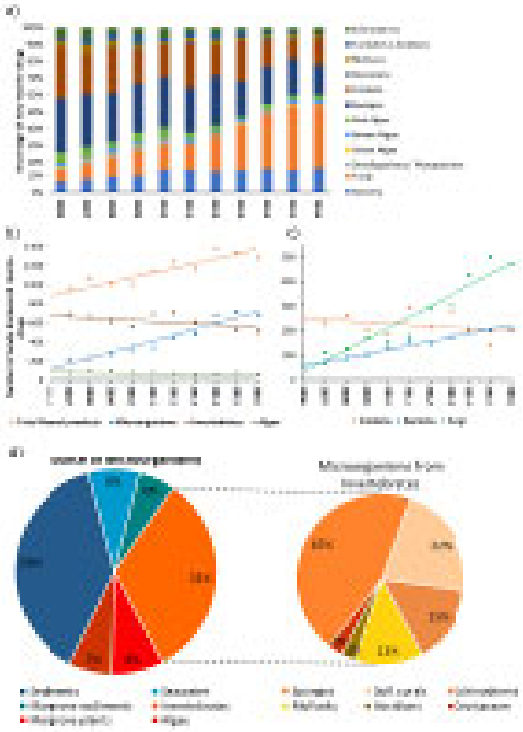
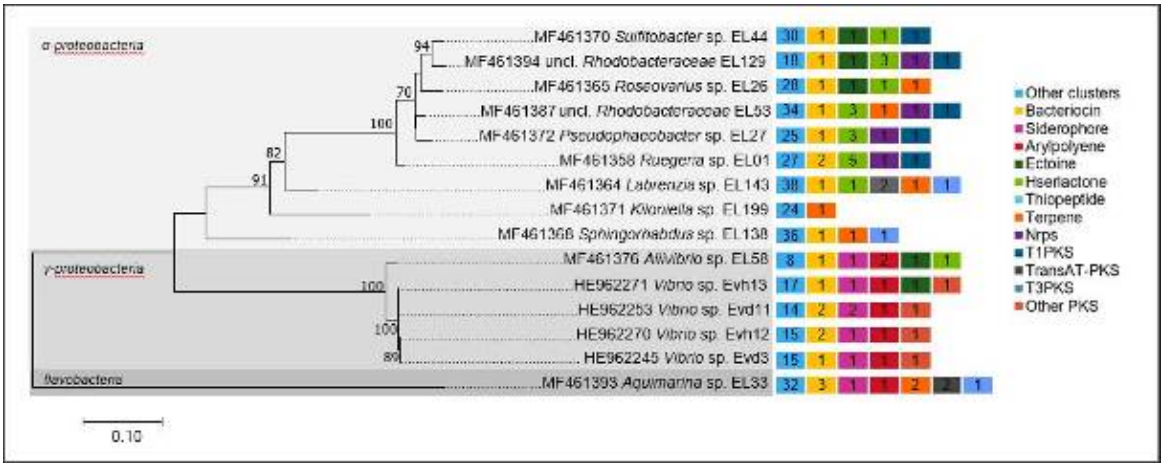


Fig. 2



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Are biphenyl 2,3-dioxygenases involved in lignin degradation?

M. Folkmanová[1], T. Veselá[1], S. Reagen[2], J. Chalupa[3], A. Kubátová[2], O. Uhlík[1]
[1]*University of Chemistry and Technology in Prague, Biochemistry and Microbiology, Prague, Czech Republic*
[2]*University of North Dakota, Chemistry, Grand Forks, ND, United States*
[3]*University of Chemistry and Technology in Prague, Biotechnology, Prague, Czech Republic*

Microbial degradation of lignin has been well studied in fungi, but much less in bacteria. Recent findings have suggested that a range of soil bacteria, often aromatics-degrading bacteria, are able to break down lignin. This led us to hypothesize that the same soil bacterial enzymes that are capable of degrading specific anthropogenic environmental pollutants, such as lower polychlorinated biphenyls, might also be able to degrade natural aromatic compounds, such as lignin derivatives. Here, we investigate the potential of three bacterial strains (Burkholderia xenovorans LB400, Pseudomonas alcaliphila JAB1, Rhodococcus jostii RH1A), each of which possesses a different type of biphenyl 2,3-dioxygenase, to degrade lignin derivatives. Each strain was cultivated separately in liquid mineral media with either synthetic ligninbuilding block (guaiacylglycerol-β-guaiacyl ether and 4-formyl-2-methoxyphenyl benzoate) as the sole carbon source. The degradation products were analyzed by GC-MS at four stages of microbial growth (0%, 25%, 50% and 100% of maximal optical density of the bacterial culture during pilot experiments; λ=600nm). Furthermore, a resting-cell-assay-like degradation products were analyzed after 0, 24, 48 and 72 hours of incubation of the fully grown culture with the respective substrates. In conclusion, our study contributes to deeper understanding of the degradation of lignin and microbes involved in it.

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Identification of novel signaling molecules produced during the cocultivation of probiotic strains and *Clostridium difficile* in *in vitro* setup

T. Gnanasekaran[1], B. Lengger[1], M. Arumugam[1]
[1]*University of Copenhagen, CBMR, Copenhagen, Denmark*

The human gastrointestinal tract harbors trillions of microorganisms referred to as the gut microbiota. Gut Microbiota play crucial role in the transformation of food into interesting bioactive metabolites (e.g., short chain fatty acids, lipids, vitamins, antimicrobials and neurotransmitters) that significantly impact human health. However, many of these metabolites are yet to be identified and in-depth knowledge of these metabolites is still far to be fully elucidated. In order to realize this, we use a specially designed fermenter that mimics the physiological conditions of human gastrointestinal tract to grow complex gut microbial communities.

One of the projects from our lab focuses on investigating the signalling molecules produced during the co-culturing of probiotic strains (reported in previous literatures) and *Clostridium difficile* in *in vitro* setup. *Clostridium difficile* is a major enteric pathogen that causes pseudomembranous colitis in humans, and the infection can be fatal if not treated promptly. Using short chain fatty acid (SCFA) analysis as surrogate for community stability, we successfully established the gut microbiota from a healthy individual in our *in vitro* system, and a subsequent colonization of the probiotic strains. Using qPCR and colony counts specific to *C. difficile*, we also verified that *C. difficile* can colonize this microbial community, thus establishing a *C. difficile* infection model. In order to identify different signalling molecules produced in the cocultivation setup, sample collections were performed at different time points and the exo-metabolites were extracted using different organic solvents from the spent growth media. The extracted metabolites were then subjected to untargeted metabolomics analysis by employing different LC/GC-MS techniques.

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Interactions between *Streptomyces rapamycinicus* and *Bacillus subtilis* modulate production of bioactive molecules

E. Kovačec[1], T. Danevčič[1], D. Vogrinc[2], M. Blažič[2], J. Horvat[2], G. Kosec[2], H. Petković[1], I. Mandić Mulec[1]
[1]*University of Ljubljana, Biotechnical faculty, Ljubljana, Slovenia*
[2]*Acies Bio d.o.o., Ljubljana, Slovenia*

Question: Microbial sociality is an emerging field of science and is a consequence of continuous adaptation of microorganisms to the presence of others. Therefore, co-cultures that mimic competition or cooperation scenarios in nature represent a novel research strategy, which combined with analytical techniques may accelerate discovery of new antibiotics and/or improvement of their production. *Bacillus subtilis* is a widespread soil bacterium and a model Gram positive organism to study physiology, multicellularity and interspecies interactions. Species of the genus *Streptomycetes* are the richest source of useful metabolites and in this study we focused on industrially important actinomycete *Streptomyces rapamycinicus* NRRL 5491, which encodes 48 secondary metabolite biosynthesis clusters in its genome. In laboratory conditions *S. rapmycinicus* actively produces 3 bioactive compounds: elaiophylin (antibacterial and cytotoxic), polyether antibiotic nigericin, and anticancer compound rapamycin. The aim of our study was to explore whether *B. subtilis* influences the synthesis of these three compounds produced by *S. rapamycinicus* on different growth media.
Methods: We have tested the wild-type strain as well as mutants with inactivated biosynthesis of each of the three bioactive compounds. Bacteria were co-cultured on three different solid media (ISP4, ISP5 and MMAM). Firstly, the morphological changes were evaluated, especially focusing on *S. rapamycinicus* sporulation. After 9 days of co-cultivation the extraction with acidic acetonitrile was made and the antibiotics quantity was determined by LC-MS.
Results: We have noticed that the production of chosen compounds mainly depends on growth media. Furthermore, we showed that their production can be drastically affected (increased or decreased) by the presence of *B. subtilis.*
Conclusion: Our results underscoring the importance of social interactions in production of antibiotics.

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The presence of Isopenicillin N synthase (IPNS) gene in Greek *Streptomyces* using new designed primers and a microwave radiation DNA extraction technique

V. Makrygianni[1], A. Savvides[1], E. Katsifas[1], A. Karagouni[1]
[1]*National and Kapodistrian University of Athens, Biology, Athens, Greece*

Isopenicillin N synthase (IPNS) mediates the oxidative conversion of the linear δ-(L-α-amino-adipate)-L-cysteine-D-valine (ACV), to isopenicillin, the precursor of all penicillins and cephalosporins, which are broad spectrum β-lactam antibiotics and protagonists against microbial infections for over 80 years. The overall sequence similarity of IPNS genes from different microorganisms was very high; in all seven sequences that have been investigated, more than 60% of the nucleotide bases and 50% of the deduced amino acid residues were identical or had conservative replacements. Among streptomycetes, in particular, the similarity rises to as much as 70-80%. Our aim was to scan the genome of more than one hundred unknown streptomycetes, isolated from various Greek soil habitats for the detection of the IPNS gene. All these isolates were tested against twelve antimicrobial agents and proved to be multi productive as inhibition zones were developed against eight to twelve antimicrobial agents simultaneously. Degenerate primers were developed in silico using multi alignment tools. To improve effectiveness, a DNA extraction technique that utilizes microwave radiation to decrease time consumption and increase DNA yield and purity was used. The IPNS gene was detected in more than 30% of the isolated strains and results are discussed in relation to several parameters.

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Attempts to induce present Non-Ribosomal Peptide Synthase (NRPS) genes in Greek streptomycete strains

C. Michalopoulou[1], A. Savvides[1], E. Katsifas[1], A. Karagouni[1]
[1]*National and Kapodistrian University of Athens, Biology, Athens, Greece*

Non ribosomal peptide synthetases (NRPS) are biosynthetic systems involved in the synthesis of a range of important biologically active compounds (like antibiotics, antiparasitic agents, antifungals, anticancer drugs, toxins and immunosuppressants) produced by microorganisms, amongst them the genus of *Streptomyces*. The biosynthetic mechanism of NRPS is based on multiple enzymes and multiple domains megasynthases with a basic common structure and includes an adenylation (A) domain, a condensation (C) domain and a peptidepeptidyl carrier protein (PCP). Our study focuses on screening NRPS gene in fifty rare streptomycete strains, isolated from four diverse soil habitats of Greece, in order to select those strains which they do not exhibit antimicrobial productivity when tested against the twelve antimicrobial agents, by using two different pairs of known primers [A3F/A7R, NRPSF/NRPSR] and two steptomycetes namely *S. rochei* DSM40231 and *S. griseus* DSM40236 as positive control. The NRPS gene was detected in almost all the non-producer strains and since it is known that this gene belongs to a group of genes that are characterized as "silent genes", we aim to induce the present gene in our strains for production of new bioactive compounds. Therefore rifampicin-resistant mutants of the non-producer streptomycetes were obtained from various concentrations of rifampicin and tested against the same twelve antimicrobial agents. The obtained results are under consideration.

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Evaluating of the metabolic versatility and biotechnological potential of the phenazine biosynthesis gene cluster of *Streptomyces* sp. isolated from the Brazilian tropical dry forest

I. Soares Melo[1], S. Nobre Santos[1,2], C. L. Cantrell[1,2,3], P. F. Long[1,2,3,4]
[1]*Embrapa Environment , Laboratory of Microbiology , Jaguariúna, Brazil*
[2]*Biodiversita Tecnologia Microbiana, Campinas, Brazil*
[3]*USDA, National Center for Agricultural Utilization Research, Oxford, MS, United States*
[4]*Kings College London, Institute of Pharmaceutical Science , London, United Kingdom*

A novel actinomycete, *Streptomyces caatingaensis* sp. nov. (CMAA 1322), was isolated from the Brazilian tropical dry forest. The biome is characterized by having singular conditions of climate, instability in rainfall regimes, high temperature, elevated UV radiation, leading its inhabitant by extremophilic organisms. The unique ecological niche of the Brazilian semi-arid points at the promising metabolic and biosynthetic potential of its microbial system. Thus, we performed an evaluation of the metabolic versatility and the biotechnological potential of this novel *Streptomyces* (CMAA 1322) species by genome mining *and* metabolomics approaches, which *revealed the presence of two* broad-spectrum phenazines antibiotics, identified as 1,6 dimethylphenazine, m/z 241.09 and 1,2,3 trimethoxyphenazine m/z 270. Likewise, the genome annotation revealed a locus to phenazine biosynthesis with 12 open reading frames, *with tag phzABCDEF*. *The homology searches revealed similarities of the 44% for corresponding phenazine gene cluster in Pseudomonas aeruginosa PA7 with (22%), S. anulatus strain 9663 (22%) and S. lomondesis strain S015 (19%).* The biosynthetic pathway is intermediate by chorismic acid and the enzymatic assembly sequence is (I) EmrB/QacA; (II) LysR; (III) SmCoG/ major facilitator transporter; (IV) FMN flavin reductase; (V) Acyl-CoA dehydrogenase type 2; (VI) *phzB*; (VII) Isochorismatase; (VIII) Isochorismatase synthase; (IX) O- methyltransferase; (X) Hypothetical protein; (XI) NAD – dependent epimare/dehydrase; (XII) ATP dependent RNAs helicase. *Two well-conserved CRISP/Cas sites precede all cluster but it is not able to provide immunity*. These findings demonstrated the feasibility and efficacy of utilizing metabolomics tools to prioritize chemically unique strains, and further, highlight the potential of the tropical dry forest as a rich source for biotechnological and agricultural industry.

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Comparative genomics reveals complex carbon metabolism and natural product biosynthesis potential across host-associated and free-living *Aquimarina* species

S. G. Silva[1], J. Blom[2], T. Keller-Costa[1], R. Costa[1]
[1]*Institute for Bioengineering and Biosciences (iBB) , Department of Bioengineering, Instituto Superior Técnico, Lisbon, Portugal*
[2]*Justus-Liebig-University Giessen, Bioinformatics and Systems Biology, Giessen, Germany*

The recently-described genus *Aquimarina* comprises strictly marine, heterotrophic bacteria with a versatile carbon metabolism. Current research is unveiling a hidden and potentially complex secondary metabolism for the few *Aquimarina* strains well studied to date. To address this hypothesis and increase our knowledge of the coding potential within the genus, in this study we compare all *Aquimarina* genomes so far available in public databases (n=26). We aimed to determine the core functions and the full extent of natural products encoded by the genus, and whether the predicted proteome of *Aquimarina* spp. changes as a function of "habitat" (e.g. host-associated *versus* free-living) or phylogeny. For that purpose, a variety of *in silico* tools were used. These included several phylogenomic parameters, functional genome annotation based on Clusters of Orthologous Groups of Proteins (COGs) and Protein famlies (Pfams), and genome mining for secondary metabolites through the use of antiSMASH and the more recent BiG-SCAPE algorithm. Genome-wise, *Aquimarina* spp. are highly versatile organisms equipped to colonize host-associated and free-living microniches. The presence of gliding motility-encoding genes coupled to type IX secretion system-dependent chitinases in all surveyed genomes likely underpins the generalist pattern of occurrence and a key participation of *Aquimarina* spp. as major mediators of carbon and nitrogen cycling in the oceans. In line with these findings, no correlation between the strains" source (or "habitat") and functional annotation was observed. Instead, we reveal that phylogenetic relationships primarily shape the functionality of *Aquimarina* genomes with implications to their secondary metabolic repertoire and presumed bioactivities. To this date, *Aquimarina* spp. can be coherently organized into three major functional groups according to the presence and organisation of biosynthetic gene clusters (BGCs). Among them, we count several polyketides synthases (PKSs), terpenes and nonribosomal peptide synthetases (NRPSs). We further demonstrate that the recently-described cuniculene (PKS) biosynthetic gene cluster is conserved among, and specific to, *A. macrocephali*, *A. megaterium* and related species. In conclusion, our unprecedented comparative genomics approach to the genus *Aquimarina* provides a fresh view of an underexplored yet emerging keystone taxon in the cycling of organic matter and secondary metabolite production in marine ecosystems.

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Activating novel biosynthetic gene clusters in antarctic bacteria

V. Waschulin[1], E. Wellington[1], C. Corre[1]
[1]*University of Warwick, Life Sciences, Coventry, United Kingdom*

Background: There is an urgent need to find new antibiotics to keep up with the increasing levels of resistance in pathogens. Soil bacteria are known as prolific antibiotic producers, but it has become increasingly difficult to find truly novel compounds as the rate of re-discovery is high. It is theorized that re-discovery can be reduced by investigating bacteria from isolated, unexplored and unique environments such as Antarctica.
A large fraction of biosynthetic gene clusters (BGCs) is not expressed under laboratory conditions. *mmyB*-like genes were identified a family of transcriptional activators closely associated with BGCs in Actinobacteria. They have successfully been used to switch on the biosynthesis of various metabolites from silent BGCs in *Streptomyces* (Corre et al, unpublished work).
Objectives: The aim of this study is to obtain diverse isolates and sequence them using short- and long-read sequencing. The identified transcriptional activators will then be used to activate the expression of promising biosynthetic gene clusters.
Materials and methods: Traditional isolation techniques for oligotrophic bacteria on soil agar plates were supplemented with microfluidic encapsulation of bacterial cells followed by in-situ cultivation. 16S regions of isolates were amplified and sequenced with primers 27F and 1492R and analysed with SILVA.
Results: A great fraction of the isolates group into the Actinobacteria phylum, which is in accordance with previous work done on Mars Oasis soil. A large number of isolates showed limited 16S similarity to known isolates in the NCBI 16S database.
Conclusions: The 16S sequence diversity of the isolated bacteria and the high proportion of Actinobacteria are a promising base for exploiting the biosynthetic potential of Mars Oasis. Several isolates were chosen for NGS and Oxford Nanopore long-read sequencing to identify transcriptional activators associated with promising BGCs.

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Aromatic ring hydroxylating dioxygenases in soil bacterium *Pseudomonas alcaliphila*

A. Zubrova[1], J. Šuman[1], E. Lopez-Echartea[1], K. Michalikova[2], T. Cajthaml[3,2], O. Uhlík[1]
[1]*University of Chemistry and Technology Prague, Dpt. Biochemistry and Microbiology, Prague, Czech Republic*
[2]*Czech Academy of Sciences, Institute of Molecular Genetics, Prague, Czech Republic*
[3]*Charles University, Institute for Environmental Studies, Prague, Czech Republic*

Aromatic ring hydroxylating dioxygenases (ARHDs) represent an important group of enzymes included in bacterial biodegradative pathways, that are able to activate aromatic ring of pollutants such as polychlorinated biphenyls (PCBs), dibenzofurans, naphthalene, carbazole etc, that are otherwise chemically persistent. ARHDs are known to possess a broad substrate specificity enabling transformation of some secondary plant metabolites (SPMs), based on their structural analogy with anthropogenic pollutants. The stimulation of bacterial metabolism in the presence of SPMs leading to better removal of aromatic pollutants in contaminated soil was already observed two decades ago. In our work we aim to elucidate the mechanism behind this phenomenon on molecular level. We hypothesize that phenolic SPMs or intermediates of their bacterial metabolization can induce transcription of ARHDs-encoding genes.

In this study, a gram-negative soil bacterium *P. alcaliphila* JAB1 was used as a model organism. The spectrum of ARHD-mediated degradation of SPMs in the JAB1 strain was determined using resting cell assay followed by GC-MS analysis. Moreover, transcription of ARHD genes in the JAB1 strain upon exposition to selected SPMs was quantified on the RNA level by qPCR.

Results obtained from qPCR indicate increased transcription ARHD genes in the presence of some tested SPMs. The JAB1 strain was not able to utilize most of tested phenolics. However, JAB1-borne ARHDs were shown to participate on transformation of such compounds and to produce intermediates which could contribute to the induction of degradative genes. This study contributes to the understanding of interaction between soil degradative bacteria and plants through the action of SPMs.

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The long-term impact of antibiotic exposure in zebrafish bacterial communities

A. R. Almeida[1], M. Alves[2], I. Domingues[1], I. Henriques[3]
[1]*University of Aveiro, Department of Biology & CESAM, Aveiro, Portugal*
[2]*Universidade Católica Portuguesa/Porto, CBQF – Centro de Biologia e Química Fina, Laboratório Associado, Escola Superior de Biotecnologia, Porto, Portugal*
[3]*University of Coimbra, CESAM & Department of Life Sciences, Faculty of Science and Technology, Coimbra, Portugal*

The interactions between the microbiome and its host have an important role in host" health. Although antibiotics are useful for pathogens control in aquaculture, they may reshape aquatic animals" microbiome, disturbing hosts" welfare. Antibiotics may also alter the microbiome of non-target animals and water bacterial communities. Due to its broad-spectrum activity, oxytetracycline (OTC) is one of the most frequently used antibiotics in aquaculture. However, the impact that OTC may have in the microbiome of the fish and the surrounding water is poorly understood.
Thus, the objective of this work is to study the long-term effect of OTC in zebrafish gut and water microbiome.
Zebrafish adults were exposed for two months to three concentrations (0; 10 and 10000 µg/L) of OTC via water exposure. DNA was extracted from gut and water samples and the V3-V4 region of the bacterial 16S rRNA gene was sequenced using Illumina technology. Changes in alpha and beta diversity were assessed.
Our results revealed a significant impact of OTC exposure on the abundance of phylotypes at class level on water (for both OTC concentrations) and gut samples (for the highest OTC concentration). For instance, Gammaproteobacteria were significantly less abundant in both water and gut exposed to the highest concentration of OTC. At the OTU (Operational Taxonomic Unit) level, cluster and PCOa analysis also suggested an impact of the highest concentration of OTC on both water and gut microbiome. Diversity of bacterial communities significantly increased in water exposed to 10 µg/L of OTC.
Therefore, our study revealed that OTC exposure impacted both water and gut bacterial communities" structure. Effects on water bacterial communities were observed even for the lowest OTC concentration. Thus, more studies considering lower concentration of antibiotics should be conducted in order to understand the impact of these compounds in bacterial communities in environmental realistic scenarios.

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Compartmentalization of immune response and microbial translocation in decompensated cirrhosis

C. Alvarez-Silva[1], R. Schierwagen[2], A. Pohlmann[2], B. Lelouvier[3], J. Trebicka[2], M. Arumugam[1]
[1]*University of Copenhagen, NNF Center for Basic Metabolic Research, Copenhagen, Denmark*
[2]*Department of Internal Medicine I, Goethe University Clinic Frankfurt, Frankfurt, Germany*
[3]*Vaiomer, Labège, France*

Translocation of the gut microbiota into the circulatory system modulates the inflammatory response in many diseases, including liver disease. The nature and composition of the microbiota that enters and circulates in different compartments of blood and ascites are unknown. Here, we used 16S-rRNA-gene-sequencing and comprehensively characterized the circulating microbiome in portal vein, liver outflow, central venous blood and peripheral venous blood using samples from seven patients with decompensated liver cirrhosis. Furthermore, we characterized the bacterial composition of ascites and peripheral blood from ten additional liver patients and its relation with systemic inflammation. Blood and ascites microbiome phylum compositions (predominantly Proteobacteria) differed from the gut microbiome, where Bacteroidetes and Firmicutes are predominant. Although overall bacterial community structure did not show a compartment-specific clustering, the abundance of several genera varied strongly in circulation and as well in ascites compared with whole blood, forming compartment-specific patterns. Bacterial infections trigger acute-on-chronic liver failure and are associated with high mortality. To investigate the relationship of gut bacteria translocation into the circulatory system and systemic inflammation, we measured cytokine levels in serum of the seven patients in three blood compartments. Our findings showed that inflammatory cytokines formed patient-specific clusters instead of blood compartments clusters, and they have robust associations with the abundance of blood microbiome genera. To go further into the association between systemic inflammation and the circulating microbiome, we extend our study to 10 additional liver patients, to determine whether levels of IL-6 and IL-8 in ascites differ from the levels in blood, and whether inflammation depends on the presence of specific bacteria DNA. There were significantly higher levels of IL-6 in ascites fluid compared to blood samples in all patients, but interestingly, IL-6 levels in blood correlated tightly with disease severity and surrogates of systemic inflammation, while IL-6 levels in ascites did not. Our data may suggest that in decompensated cirrhosis, while a relationship between systemic inflammation and microbiota composition seems to exist in blood, this is not the case for ascites

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Microbial processes at the root-soil interface in the forest soil are shaped by seasonal variation of metabolomes

Z. Human[1], M. Štursová[1], T. Větrovský[1], D. Navrátilová[1], L. M. Rubén[1], A. Howe[2], H. Kauserud[3], C. Pennacchio[4], I. Grigoriev[4], S. Callister[5], Y. M. Kim[5],
M. Lipton[5], P. Baldrian[1]
[1]*Institute of Microbiology of the CAS, Laboratory of Environmental Microbiology, Prague, Czech Republic*
[2]*Iowa State University, Ames, ID, United States*
[3]*Oslo University, Oslo, Norway*
[4]*Joint Genome Institute, Walnut Creek, CA, United States*
[5]*Environmental Molecular Sciences Laboratory, Richland, WA, United States*

In forest soils, bacteria and fungi are important drivers of soil processes, mediating both decomposition and nutrient transfer from and to trees, the main primary producers. It was demonstrated recently, that microbial activity varies among seasons. Here we have explored the contribution of bacteria and fungi to soil processes in a coniferous forest in the context of the seasonal differences among metabolomes. The combination of metabolomics, metagenomics, metatranscriptomics and microbial community sequencing was used to assess the seasonality of nutrient availability at the root/soil interface and its effects on the composition of the microbiomes and their contribution to C and N cycling in early and late vegetation season and early and late winter. Profound differences in metabolome profiles were found between periods of tree activity during the vegetation season and in summer. While the communities of bacteria and fungi were similar across all seasons, their activity differed seasonally. The rhizosphere and root compartments were the most dynamic, but roots, comprising ectomycorrhizal symbionts of tree roots showed higher activity of fungi, especially in summer. The C-cycle processes were dominated by bacteria in soil, but by fungi in litter and the composition of the seasonal metabolomes had further effects on the intensity of utilization of individual C compounds across seasons. In all compartments, seasons of vegetation activity were associated with microbial growth and turnover of cell wall components while in winter, reserve compounds including trehalose, glycogen and mannitol represented important C resources. Our results show that the understanding of soil microbiome functioning is impossible without considering plant activity, demonstrated by the seasonality of metabolomes of soil microhabitats. Forest soils represent highly complex systems where bacteria and fungi locally dominate and contribute together to the C and N cycling in the ecosystem.

Fig. 1

P 204

Revealing the uncultured bacteria inside the giant snail Achatina fulica

A. Cardoso[1]
[1]*UEZO, Rio de Janeiro, Brazil*

The giant snail *Achatina fulica* is an agricultural pest whose biotechnological potential has been largely untested. Here, the composition of the microbial population within the crop of this invasive land snail, as well as key genes involved in various biochemical pathways, have been explored. In a high-throughput approach, 318 Mbp of 454-Titanium shotgun metagenomic sequencing data were obtained. The predominant bacterial phylum found was *Proteobacteria*, followed by *Bacteroidetes* and *Firmicutes*. *Viruses*, *Fungi*, and *Archaea* were present to lesser extents. The functional analysis reveals a variety of microbial genes that could assist the host in the degradation of recalcitrant lignocellulose, detoxification of xenobiotics, and synthesis of essential amino acids and vitamins, contributing to the adaptability and wide-ranging diet of this snail. More than 2,700 genes encoding glycoside hydrolase (GH) domains and carbohydrate-binding modules were detected. When we compared GH profiles, we found an abundance of sequences coding for oligosaccharide-degrading enzymes (36%), very similar to those from wallabies and giant pandas, as well as many novel cellulase and hemicellulase coding sequences, which points to this model as a remarkable potential source of enzymes for the biofuel industry. Furthermore, this work is a major step toward the understanding of the unique genetic profile of the land snail holobiont.

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Phylogenetic identification and characterization of microbial diversity in Moroccan and Portuguese rivers using a metagenomics approach

S. Ettamimi[1]
[1]*CCMAR Ualg, CCMAR Ualg, Faro, Portugal*

The microbial communities in fresh and mine contaminated water has an important role in the biogeochemical cycling of nutriments and the clean-up of pollution. Mine sites are an example of extreme environment that contains a large number of diverse and specific bacteria. only 10% of these microbes can be studied by classical culture. Metagenomics analysis gives the opportunity to study better the functioning of these natural ecosystems.

The aim of this project is to exploit the metagenomics approach for exploration of microbial diversity of three Moroccan rivers (Sebou, Moulouya end Oum Errabiaa) We characterize and compare microbial community composition and structure in these populations using bioinformatics and computational tools to identify taxonomic groups and genes (biomarkers) to suggest which environmental factors influence the selection of microorganisms.

Metagenomics data generated by this work are used to make comparison with Portuguese ecosystems to suggest which environmental factors influence microbial diversity in this site and the selection of microorganisms that might have an impact on the environmental and human health.

P 206

Fingerprinting microbial communities through flow cytometry and Raman spectroscopy

C. García-Timermans[1], P. Rubbens[1,2], F. M. Kerckhof[1], W. Waegeman[2], N. Boon[1]
[1]*Ghent University, CMET, Center for Microbial Technology and Ecology, Ghent, Belgium*
[2]*Ghent University, KERMIT, Department of Data Analysis and Mathematical Modelling, Ghent, Belgium*

Natural and engineered communities can be more accurately characterized –and therefore managed- with single-cell technologies. Raman spectroscopy is an emerging tool for single-cell analysis. It is capable of making a fingerprint for a single bacteria, using the information of the (bio)molecules that are present in the cell. This fingerprint can be acquired without using a dye, and without damaging the sample, that can be further used.

As Raman spectroscopy becomes more widely used in microbial ecology, there is a need to ameliorate the standardization of data acquisition. Our group has demonstrated how different sources of noise due to improper sample manipulation can result in artificial subpopulations. Therefore, we have proposed a metadata aid to report measurements. We are also developing the R package "MicroRaman" to assist users in the analysis of microbial Raman data. In this way, we hope to improve experimental reproducibility and sharing of spectral data between research groups.

We compared Raman spectroscopy to another technology for microbial fingerprinting: flow cytometry. This single-cell technology is lower in resolution, but much more high-throughput than Raman spectroscopy. To compare their capacity to identify bacterial phenotypes, we harvested *E. coli* in different growth stages and analyzed them using both techniques. Our results indicate that flow cytometry allows to quantify shifts in the community fingerprint at the population level, while Raman spectra contained sufficient resolution to detect separated subpopulations at the single-cell level.

Phenotypic heterogeneity allows bacteria to adapt to a changing environment, as well as to divide the labor within the community. As both Raman spectroscopy and flow cytometry allow to quantitatively measure phenotypic heterogeneity, these technologies may become an important tool to link bacterial fingerprints with functionality. Ultimately, these tools might allow to steer microbial communities to their most optimal phenotype.

P 207

Tracking antibiotic resistance genes in Arctic – from genes to metagenomes and back

A. Gorecki[1], P. Decewicz[1], M. Dziurzynski[1], P. Ostapczuk[1], M. Musialowski[1], L. Dziewit[1]
[1]University of Warsaw, Department of Bacterial Genetics, Warsaw, Poland

Introduction: An accelerated usage of antibiotics in various clinical and environmental settings brings us to the dusk of antibiotic era. Nowadays, novel emerging pollutants, i.e. multi-drug resistant bacteria and antibiotic resistance genes (ARGs) relentlessly arise. However, the antibiotic resistance is not a recent phenomenon, and analysis of pristine, not influenced by human, environments enable insight into its origin and evolution. To analyze this complex phenomenon, novel tools that enable thorough studies of the prevalence and spread of ARGs are needed.
Objectives: The aim of this work was to develop a database of primer pairs suitable for detection of ARGs in various environments. Applying the novel bioinformatic tool (UniPriVal) and metagenomics approach a set of PCR primers were chosen and then used to analyze ARGs in pristine Arctic environment.
Materials & methods: Soil samples were collected in Svalbard. Standard methods of molecular biology were used, including high-throughput DNA sequencing, gene cloning, PCR and bioinformatic analyses. The UniPriVal software was developed in Python v3.4.
Results: The UniPriVal has been used to test over 600 primers pairs against various environmental metagenomes datasets including anthropogenically-shaped and pristine ones. The results of this analysis were summarized in the LCPDb database (*lcpdb.ddg.biol.uw.edu.pl*). Best primers were used for detection of ARGs in Arctic soil. Obtained results were analyzed in relation to obtained shotgun metagenomic data for soil samples from Arctic. Genes conferring resistance to aminoglycosides, β-lactams, glycopeptides, sulfonamides, tetracyclines and these encoding multidrug resistance pumps were found.
Conclusions: The LCPDb is a user-friendly web application, gathering information concerning PCR primer pairs useful in detection of ARGs in various environments. Combined application of PCR screening and metagenomic analysis enabled an insight into Arctic resistome.

P 208

The dynamics of the faecal microbiota in the swine following therapeutic administration of oxytetracycline

A. Köstelbauer[1], M. Ghanbari[1]
[1]BIOMIN, Tulln, Austria

Question: Part of discovering and implementing alternatives to in-feed antibiotics is understanding the effects of antibiotics on the composition of the gut microbiota in livestock animals. Oxytetracycline is an in-feed antibiotic that is widely used in swine production to prevent enteric infections and to improve feed efficiency. In this study, we used whole metagenome shotgun sequencing, to monitor the effect of antibiotic feed additive at therapeutic level on the dynamics of gut microbiota composition in weaned swine over a 21 day period.
Methods: Eight pigs of the control group received basal diet for the duration of the trial. Eight pigs in the treatment group received feed containing oxytetracycline. After 1-week of continuous administration, the pigs were switched to a maintenance diet without oxytetracycline. DNA was extracted from faeces taken before, during, and following (2-week withdrawal) oxytetracycline treatment.
Results: The therapeutic dose of oxytetracycline caused a reduction in overall species richness and diversity in the treated animals. The reduction lasted even after antibiotic administration was discontinued. The most notable change in bacterial abundance was the increase in the abundances of Bacteroidetes and Proteobacteria during the first 8 days of oxytetracycline exposure, which was mainly observed as increased *Prevotella*, *Parabacteroides*, *Paludibacter* (Bacteroidetes) and *Escherichia* (Proteobacteria) abundances.
Conclusion: The increase in the abundances of *Prevotella*, *Parabacteroides*, and *Paludibacter* in the medicated animals in the present study might be explained by the potential of these taxa to harbor antimicrobial resistance genes that may be transferred to other susceptible intestinal strains. These findings will help to optimize therapeutic schemes of the antibiotic usage in swine production and may inform alternative strategies that preserve the performance benefits, while reducing potential risks to human and animal health.

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Metagenomics and stable isotope probing reveal the active contribution of bacterial taxa in the recycling of dead biomass in forest soil

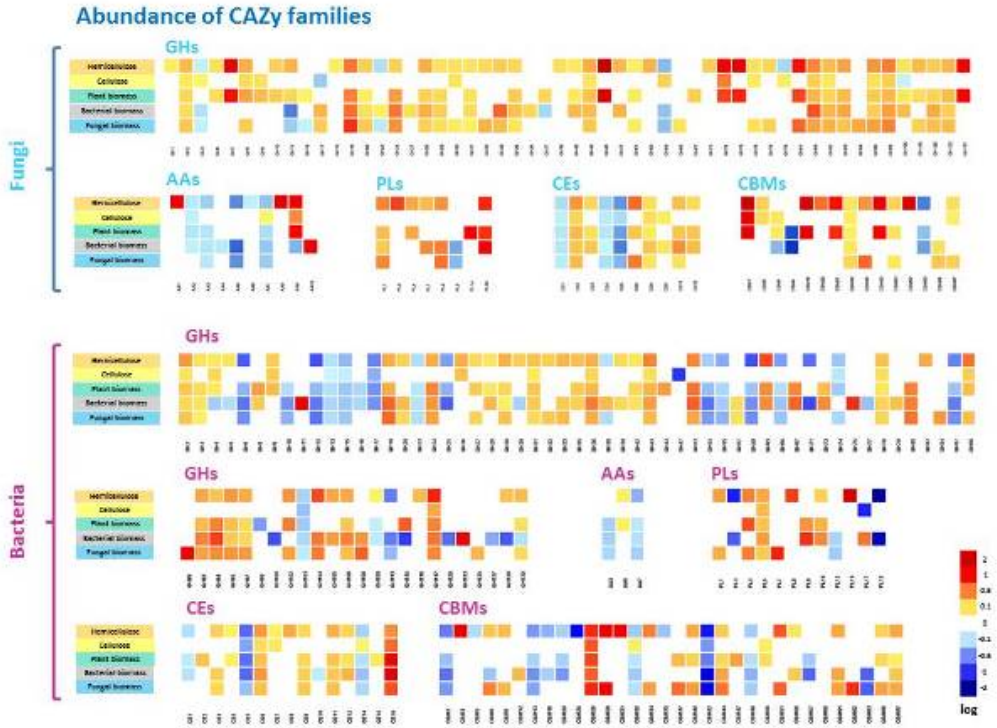
R. López-Mondéjar[1], V. Tláskal[1], T. Větrovský[1], M. Štursová[1], R. Toscan[2], U. Nunes da Rocha[2], P. Baldrian[1]
[1]*Institute of Microbiology of the Czech Academy of Sciences, Prague, Czech Republic*
[2]*Helmholtz Centre for Environmental Research – UFZ, Department of Environmental Microbiology, Leipzig, Germany*

Forest soils represent important terrestrial carbon (C) pools, where C is primarily fixed in plant biomass and then it flows further through the biomass of fungi and bacteria in soil. Although classical concepts assume that fungi are the main decomposers of the recalcitrant organic matter within plant and microbial biomass, whereas bacteria are often considered to mostly utilize simpler compounds, recent studies have shown that fungi and bacteria may overlap in substrate utilization[1]. Here, we have studied microbial contribution to the recycling of dead biomass by analyzing the bacterial and fungal community in soil microcosms upon the addition of ^{13}C-labeled biomass of plant, fungal, and bacterial origin by combining DNA-stable isotope probing and metagenomics. The results show that both fungi and bacteria contribute actively to the degradation of complex components of plant and microbial biomass. Moreover, the analysis of the CAZyme pool identified those major CAZy families involved in the degradation of each biomass type. Interestingly, the analysis of four bacterial metagenome-assembled genomes (MAGs), indicated the key role of some bacterial genera in the degradation of plant (*Cytophaga* and *Asticcaulis*) and microbial (*Heminiimonas*) biomass. These findings reveal not only new insights into the high diversity of functional genes and the complex enzymatic systems encoded by soil microorganisms, but also recognize the active role of bacteria as important decomposers of complex organic matter in forest soils.

References:

1. López-Mondéjar R, Brabcová V, Štursová M, Davidová A, Jansa J, Cajthaml T, Baldrian P (2018). Decomposer food web in a deciduous forest shows high share of generalist microorganisms and importance of microbial biomass recycling. The ISME Journal, 12,1768–1778.

Fig. 1



P 210

Analysis of multiple fungal sequence repositories highlights shortcomings in microbial databases

S. Mangul[1]

[1]UCLA QCBio and Computer Science Department, UCLA QCBio and Computer Science Department, Santa Mónica, CA, United States

Method. Reference genomes are essential for metagenomics studies, which require comparing short metagenomic reads with available reference genomes to identify organisms within a sample. Current efforts promise to extend genomic representation of microbial organisms across the tree of life. However new efforts are not always integrated into existing databases. Lack of comprehensive microbial databases complicates the choice of which database to use and potentially results in failing to classify portions of the sample due to missing database organisms. To illustrate, we considered fungal species and genera across three reference databases: Ensembl, RefSeq, and JGI"s 1000 fungal genomes project (1K).

Results. We identified 64% of genera that were present only in a single database. On the species level, we observed an even larger discrepancy across databases with 77% of species being present only in a single database. Using only a single reference database for analysis of metagenomic samples possibly results in the failure to identify some organisms in the sample. We observed a shorter length of genomes in the 1K database. The shorter overall length of genomes in the 1K database can be attributed to the lower amount of complete genomes in the 1K database compared to other databases. The percent of species represented as complete genomes are 23.5% (RefSeq), 1.4% (JGI), and 18.5% (Ensemble). The percentage of species containing both contigs and complete chromosomes are 6.8% (RefSeq), 6.3% (JGI), and 5.3% (Ensembl). Additionally, for the same species, RefSeq and Ensembl had mitochondrial reference genomes (Figure 1e). None of the complete and incomplete genomes in the 1K database were annotated as mitochondrial reference genomes. Finally, 9.5% of references in Ensembl contained plasmid genomes as did 0.36% of references in RefSeq, while 1K did not contain any plasmid genomes

Conclusions. We have identified an emerging need to integrate and disambiguate such databases. The current state of microbial databases is hampering metagenomic research and it is time to establish an effective dialogue between all the parties involved in creating microbial databases, where any new database will incorporate the information from the previous efforts in a consistent manner to avoid discrepancies between the databases and hence aid in metagenomic tool development.

The preprint is available at https://www.biorxiv.org/content/early/2018/12/17/497867

P 211

Application of Raman spectroscopy for *in-situ* identification of drought-tolerant diazotrophs in the rhizosphere

J. H. No[1], T. K. Lee[1]

[1]Yonsei University, Wonju, South Korea

Biological nitrogen fixation is dramatically affected under drought stress. The effect of drought on biological nitrogen fixation is considered to be the most important environmental factor resulting in crop yield loss. However, the number of the drought-tolerant diazotroph is limited due to the technical challenge of isolation using conventional culture-dependent methods. In this study, a culture-independent and non-destructive phenotyping approach, called Raman deuterium stable isotope probing, was developed to identify drought-tolerant diazotrophs in rhizospheric soil samples. The representative diazotrophs were incubated for 24 h in medium containing 40% deuterium supplemented with 25% Polyethylene glycol (PEG). Although all spectrums under drought stress were altered at broad ranges of raman shifts (1250, 1312, 1375, 1437 and 1562 cm^{-1}) in height and area, there are no specific raman features among all test species. However, the intensity of C-D bands (2040-2300 cm^{-1}) were significantly different between drought-sensitive and -tolerant diazotrophs. These features allowed us to identify and sort unculturable drought-tolerant diazotrophs for further studying evolution of its genomes at single cell level using Raman activated cell sorting technology.

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Is the environment shaping the *Serratia plymuthica* genome and bacteriophages content?

D. N. Proença[1], P. V. Morais[1]
[1]*CEMMPRE, University of Coimbra, Department of Life Sciences, Coimbra, Portugal*

Question: This study aims to evaluate the bacteriophages signatures present in *Serratia plymuthica* strains in order to understand if their number and type i) are dependent of habitats of *S. plymuthica*; ii) shared the same phylogenetic relationships as showed by pan-genome analysis; and/or iii) can be used as biological markers.
Methods: Fourteen genomes of *S. plymuthica* were retrieved from IMG database. These strains were isolated from pinewood nematode, plant leaves, soil, rapeseed roots, pasteurizer, and water; from eight different countries. The pangenome of these strains was analyzed by using BPGA software. PHASTER was used to identify and annotate the bacteriophages present in these 14 genomes. The identified regions were retrieved in VIRFAM. Phylogeny was analyzed using: i) 16S rRNA gene; ii) core genome; iii) bacteriophages regions; iv) head-neck-tail modules of bacteriophages. Protein sequences were queried into the genomes that are involved in i) virulence and nematode, ii) nematicidal, iii) toxins of *Serratia plymuthica*, and iv) plant growth promotion. NMDS was calculated based on the four categories *per si* and all together.
Results: The genomes sizes ranged from 5.25 to 5.55 Mbp and from 50.1 to 56.2 G+C mol%. The ANI between all these strains varies from 75.3 to 100. Core genome (2,244 genes) phylogeny analysis grouped the strains M24T3 and Leaf50 in one cluster, both related to plants, close with strain PRI-2C, and the remaining grouped in one cluster. This finding was also observed in all the NMDS analyses. Bacteriophages regions range 1 to 6 per genome. Phylogenetic analysis of these regions showed five clusters related to the viral family. This finding is also supported by head-neck-tail modules phylogenetic analysis and not related to the environment of the strain. The gene encoding for cI repressor protein (from family Myoviridae) was present in 12 strains and a similar strains-phylogenetic relationship was obtained when compared with the core genome analysis.
Conclusions: *S. plymuthica* strains isolated from different sources contain different genes, influenced by environmental origin or the functional role of the strain. Phylogeny of core genome and NMDS analysis showed the same relationship between strains. Phylogenetic analysis showed that regions of bacteriophages grouped according to their family, and are not related to strains geographical location or environment. The cI repressor protein might be used as biological marker.

P 213

A metagenomic insight into the 2 km-deep subsurface aquifer revealed multiple pathways of organic matter decomposition performed by uncultured bacterial lineages

V. Kadnikov[1], A. Mardanov[1], A. Beletsky[1], Y. Frank[2], O. Karnachuk[2], N. Ravin[1]
[1]*Research Center for Biotechnology RAS, Institute of Bioengineering, Moscow, Russian Federation*
[2]*Tomsk State University, Tomsk, Russian Federation*

Our knowledge of taxonomic and functional diversity of microorganisms inhabiting deep subsurface habitats is very limited. We studied microbial communities of the deep thermal aquifer (Western Siberia region, Russia) by metagenomic approach. Our goal was the recovery of near-complete genomes of the community members to enable accurate reconstruction of metabolism and ecological roles of the microbial majority, including uncultured lineages. Thermal water (40-45°C) flowing out from a depth of about 2 km was collected from the exploration borehole 1-R. Microbial community, as revealed by 16S rRNA gene profiling over 2 years, mostly consisted of sulfate-reducing Firmicutes and Deltaproteobacteria, and uncultured lineages of Chlorofexi, Ignavibacteriae, Aminicenantes, and Riflebacteria. 25 near-complete metagenome-assembled genomes were obtained and used for metabolic reconstruction. Members of Chlorofexi and Ignavibacteriae are likely involved in degradation of carbohydrates by fermentation, and are also capable of aerobic and anaerobic respiration. Metabolic reconstruction of a member of recently identified candidate phylum Riflebacteria predicted that it is an anaerobe capable to grow on carbohydrates by fermentation or dissimilatory Fe(III) reduction. We obtained first closed 3.3 Mbp-long genome of a member of candidate phylum BRC1, a yet uncultured lineage found more than 15 years ago in rice field soil. Genome analysis revealed pathways enabling the fermentation of various polysaccharides including chitin, as well as their oxidation through aerobic and anaerobic respiration. Fermentative saccharolytic lifestyle was predicted for a member of the candidate phylum Aminicenantes (OP8). The fossil organic matter from Mesozoic sediments provides substrates for respiratory organisms and fermenters. The latter produces hydrogen, acetate and other simple organic compounds that can be used by sulfate reducers. This work was supported by the Russian Science Foundation.

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A metagenomic catalog of short genes enables identification of ecologically relevant ribosomally transcribed peptides

C. Saenz[1], A. D. Brejnrod[1], G. Fernandes[2], M. Arumugam[1]

[1]*The Novo Nordisk Foundation Center for Basic Metabolic Research - University of Copenhagen, Human Genomics and Metagenomics in Metabolism, Copenhagen, Denmark*

[2]*Instituto René Rachou Fiocruz Minas, Informática de Biossistemas & Genômica, Belo Horizonte, Brazil*

Bacteria develop different antagonism strategies to gain ecological advantages over other bacteria. These can be direct, like competitive removal of essential substrates (Garcia-Gutierrez et al., 2018). Microbial natural products are known to mediate microbe-microbe and microbe-host interactions. Among them bacteriocins by preventing pathogen colonization of the same environmental niche (Garcia-Gutierrez et al., 2018; Noda et al., 2018) (Noda et al., 2018). Bacteriocins are small-peptide ribosomally translated compounds (10-50 amino acids) which interacts with bacterial membranes and cell walls causing the death of the bacterial cells.

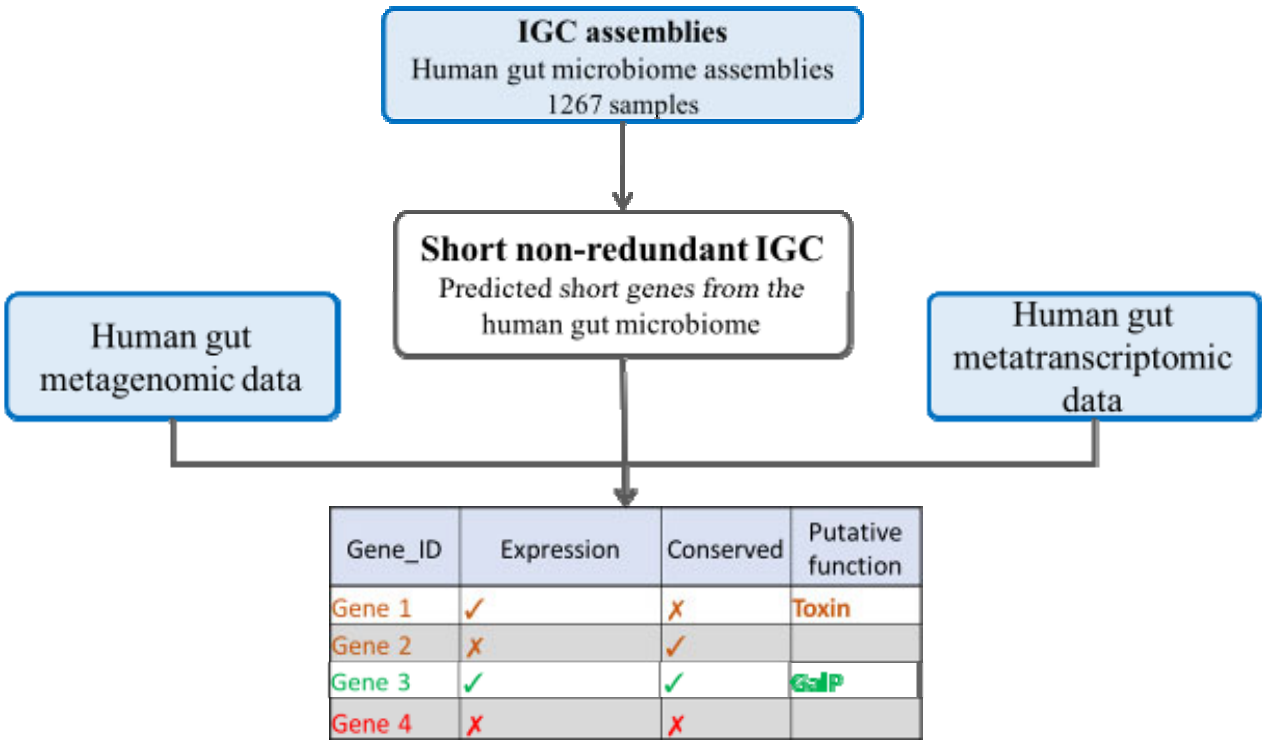
The continuous accumulation of antibiotic resistance genes in clinically relevant pathogens and the rising demand for safe foods have increased interest in replacing conventional antibiotics with bacteriocins. Bacteriocins resistance is not easily developed, they have low toxicity, are target-specific and offer the possibility of being bioengineered to improve properties such as specific activity or stability (Chikindas, Weeks, Drider, Chistyakov, & Dicks, 2018).

The human gut microbiome is a complex microbial community (Sekirov, Russell, Antunes, & Finlay, 2010) and represents an increasingly relevant source for novel antimicrobial agents, but is complicated by difficulties in annotating bacteriocin-encoding genes

The Integrated Gene Catalog (IGC) (Li et al., 2014), containing 9.9 million gut microbial genes describing the diversity of the human gut microbiome functionality. However, calling genes from metagenomics contigs is not trivial and the shortest protein encoded by the genes annotated in the IGC database is 33 amino acids long for technical reasons. An extension to IGC of carefully identified short genes is needed.

We have identified short genes in the IGC from the original metagenome assemblies used to build the IGC. To avoid false positive gene identifications, we will search genomes from human gut to establish conservation and publically available metatranscriptomes to substantiate expression. The synteny of genes near positively identified bacteriocins can be further investigated for antibiotic activity thus contributing to the discovery of new antimicrobial agents in the human gut microbiome.

Fig. 1



P 215

Amberlite binds characteristic soluble compounds during exposure in forest soil

M. Sagova-Mareckova[1], J. Kopecky[1]
[1]*Czech University of Life Science, Dept. of Microbiology, Nutrition and Dietetics, Prague, Czech Republic*

The study aimed in finding if soluble soil metabolites can be studied directly without further sample processing, which typically requires separation from insoluble polymers. The applied method was based on polyester bags of size 6 x 6 cm with mesh size of 0.1 mm filled with 5 g of Amberlite washed with methanol and dried. We selected four sites differing in soil conditions; Oblik and Srbsko are high pH sites with nutrient rich soils, Trebon and Zakopana are low pH sites with barren soils. Sites differ in climate and vegetation cover and previous work also determined differences in soil microbial communities. At each site, 14 Amberlite samples were placed about 5 cm deep to the organic horizon. Seven samples were collected after two and three months always together with seven samples of surrounding soil. That was collected to glass bottles with methanol: water: acetic acid (80v:19v:1v) and also to Eppendorf tubes for analysis of microbial communities. Amberlite samples were extracted with the same solvent, the extract was then evaporated to dryness on a rotary vacuum evaporator, weighed, dissolved in methanol, and analyzed by LC-MS. Results were evaluated by non-metric multidimensional scaling to compare metabolic profiles between sites and sampling times but also by individual compounds characterizing soil metabolism processes at the individual sites. The sites clearly differed by their metabolic profiles, however, there was a large local variability preventing statistical analysis at this scale. Several typical compounds were identified at each site and their character could be related to humus type and soil chemical conditions. It was concluded that this approach is valuable in detail studies of soil metabolites and the method shall be further improved with respect to ideal time and space scale, for which it is applicable.

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***Mycobacterium tuberculosis* genotypes causing extrapulmonary tuberculosis in Southwest Ethiopia**

M. T. Jano[1], G. Abebe[1], P. de RIJK[1], C. Meehan[1], B. de Jong[1], L. Rigouts[1], M. Tadesse[1]
[1]*Jimma University, Mycobacteriology Research Center, Jimma, Ethiopia*

Background: Ethiopia is hotspot for tuberculosis infection and ranks 10th in the world in terms of the number of TB. Understanding the genetic diversity and transmission dynamics of the circulating *M. tuberculosis* strains is very important to propose more effective preventive strategies. However, little is known about *Mycobacterium tuberculosis* (Mtb) strains responsible for extrapulmonary TB in Southwest Ethiopia.
Methods: A total of 304 Mtb complex strains from extrapulmonary TB patients in Southwest Ethiopia were genotyped primarily by spoligotyping. Isolates of selected spoligotypes were further analyzed by 15-loci mycobacterial interspersed repetitive unit–variable number tandem repeat (MIRU-VNTR). Isolates were classified into phylogenetic lineages and families by using the reference strain collections available at www.miru-vntrplus.org.
Results: Of the 304 *M. tuberculosis* isolates, 248 (81.6%) were belonged to the Euro-American lineage (Lineage 4). The recently described and poorly defined Ethiopian lineages were the most predominate comprising 108 (35.7%) isolates, of which 44 isolates were Ethiopia_2, 34 isolates Ethiopia_3 and 30 isolates Ethiopia_H37Rv-like.The next predominant lineages were the Delhi/CAS (14.5%) followed by Haarlem (14.1%). Interestingly, six isolates (2%) were belonged to the newly defined Ethiopian lineage called lineage 7. *M. bovis* was identified in only two (0.7%) of TBLN cases. The overall clustering rate was 35% indicating high rate of recent transmission. Delhi/CAS lineage strains were significantly associated with rifampicin resistance.
Conclusion: This study revealed a high diversity of *M. tuberculosis* complex genotypes responsible for TB lymphadenitis in Southwest Ethiopia. We reported the presence of a new phylogenetic lineage called Lineage 7 from TB lymphadenitis patients in Southwest Ethiopia. Zoonotic transmission of *M. bovis* infection has been excluded as a major factor in TB lymphadenitis.

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Optimization of metagenomic assembly from high-throughput data using a linear regression model

R. Toscan[1], F. Côrrea[1], J. P. Saraiva[1], U. Nunes da Rocha[1]
[1]*Helmholtz Centre for Environmental Research - UFZ, Department of Environmental Microbiology, Leipzig, Germany*

Metagenome assembly is a key step in genome recovery from genetic material from environmental samples. It consists of elongating the input sequences (sequenced reads) into larger sequences: contigs (overlapping reads) and scaffolds (or supercontigs). The assembly of highly complex communities may need up to 1 Pb of memory, which is the memory found in 125 personal computers (considering regular computers with 8 Gb of RAM). Thus, it usually involves the use of High Performance Computers (HPCs).
This work aims to determine which parameters regulate metagenome assembly resources and present a model that can predict the computational power required for such task.
We randomly selected 100 metagenomes from the Sequence Read Archive. Each metagenome was individually assembled (metaSPades) after a quality control step (TrimGalore). For each assembly, we measured a set of parameters: size of input data (in base pairs), unique k-mers of size 21 bp, 33 bp and 55 bp (KHMER), number of input sequences, GC-content, maximum memory usage (hereafter, *maxmem)*, time of execution and number of threads. We then performed an exploratory analysis by calculating the correlation of the numerical values of different parameters (e.g., k-mer, time and *maxmem*) among each other and developed a linear regression model, correlating the number of unique k-mers with time and *maxmem*.
Among the different parameters studied, k-mers showed the maximum correlation coefficient with *maxmem*, varying from 0.82 to 0.90. K-mers also correlate with time needed to finish the assemblies with a coefficient varying from 0.80 to 0.84. Data size and the number of input sequences correlation coefficients were 0.65 and 0.61, respectively. Multi-threading presented significant time speedup only when applied to highly complex communities.
The observed results reveal that sample complexity (indicated by the number of unique k-mers) can be used as an indicator of time of execution and maximum memory usage. Our linear model allows the prediction of computational resources that can improve time management of projects with a large number of samples.

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Contributions of high throughput qPCR in the field of antimicrobial resistance – trends, inconsistencies and disparities

H. Waseem[1], M. I. Ali[2]
[1]*Michigan State University, Environmental Engineering, East Lansing, MI, United States*
[2]*Quaid-i-Azam University, Microbiology, Islamabad, Pakistan*

Questions: qPCR technology has contributed significantly in environmental safety and public health. The expansion in whole genome sequencing and subsequent increase in antibiotic resistance targets have paved the way of high throughput qPCR (HT-qPCR) for measuring hundreds of antimicrobial resistance genes (ARGs) at once. The main goal of our study is to evaluate the contributions of HT-qPCR technology in the field of antimicrobial resistance and to highlight major trends in antimicrobial resistance (AMR) and discripancies in the HT-qPCR data analysis.
Methods: Meta-analysis of 51 selected studies is performed to evaluate the trends of ARGs abundance as evaluated by HT-qPCR over the last 7 years. Around 1000 environmental samples (excluding biological replicates) from different parts of the world analyzed on HT-qPCR were included in the analysis to highlight important trends.
Results:
WaferGen"s SmartChip qPCR platform is most frequently used (75 %) among others for evaluating ARGs. About two third of the studies selected for meta-analysis were from China. Calculated detection frequency and normalized ARGs abundance (ARGs/ 16S rRNA gene) reported in gut microbiome studies have shown a trend of low ARGs as compared to other environmental matrices. Major inconsistencies and disparities in the data analysis have been identified and a possible way for resolving them is also suggested.
Conclusion:
Over all the study has provided an updated information regarding the role, current status and future perspectives of HT-qPCR in the field of antimicrobial resistance. There is also an urgent need to steer the focus of the HT-qPCR based studies from just broadening the scope of the technology towards more in depth studies of validatory nature.
Figure 1: Graphical abstract showing the main theme of the study. It shows different HTqPCR platforms with their high throuput capacity and reaction volume.
Figure 2: World map showing no. of samples of different environmental matrices analysed by HT-qPCR over last 7 years in different regions of the world.

Fig. 1

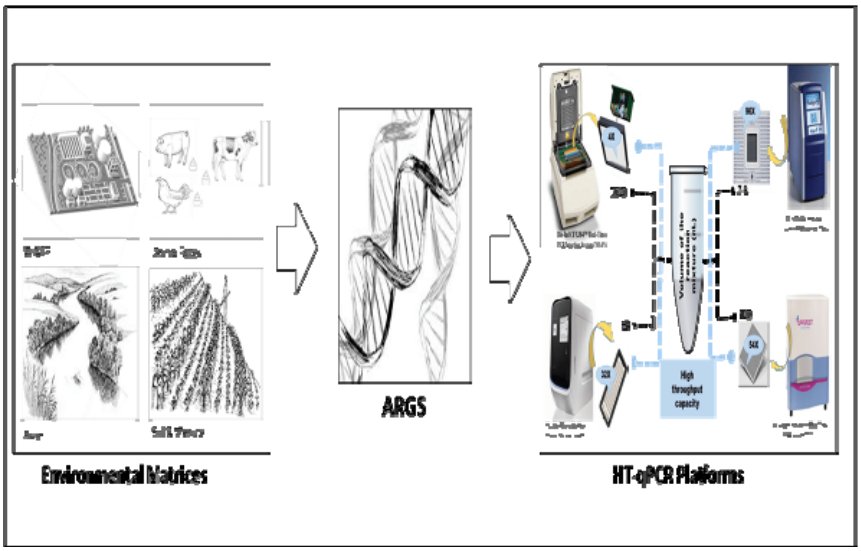
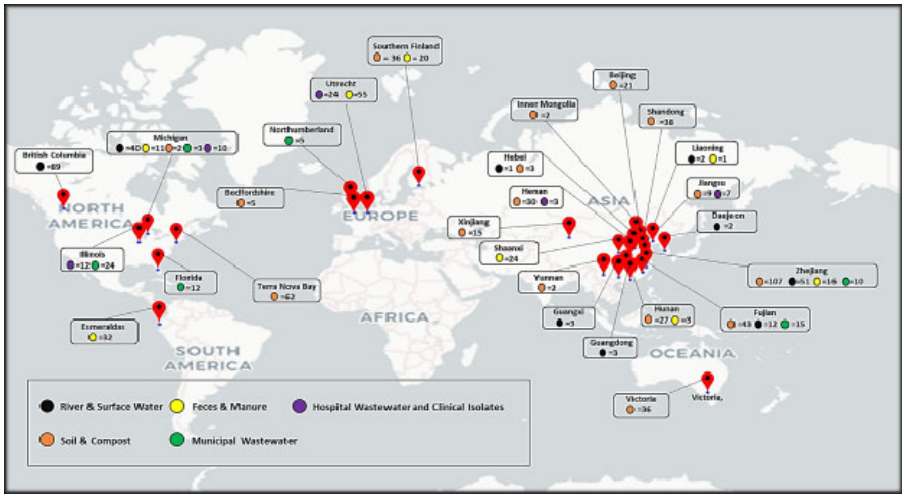

ARGS
Environmental Matrices
HT-qPCR Platforms

Fig. 2


NORTH AMERICA
EUROPE
ASIA
AFRICA
SOUTH AMERICA
OCEANIA
River & Surface Water
Feces & Manure
Hospital Wastewater and Clinical Isolates
Soil & Compost
Municipal Wastewater

P 219

Occurrence and diversity of *Legionella* contaminations and bacterial community composition in building"s plumbing and cooling towers

E. Ceci[1], C. Casagrande[1], R. Bellini[1], E. Federici[1]
[1]*University of Perugia, Chimica Biologia Biotecnologie, Perugia, Italy*

Introduction: The presence of opportunistic bacterial pathogens, such as *Legionella*, in building"s plumbing and cooling towers represents a serious concern for human health. Despite the improvements in *Legionella* monitoring, a deeper understanding of the microbial ecology of engineered water systems is needed to develop more efficient prevention strategies.
Objectives: We have studied the occurrence and diversity of *Legionella* contaminations and the composition of the associated bacterial communities in building"s water distribution systems and cooling towers.
Materials & methods: More than 400 water samples from 42 sites were collected and analyzed by culture and qPCR to assess *Legionella* contaminations. *Legionella* isolates were identified by *mip* gene sequencing and *L. pneumophila* strain diversity was investigated with sequence-based typing of seven genes (*flaA, pilE, asd, mip, mompS, proA, neuA*). Bacterial communitie were characterized with Illumina sequencing of 16S rRNA genes.
Results: *Legionella* contamination rates assessed by qPCR were much higher than those found by the culture-based method, suggesting the occurrence of cells in the viable but non-culturable state. Contaminations were mainly due to the presence of *L. pneumophila* sg 1, identified as strains ST1, ST23, ST356 and ST445. Nevertheless, other serogroups (6, 7, 8, 9, 10), as well as other species, namely *L. tauriniensis, L. anisa* and *L. rubrilucens* were also found. Bacterial communities were very diverse among samples and strongly depended on the system (plumbing vs. cooling towers), as well as on the water network and heating-system characteristics.
Conclusion: Our data indicate that engineered water systems can host complex bacterial communities and can be contaminated by highly diverse Legionellapopulations, suggesting how ecological investigations of the diversity and distribution of Legionellain the built environment should also be considered for prevention and control of outbreaks.

P 220

Bacillus species prevent *Staphylococcus aureus* colonisation in the nasal microbiome

D. Y. K. Ng[1]
[1]*Statens Serum Institut, Microbiology, Parasitology & Virology, Copenhagen, Denmark*

Question: We have a few isolates from nasal samples. Through culturing methods, we demonstrate the isolates, *Bacillus* pumilus, inhibit Staphylococcus aureus by producing amicoumacin. Amicoumacin was characterised in 1982 and shown to possess a strong inhibitory effect on *S. aureus*. Since the bacteria were isolated from the nose, we wanted to know if the presence of amicoumacin could prevent the colonisation of S. aureus in the nasal microbiome.
Method: A comparative genomic and genome mining approach is used to identify the gene cluster responsible for the production of amicoumacin. Microbiome data from a large nasal study is used to show the prevalence of Bacillus and their relations to Staphylococcus.
Results: By using a combination of antiSMASH and Roary, we identified Bacillus species that harbour the full gene cluster corresponding to amicoumacin. Our preliminary phylogenetic tree containing only *B.* pumilus suggest that amicoumacin entire gene cluster is present in more than half (26/46) of the sequenced genome on RefSeq. Some species of *Bacillus subtilis* are known to produce amicoumacin too.
Next, we looked into the microbiome data. After filtering erroneous samples, a total of 798 samples remain. Out of these, 100 samples (12.5%) contains Bacillus species. We define it by a count higher than 50. 702 (88%) of these sample contains Staphylococcus species. A Fisher"s exact test is performed and shown that the presence of Bacillus sp. has a significant difference (p=0.032) on the presence of Staphylococcus sp. The sequence of OTUs corresponding to Bacillus sp. and Staphylococcus will be examined and attempt to identify to species level to further validate the hypothesis.
Discussion: Our study suggests the presence of Bacillus species can prevent the colonisation of *S. aureus* in the nasal microbiome. However, it would require further validation.

P 221

Interactions of antimicrobial substances from bacteria against pathogenic microbes

D. S. Lee[1], H. G. Song[1]

[1]Kangwon National University, Biological Sciences, Chuncheon, South Korea

Question: Proliferation of microorganisms in cosmetics and foodstuffs not only deteriorates the quality of the products but also causes various diseases in human and therefore it should be prevented. Chemical antiseptics such as parabens have been widely used for this purpose, but they showed several problems including harmful side effects and persistence on human skin. Antimicrobial substances produced by microbes can solve these problems, but they should have broad spectrum and environmental stability of antimicrobial activity. Aim of this study is to evaluate the activity of antimicrobial substances produced by isolated bacteria against some human pathogens and mycotoxigenic fungi.

Methods: Bacterial strains showing antimicrobial activity were isolated by agar well diffusion test. Their antimicrobial substances were extracted and analyzed, and their antimicrobial activity and synergistic effects between them were examined.

Results: Strains DS381, DS518, DS620 and DS1515 showed 15.3 to 35.3 mm inhibition zone diameter against most bacteria and yeast, and inhibited mycelial growth (~66.7%) and sporulation (~85.7%) of target fungi. The purified antimicrobial substances (lipopeptide, chitinase, siderophore, protease and anthracyclic antibiotics, etc.) from isolated bacteria exhibited low minimum inhibitory concentrations (0.0078-10,000 mg/ml) on target organisms. When the synergistic effect of antimicrobial substances was investigated, combinations of antimicrobial substance displayed synergistic effects against each different target organism (0 < fractional inhibitory concentration index < 0.75). In time-kill assays, most combinations reduced more than 105 in colony count of bacteria and yeast during 24 h. Also, combination of antimicrobial substances showed spore degradation (28.2~91.6%) and spore germination inhibition (97.2~100%).

Conclusion: These results suggest that isolated bacteria and antimicrobial substances may be utilized as an environment-friendly preservative and biocontrol agent against human pathogens and mycotoxigenic fungi.

Fig. 1

Table 4. Checkboard assay and spore germination inhibition-synergistic effect of antimicrobial substances of DS381, DS518, DS620 and DS1515.

Target organism	DS381+DS620		DS381+DS1515		DS518+DS620		DS620+DS1515	
	FICI	SGI (%)	FICI	SGI (%)	FICI	SGI (%)	FICI	SGI (%)
A. flavus	0.31	100.00	0.31	100.00	-	-	0.63	100.00
A. awamori	2.00	-	0.63	100.00	-	-	2.00	-
A. almaceus	0.63	100.00	0.75	99.60	-	-	0.50	100.00
A. niger	0.63	99.90	1.13	100.00	0.51	89.95	0.50	100.00
F. fugikuroi	0.75	100.00	0.75	100.00	0.13	100.00	0.75	97.20
F. proliferatum	0.75	100.00	1.00	-	0.13	100.00	0.75	100.00
F. verticillioides	0.75	100.00	0.75	100.00	0.13	100.00	0.50	100.00

*SGI: spore germination inhibition

P 222

A conceptual framework for the phylogenetically-constrained assembly of microbial communities

D. Aguirre de Cárcer[1]
[1]*Universidad Autónoma de Madrid, Biology, Madrid, Spain*

Most microbial communities present well-supported characteristics: their assembly is significantly deterministic, and show significant phylogenetic clustering. Also, traits/ecological coherence are somewhat phylogenetically conserved. From these ideas we can predict that:
1) We will find discrete portions of the phylogeny of varying depth present in all instances of a given ecosystem (phylogenetic core groups; PCGs).
2) Their existence relates to the occupancy of niches requiring specific sets of phylogenetically conserved traits (A-niches).
3) Resident microbes not belonging to any PCG occupy niches requiring sets of traits not showing strong phylogenetic signal (B-niches).
These predictions are in line with recent reports. Moreover, the first prediction has so far been substantiated by the deliberate analysis of various datasets and ecosystems.
Hence, simplistically, each instance of the ecosystem should present populations from each PCG (occupying A-niches), and non-phylogenetic-core populations (occupying B-niches). Populations occupying each A and B-niche should be (partially) ecologically redundant, their intra-niche structure governed by neutral processes, and likely show intra-group competition.
The existence of microenvironments and patches within macroscopic samples will foster the co-occurrence of theoretically competing populations, producing the observed larger-than-expected species richness. Finally, the existence of intra/inter-niche co-evolved populations, would reduce the system's niche-based modularity and foster the emergence of social and rival groups.
PCGs' identification is feasible using 16S sequencing. By pinpointing the plausible existence of A-niches, this framework should enhance our understanding of community assembly, with the ratio of A to B-niches determining its usefulness for each ecosystem. Lastly, alternative community assembly rules must adequately explain the abovementioned microbial communities' characteristics.

P 223

Association of skin microbiome and skin sensitivity

H. Keum[1], W. J. Sul[1]
[1]*Chung-Ang University, Systems biotechnology, Anseong, South Korea*

Sensitive skin is a syndrome occurring from unpleasant sensations such as burning, pain, pruritus, and tingling in response to irritation which normally should have not provoked such sensations. Sensitive skin has been explained by cutaneous, environmental, lifestyle, and endogenous factor. But until now, there has not been much research on skin sensitivity from the perspective of a skin microbiome. We focused on the relationship between skin sensitivity and skin microbiome. Bacterial and fungal community were analyzed by 16S rRNA gene and ITS1 region amplicon sequencing. Principal coordinate analysis (PCoA) identified a significant difference between non-sensitive and sensitive skin microbiome in both bacterial (ANOSIM, p=0.004) and fungal community (ANOSIM, p=0.008). In fungus, sensitive skin group was significantly diverse than non-sensitive skin group. *Mucor racemosus* and *Phanerochaete* were significantly increased fungi in sensitive skin. The co-occurrence skin microbiome network more collapsed in sensitive skin group compared to non-sensitive skin group. A multivariate analysis suggested self-assessment sensitivity of individuals was related to the skin microbiome. The factors such as allergic experience, unpleasant feeling, and the extent of pimple also correlated with the bacterial and fungal community of sensitive skin. These observations show that skin microbial community shifts are related to skin sensitivity. This will provide the basis of the microbiome-based remedy on sensitive skin.

P 224

Assessment of skin microbiomes assembly process for human skin health

H. J. Kim[1], S. An[2], W. J. Sul[1]
[1]*Chung-Ang University, Systems Biotechnology, Seoul, South Korea*
[2]*Amorepacific, R&D center, Yongin, South Korea*

Given the higher incidence of skin diseases in more urbanized populations and their associations with the skin microbiome, we questioned how the skin microbiome differs depending on the degree of urbanization. Assessing the impact of residential environment on skin microbes is important to addressing its association with human skin health. We focused on addressing the skin microbial community assembly process by implementing microbial ecological metacommunity theory. Skin microbiomes of 231 healthy subjects in five large cities in China varied mainly depending on the city"s environment and socioeconomic status. The differences among microbiomes could be explained by the predominantly niche-based assembly of the microbial communities, which was supported by a dominance test, β-null deviation, and edge-length abundance distribution. Networks among microbes in larger cities were more fragile, which may contribute to the higher incidence of skin diseases in more urbanized environments. These results suggest that microbial ecological theory can provide a framework for understanding crucial health-associated features of the human microbiome.

P 225

Microbial lifestyles in preferential flow paths of a clayey till

F. Bak[1], C. Keuschnig[2], O. Nybroe[1], J. Aamand[3], M. H. Nicolaisen[1], T. M. Vogel[2], C. Larose[2]
[1]*University of Copenhagen, Plant and Environmental Sciences, Frederiksberg, Denmark*
[2]*Ecolé Centrale de Lyon, Lyon, France*
[3]*Geological Survey of Denmark and Greenland, Geochemistry, Copenhagen, Denmark*

Clayey tills contain preferential flow paths such as biopores and fractures that harbour distinct microbial communities compared with the adjacent matrix sediments. Currently, little is known about the life styles of microbial communities in these flow paths at different depths, and their adaptations to environmental stresses derived from changing exposure to nutrient, oxygen and water. Here, we applied amplicon sequencing of the 16S rRNA gene and the ITS region, and shotgun metagenomics to DNA extracted from biopores and fractures in a Danish agricultural clayey till to a depth of 4 meter below ground surface. We found that communities changed with depth for both bacteria and fungi in the preferential flow paths. Analysis of metagenomics sequences showed that communities in biopores had higher abundance of genes related to flagellar motility and plant material degradation than communities in the surrounding matrix sediments. Additionally, the shallow biopore microbial communities had more functions related to protection against desiccation and oxygen stress than communities in deeper fractures. In the fracture communities, functions enabling microorganisms to resist environmental variation in pH, salinity and low nutrient availability were abundant. Abundant functions in the surrounding matrix sediment communities indicated that microbes in this habitat rely on degradation of amino acids and peptides for carbon and nitrogen sources, and on the synthesis of essential vitamins. Our results indicate that more microorganisms in preferential flow paths are motile and they are better adapted to resist environmental stress than microorganisms in the surrounding matrix sediments.

P 226

Marine sponges as *Chloroflexi* hot spots – genomic insights and high-resolution visualization of an abundant and diverse symbiotic clade

K. Bayer[1], M. T. Jahn[1,2], B. M. Slaby[1], L. Moitinho-Silva[3], U. Hentschel[1,4]
[1]*GEOMAR-Helmholtz centre for ocean research Kiel, RD3-Marine Ecology, RU-Marine Microbiology, Kiel, Germany*
[2]*University of Wuerzburg, Imaging Core Facility at the Theodor Boveri Institute of Bioscience, Wuerzburg, Germany*
[3]*University of New South Wales, Centre for Marine Bio-Innovation, Sydney, NSW, Australia*
[4]*Christian-Albrechts University of Kiel, Kiel, Germany*

Members of the widespread bacterial phylum *Chloroflexi* can dominate high-microbial-abundance (HMA) sponge microbiomes. In the Sponge Microbiome Project, *Chloroflexi* sequences amounted to 20 to 30% of the total microbiome of certain HMA sponge genera with the classes/clades SAR202, Caldilineae, and Anaerolineae being the most prominent. We performed metagenomic and single cell genomic analyses to elucidate the functional gene repertoire of *Chloroflexi* symbionts of Aplysina aerophoba. Eighteen draft genomes were reconstructed and placed into phylogenetic context of which six were investigated in detail. Common genomic features of *Chloroflexi* sponge symbionts were related to central energy and carbon converting pathways, amino acid and fatty acid metabolism, and respiration. Clade-specific metabolic features included a massively expanded genomic repertoire for carbohydrate degradation in Anaerolineae and Caldilineae genomes, but only amino acid utilization by SAR202. While Anaerolineae and Caldilineae import cofactors and vitamins, SAR202 genomes harbor genes encoding components involved in cofactor biosynthesis. A number of features relevant to symbiosis were further identified, including CRISPR-Cas systems, eukaryote-like repeat proteins, and secondary metabolite gene clusters. Chloroflexi symbionts were visualized in the sponge extracellular matrix at ultrastructural resolution by the fluorescence in situ hybridization-correlative light and electron microscopy (FISH-CLEM) method. Carbohydrate degradation potential was reported previously for "Candidatus Poribacteria" and SAUL, typical symbionts of HMA sponges, and we propose here that HMA sponge symbionts collectively engage in degradation of dissolved organic matter, both labile and recalcitrant. Thus, sponge microbes may not only provide nutrients to the sponge host, but they may also contribute to dissolved organic matter (DOM) recycling and primary productivity in reef ecosystems via a pathway termed the sponge loop.

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Substrate characteristics and environment shape prokaryotic community dynamics of marine biofilms

T. Pollet[1], E. Catao[1], B. Misson[2], C. Garnier[2], C. Bressy[1], J. F. Ghiglione[3], J. F. Briand[1]

[1]*University of Toulon, MAPIEM, Toulon, France*

[2]*University of Toulon, MIO, Toulon, France*

[3]*UPMC, LOMIC, Banyuls-sur-Mer, France*

Marine artificial facilities (ship hull, aquaculture net, …) are colonized by biofilms, threatening their integrity and leading to economic loss. Biofilm community dynamics were studied in both static and dynamic mode in two coastal French Mediterranean sites (Toulon and Banyuls bays) during a year, on two antifouling coatings in comparison with a reference surface (PVC). One coating release copper (SPC) whereas the other was based on a low surface energy and elastic modulus (FRC).

Although pioneer taxa were dissimilar on all the three surfaces, the communities on PVC and FRC tend to converge with time whereas biocidal coating ones tends to diverge. If Flavobacteriaceae and Rhodobacteraceae represented critical members of highly diversified biofilms on PVC, *Alteromonas* remarkably accounted for more than 90% of the community on SPC until 12 days of static immersion. Then, bacterial diversity increased progressively, keeping *Alteromonas* as one of the co-dominant taxa until 75 days of immersion. SPC diversity remained lower and promoted the co-dominance of other common (Rhodobacteraceae) or site specific bacterial families (Flavobacteriaceae and Erythrobacteraceae at Toulon, Hyphomonodaceae at Banyuls). After one year, a significant impact of shear stress could be observed on taxonomic community structure for each surface. Roughness and wettability shaped prokaryotic community without shear stress magnification. Conversely, biocidal effect, already major in static mode, was amplified. SPC was 60% dissimilar from others, distinctly colonized by Sphingomonadaceae ((Alter)Erythrobacter). At Banyuls, prokaryotic communities were more similar within surfaces, possibly due to a masking effect of environmental constraints over surface. Predicted functions confirmed some of the hypothesis regarding the taxa developed over each surface. Community shaping appeared to be complex, driven by substrate and environment.

Fig. 1

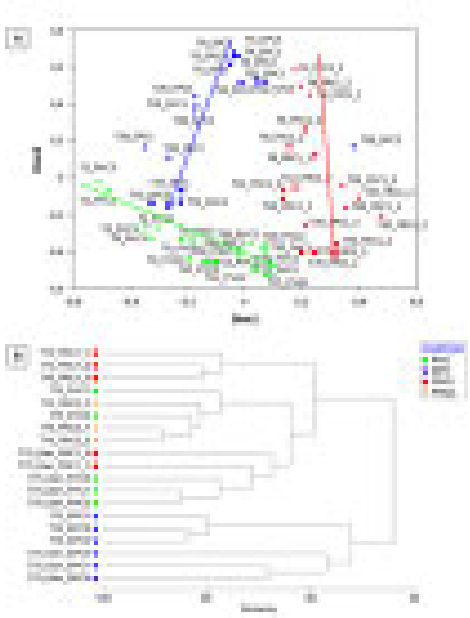
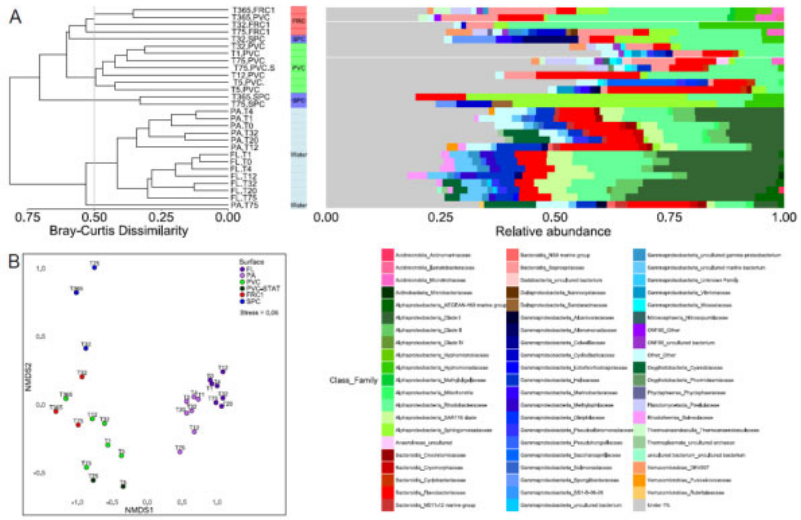


Fig. 2



P 228

Exploring Baltic Sea cyanobacteria for small-molecule inhibitors of microRNA 92b-3p function

P. Brzuzan[1], H. Mazur-Marzec[2], F. Stefaniak[3], M. Florczyk[1], P. Budzińska[1], M. Babatunde[1], M. Woźny[1]
[1]*University of Warmia and Mazury in Olsztyn, Department of Environmental Biotechnology , Olsztyn, Poland*
[2]*University of Gdańsk, Division of Marine Biotechnology , Gdańsk, Poland*
[3]*International Institute of Molecular and Cell Biology,, Laboratory of Bioinformatics and Protein Engineering, Warsaw, Poland*

Introduction: Cyanobacteria constitute a rich source of biologically active and structurally diverse compounds. The pharmacological potential of these compounds resides, in part, in their ability to control the proliferation and growth of cancer cell lines and potent disease-causing microbial agents. Because they can modulate regulatory elements of cell processes (proteins and functional RNAs), bioactive cyanobacterial metabolites could be important tools for clarifying the mechanisms of these processes and serve as lead structures for the development of new therapeutic agents.
Objectives: -to prepare a *de novo* library of bioactive and non-toxic peptides from Baltic Sea cyanobacteria, and
-to create and use a cell sensor to find compounds with strong and selective affinity for MiR92b-3p, a liver-cancer–specific microRNA.
Materials and Methods: Our small-molecule screen employs a Huh7 human hepatoma cell line stably transfected with a pmirGLO dual luciferase sensor for endogenous MIR92b-3p, which is involved in hepatocellular carcinoma (HCC) development and hepatitis C virus infection. The assay was optimized and validated using MiR92b-3p antisense and MiR92b-3p mimicking agents, followed by measurements of luciferase mRNA levels (qPCR) in the engineered Huh7 cell line. This reporter system was then used to screen cyanobacterial peptides from a *de novo* library, which was prepared on the basis of the peptides" activity against key metabolic enzymes (proteases and protein phosphatases) and against selected cell lines (MTT test).
Results: Exposure of the Huh7-pmirGLO-MIR92b-3p cells to increasing concentrations of an initial group of peptides that were isolated from *Nostoc edaphicum* (CCNP 1411) did not lead to a dose-dependent restoration of the luciferase signal. Instead, the Renilla signal was reduced at higher peptide concentrations (>5µM), suggesting non-target effects of the peptides on the cells.
Conclusions: Although the first use of this reporter system to screen peptides from Baltic Sea cyanobacteria failed to identify inhibitors of MIR92b-3p, this method provides a means to identify functional miRNA regulators and could be readily extended to other compounds.
The study was funded by the National Science Centre of Poland (DEC-2017/25/B/NZ9/00202).

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Bacterial pathogens in output material from biogas plants processing livestock manure: A real threat?

L. Čermák[1], J. Vlčková[1], M. Marounek[1], V. Skřivanová[1]
[1]*Institute of Animal Science, Nutritional Physiology and Animal Product Quality , Prague, Czech Republic*

Biogas plants represent currently an important part of agricultural production management. While the main feedstock for anaerobic digestion is corn and grass silage, they can be fed also with bio-waste materials, such as cattle, swine or poultry manure. Fugate, the waste product of biogas production, is regularly used in agriculture as fertiliser. But it can contain bacterial pathogens commonly related to animal husbandry.
The aim of the study was (i) to compare different types of manure with regard to the content of selected zoonotic agents after anaerobic digestion process and (ii) examine the effect of a stage of treating fugate after anaerobic digestion. The numbers of coliform bacteria, *Salmonella* spp., *Clostridium perfringens*, *Campylobacter* spp. and total anaerobes were investigated by cultivation in fugate samples and evaluated as log CFU/g of a dry matter. Furthermore, a comparison of dendrograms based on partial 16S rRNA gene sequences was done.
Our results showed that there were significant differences in numbers of all monitored groups of bacteria between samples from biogas plants processing different type of manure. The fugate from swine manure showed higher counts of Clostridium perfringens than those from poultry and cattle manure (by one and two order of magnitude respectively; $P < 0,0001$). The lowest numbers of pathogens were detected in fugate from cattle manure, with undetectable counts of coliforms after anaerobic digestion process (prior log CFU = 5).
The study should help improve the hygiene of agricultural production and raise awareness of zoonotic infections originating from animal husbandry.
Acknowledgments: This study was supported by the Ministry of Agriculture of the Czech Republic (Project No. MZeRO0719).

P 230

Compartmentalization of biosynthetic enzymes in bacterial cells: the histidine metabolic pathway case

S. Chioccioli[1], S. Del Duca[1], P. Bogani[1], R. Fani[1], L. M. Castronovo[1], A. M. Puglia[2], A. Vassallo[2], L. Pazzagli[3], P. Paoli[3]
[1]*University of Florence, Department of Biology, Sesto Fiorentino, Italy*
[2]*University of Palermo, Department of Biological, Chemical and Pharmaceutical Sciences and Technologies , Palermo, Italy*
[3]*University of Florence, Department of Experimental and Clinical Biomedical Sciences, Florence, Italy*

Introduction: It is known that the inner concentration of proteins within the cell cytoplasm is so high that limits the diffusion of enzymes and metabolic intermediates, leading to a loss of time and energy. Therefore, the organization of genes in operons would have enabled to have enzymes involved in the same metabolic pathway physically close to each other. A corollary to this hypothesis in the possibility of physical interactions between the enzymes of the same metabolic pathway, resulting in the formation of a supramolecular complex capable in channeling the intermediates from one enzyme to a physical adjacent one, with restricted diffusion in the surrounding milieu.
Objectives: The aim of this study is to try to demonstrate the existence of the compartmentalization of proteins involved in the same metabolic pathway, and the formation of multi-enzymatic complexes in bacterial cells, using as model system the histidine biosynthesis. In *Escherichia coli*the genes involved in this metabolic pathway are organized in a compact operon. At least 7 of the 10 genes of the pathway underwent different gene fusions in different evolutionary lineages, suggesting a compartmentalization of the biosynthetic enzymes.
Materials & Methods: To evaluate the possible interactions between the enzymes of this pathway a combination of different approaches has been used: i) the Bacterial Adenylate Cyclase Two-Hybrid (BACTH) system, ii) the β-galactosidase assay, iii) the His-tag method, and iv) microscopy analyses.
Results: So far, using the BACTH system, the interaction between the enzymes His-H and His-F has been validate, as a positive control of the system.
Conclusion: The testing of all the other possible interactions between the enzymes of the histidine biosynthetic pathway, through the proposed multidisciplinary approach, is in progress to evaluate if they form a single big multi-enzymatic complex.

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Experimental systems to study marine microbiomes

G. Duarte[1,2], D. Silva[1], H. Villela[1], H. Santos[1,3], P. Rosado[1], J. G. Rosado[1], A. Rosado[1,2], E. Ferreira[1], A. Soriano[4], R. Peixoto[1,2]
[1]*Federal University of Rio de Janeiro, Institute of Microbiology Paulo de Góes, Rio de Janeiro, Brazil*
[2]*IMAM-AquaRio, Rio de Janeiro Aquarium Research Center, Rio de Janeiro, Brazil*
[3]*Fluminense Federal University, Current affiliation: Department of Marine Biology, Rio de Janeiro, Brazil*
[4]*Petrobras, Biotechnology Section, Leopoldo Américo Miguez de Mello Research Center - CENPES, Rio de Janeiro, Brazil*

Few studies have been carried out to understand the effects of oil spills on coral reefs, and, especially to develop efficient remediation strategies. This limitation is partly explained by the difficulties in developing experiments with contaminants, such as crude oil, or to simulate global changes, such heat stress, and keep marine organisms, as corals, in good conditions. In this case, these micro-and mesocosms systems need to allow safe handling and, at the same time, provide the marine organism requirements, reproducing field conditions. This study aimed to describe a randomizable flow-through mesocosm with true replicates (up to 52) and a waste-treatment system for studies simulating ecotoxicology studies as oil spills, climate change scenarios and their effects on corals or other marine invertebrates. This adaptable system can be configured to (i) have continuous flow-through; (ii) operate as an open or closed system; (iii) be fed by gravity, avoiding pump clogging; (iv) have separate mesocosm sections that can be used for individual and simultaneous experiments, including the use of different marine organisms and their associated microbiomes; and (v) simulate the migration of oil from ocean oil spills to the nearby reefs. The mesocosm performance was assessed with 2 batch experiments that used different configurations to simulate 2 magnitudes of oil spills reaching coral reefs. Physical and chemical parameters including pH and salinity remained stable in all replicates throughout the experiments, indicating that the mesocosm system reliably mimicked the desired conditions. Regarding the biotic parameters, in the second experiment it was possible to follow the decrease in the maximum quantum yield of photosystem II (Fv/Fm) of the photosynthetic algae associated with the hydrocoral Millepora alcicornis, in response to the 1% crude oil applied in the system. This mesocosm facility is customizable and adjustable for these and other experiments, replicating marine ecosystems, and proved to be efficient for studies of oil spills and heat stress, with the associated use of Beneficial Microorganisms for Corals (BMCs). Many of the features of this adaptable mesocosm are being used for the construction of a new deep sea simulator mesocosm system, at the AquaRio Research Center. The new system is also composed by a deep sea microorganisms chemostat, with oxygen and temperature control, and is able to simulate important deep sea environmental conditions.

Fig. 1

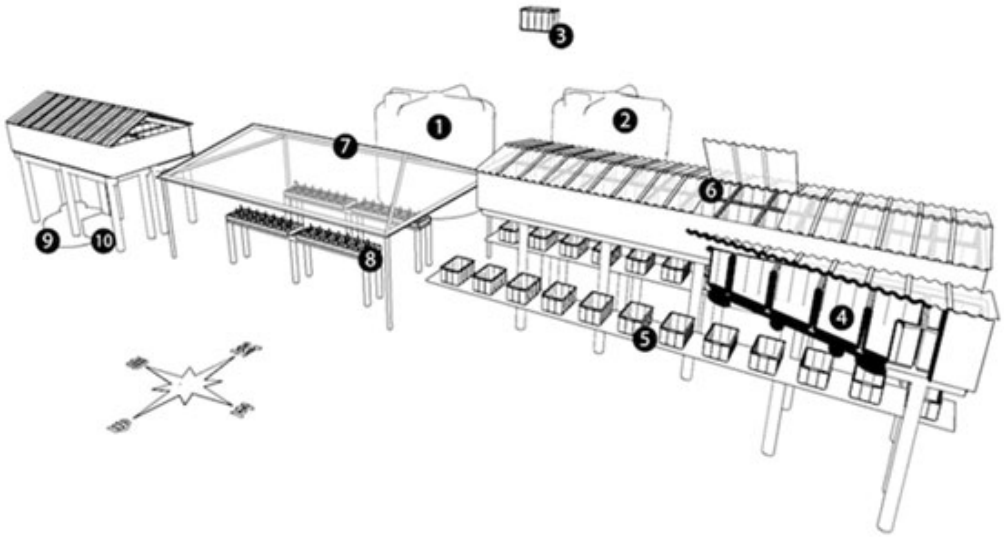
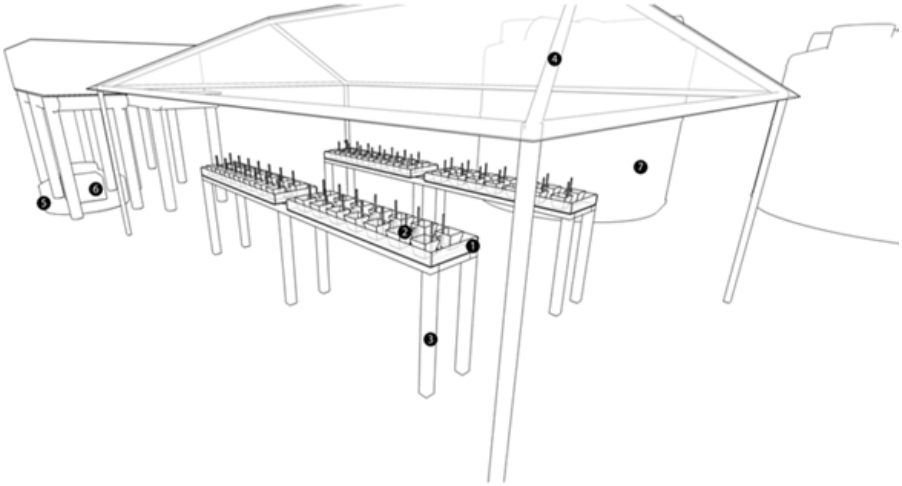


Fig. 2



P 232

Understanding global transcription patterns of industrial *streptomyces* strains in soil

L. T. Fernández Martínez[1]

[1]*Edge Hill University, Biology, Ormskirk, United Kingdom*

Understanding the regulation of antibiotic gene clusters under soil conditions, particularly in industrially relevant strains, will help us understand how the production of these compounds is regulated in their natural environment. Expression of most of these antibiotic gene clusters appears dormant (cryptic) under laboratory conditions but the clusters are maintained in the genomes of these strains, therefore indicating that they must play important roles in adaptation and survival within their ecological niches. These cryptic pathways represent an untapped resource in terms of new metabolites and novel chemistry that could be very useful in the clinic. By growing industrial strains on soil for the first time, we aim to understand the pathways which lead to the activation of transcription of these antimicrobial clusters in order to design new strategies to find novel metabolites. This will allow us to establish global regulatory pathways which lead to the production of these compounds. Moreover this knowledge can then be used to generate genetically modified industrial strains in order to increase the yield of both well characterised and unknown compounds under industrial fermentation conditions.

P 233

Cost and benefits of harboring metal resistant genes

J. Gannon[1]
[1]*American University of Sharjah, Biology, Chemistry and Environmental Sciences, Sharjah, United Arab Emirates*

A thermodynamic approach was used to analyze the response of a metal-tolerant soil bacterium to metal resistant gene deletions in cadmium-amended media. Under cadmium stress, the wild-type strain benefited from the presence of resistance genes by entering the exponential growth phase significantly earlier than knockout strains which lacked functional resistance genes. In the absence of cadmium, strain KT1, carrying a deletion in the main component of a Cd/Zn chemiosmotic efflux transporter grew more efficiently than the wild type and released 700 kJ (per mole of biomass carbon) less heat than the wild-type strain, demonstrating the energetic cost of maintaining resistance in the absence of cadmium. A second mutant strain (KT4) carrying a different gene deletion, cadA2, which encodes the main Cd/Pb efflux transporter (a P-type ATPase), did not survive beyond moderate cadmium concentrations and exhibited a decreased growth yield in the absence of cadmium. Therefore, CadA2 plays an essential role in cadmium resistance and perhaps serves a secondary function. The results of this study provide direct evidence that heavy metal cation efflux mechanisms facilitate shorter lag phases in the presence of metals and that the maintenance and expression of tolerance genes carry quantifiable energetic costs and benefits.

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Proteomics – an efficient and reliable tool for research on alphaproteobacterial methanotrophs

A. Hakobyan[1], J. Zhu[1], T. Glatter[2], W. Liesack[1]
[1]*Max Planck Institute for Terrestrial Microbiology, Research group of Methanotrophic Bacteria, Environmental Genomics and Transcriptomics, Marburg, Germany*
[2]*Max Planck Institute for Terrestrial Microbiology, Core Facility of Mass Spectrometry and Proteomics, Marburg, Germany*

Microbial methane oxidation is one of the fundamental processes in global methane cycling. Our model organism, *Methylocystis* sp. strain SC2, is a representative of type IIa methanotrophs. Using recent developments in sample preparation strategies and improvements in mass spectrometry, we developed an optimized procedure to characterize the proteome of *Methylocystis* sp. strain SC2. It tackles the major challenges related to the high amount of integral membrane proteins that need to be efficiently solubilized and digested for downstream analysis. Therefore, each step of the workflow, including cell lysis, protein solubilization and digestion, and MS peptide quantification, was assessed and optimized. Our new crude-lysis approach proved to increase protein quantification accuracy and proteome coverage of strain SC2. It captured 62% of the predicted SC2 proteome, with up to 10-fold increase in membrane-associated proteins relative to less effective conditions. We further validated our workflow efficiency using contrasting nitrogen conditions. Upon exposure to high ammonium- and nitrogen-limiting conditions, we observed a concurrent trend of major abundance changes between key proteins and their transcripts. Therefore, we conclude that our crude-lysis proteomics workflow is a fast and reliable way to accurately analyze the response of strain SC2 to environmental change.
We applied our proteomics workflow to study the response of strain SC2 to hydrogen as an alternative energy source. A genomic survey had revealed the genetic potential of strain SC2 for hydrogen oxidation. The addition of hydrogen had a significant effect on both specific growth yield and methane consumption rate of strain SC2 under 6% CH_4 and 3% O_2. Proteomics showed that the hydrogen effect was correlated with a significant increase in the enzyme level of the low-affinity Group 1d hydrogenase, Group 2b regulatory hydrogenase, nitrogenase, and Fix protein complex. In conclusion, strain SC2 has the metabolic capacity to utilize hydrogen as an energy source, which may represent a particular adaptation to environments with fluctuations in the methane and oxygen concentrations.

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Ecology, dynamics and future prospects of lignocellulose-degrading microbial consortia

D. Jiménez[1]
[1]*Universidad de los Andes, Biological Sciences, Bogotá, Colombia*

Agricultural residues are key sources for commodity chemicals production. However, an efficient saccharification of plant biomass is still a challenge in biorefineries. The use of enzyme cocktails produced from lignocellulose-degrading microbial consortia is a promising approach to optimize this process. Nevertheless, the proper use of these consortia depends on a sound understanding of the ecology, dynamics and their enzymatic mechanisms (Jiménez *et al*. 2017). In a recently published manuscript, we have explored the temporal expression profile of carbohydrate-active enzymes in a synthetic bacterial consortium growing on sugar cane bagasse. Using a metatranscriptomics-based approach, we observed a clear successional profile, where endoglucanases and endoxylanases were significantly expressed at 12 hours of growth. In contrast, enzymes acting on external-side linkages of plant polysaccharides, as exoglucanases and alpha-arabinosidases/beta-xylosidases, were highly expressed after 48 hours. Here, *Paenibacillus*, *Brevundimonas* and *Chryseobacterium* were the most important contributors, whereas *Stenotrophomonas* was highly active at the end of the culture without contributing largely to the expression of lignocellulolytic enzymes (Jiménez *et al*. 2018). Finally, within this topic, I would like to show new ecological and biotechnological questions or prospects. For instance, *i*) it is possible to build a minimal and versatile consortium using two approaches of selection (dilution-to-stimulation and dilution-to-extinction)? *ii*) how design an effective enzyme cocktail using a consortial secretome plus specific fungal enzymes?, and *iii*) can we evaluate stochasticity events within the selection process of these consortia? I believe that these perspectives can help to our ecological understanding and could improve the saccharification process in second-generation biorefinery applications.

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P 236

Exploring the potential of *Micrococcus luteus* culture supernatant for enhancing the culturability of soil bacteria

M. A. Lopez Marin[1], M. Strejcek[1], P. Junkova[1], J. Santrucek[1], O. Uhlík[1]
[1]*University of Chemistry and Technology Prague, Biochemistry and Microbiology, Prague, Czech Republic*

While isolation in pure culture remains the best way to characterize most bacterial species, knowledge of the attributes of soil bacteria is hampered by their basic unculturability. However, it is possible that culturability can be improved by the use of growth-promoting factors. With the aim of increasing the number of bacteria that can grow on solid medium, we investigate the potential of *Micrococcus luteus* resuscitation-promoting factor (Rpf) as a growth-promoting factor for soil bacteria. The Rpf was tested in fresh soil and in sterile soil individually bioaugmented with one of three different bacterial strains, including *M. luteus*. These strains remained in the sterile soil for several weeks prior to the experiment start. PBS and enzyme-inactivated supernatant were added to the control soils. After agitation at 28 °C, the soil suspension was diluted and plated. The effectiveness of each treatment was evaluated by the number of CFUs formed on solid medium and by changes in diversity, the latter being assessed by 16S rRNA amplicon sequencing and MALDI-TOF MS. In all cases, both diversity and the number of CFUs were higher for the extraction with Rpf. Thus, our results suggest that Rpf can enhance the growth of poorly-known soil bacteria. Funding is acknowledged of the Czech Science Foundation grant no. 17-00227S and specific university research (MSMT No 21-SVV/2019).

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Exploring the *in vitro* activity of the preservative ethoxyquin and its oxidation derivatives as nitrification inhibitors against ammonia and nitrite-oxidizers

E. Papadopoulou[1], E. Lampronikou[1], E. Mpaxtsebani[1], E. Adamou[1], A. Katsaouni[1], C. Thion[2], G. W. Nicol[2], U. Menkissoglu-Spiroudi[3], S. Vasileiadis[1], D. Karpouzas[1]

[1]*University of Thessaly, Department of Biochemistry And Biothechnology, Larissa, Greece*

[2]*Ecole Centrale de Lyon, Lyon, France*

[3]*Aristotle University of Thessaloniki, Thessaloniki, Greece*

Introduction: Nitrification inhibitors (NIs) are used in agricultural practice to reduce nitrogen losses from agro-ecosystems. Apart from the characterized NIs, other agrochemicals can also affect the organisms participating in nitrification. Previous soil microcosm studies showed that ethoxyquin (EQ), used as a preservative in fruit industry, is rapidly transformed to quinone imine (QI) and 2,4-dimethyl-6-ethoxyquinoline (EQNL), and suggested a strong inhibitory effect on ammonia oxidizers (AOs).

Objectives: We aimed to explore the inhibitory potency of EQ and its derivatives on soil nitrifying isolates *in vitro*, and identify their spectrum of activity and their inhibition thresholds, compared with widely used NIs (nitrapyrin, DCD, and DMPP).

Materials and Methods: The growth and activity of two ammonia oxidizing-bacterial (AOB), two ammonia-oxidizing archaeal (AOA), and a nitrite-oxidizing bacterial (NOB) terrestrial isolates were monitored in liquid cultures, via qPCR of phylogenetic markers and nitrite production/consumption, respectively, for a broad range of NIs concentrations. The dissipation and metabolism of the tested NIs in the liquid cultures were determined via HPLC.

Results: EQ was transformed to the short-lived QI (major metabolite), and to the persistent EQNL (minor metabolite). AOA were more sensitive than AOB and NOB to EQ, QI and EQNL. AOs were susceptible to lower concentrations of QI compared to EQ and EQNL, pointing to QI as the main responsible for the persistent reduction in growth and activity of AOs cultures treated with EQ. The EC50 values for the tested NIs in AOB cultures increased in the order nitrapyrin<DMPP<QI<EQ<EQNL<DCD. Results on the effects of DCD, nitrapyrin and DMPP on AOA are pending.

Conclusion: The differential sensitivity of AOA and AOB to EQ and its derivatives may reflect differences in their ammonia oxidation mechanisms/pathways or physiologies. Our findings could be exploited for the development of novel, universal NIs.

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Short-term effects of *Eucalyptus* plantations renewal on soil microbial community and greenhouse gas fluxes

C. Rachid[1], C. Cuer[2], D. Monteiro[1], R. Rodrigues[3], J. Jesus[3], F. Balieiro[3], E. Silva[3], B. Alves[3]

[1]*UFRJ, General Microbiology, Rio de Janeiro, Brazil*

[2]*University of Strasbourg, Strasbourg, France*

[3]*Embrapa, Brasil, Brazil*

Soil greenhouse gases (GHG) emissions are a major environmental problem which come from N and C cycling microbial processes. Agriculture, forestry and other land uses are the third-highest sources of anthropogenic GHG, mainly through crop cultivation and tropical deforestation. Owing to high levels of deforestation, land use change, and improper land use practices. Planted forests, however, are thought to play many positive roles in the context of climate change and deforestation through restoration of degraded land, soil conservation, CO_2 sequestration, and protection of biodiversity. This study aimed to investigate the impact of *Eucalyptus* plantations renewal on soil microbial community structure and function and how these shifts are linked to GHG fluxes, comparing to native vegetation. We used 16S rRNA gene sequencing and qPCR amplifications of key nitrogen and methane cycle genes: nifH, nirK, archaeal amoA, bacterial amoA, nosZ and mcrA to characterize microbial structure and function. The results were then linked with soil parameters and GHG fluxes. Both microbial community structure and function were significantly affected by land use and renewal of *Eucalyptus* plantations. Higher alfa and beta microbial diversity were found in *Eucalyptus* plantation compared to native forest. nosZ and archaeal amoA genes were significantly more abundant in native forest than in *Eucalyptus* plantations. Statistical analyses suggest that land use had a larger impact on microbial community structure and function than managing practices, but significant effects caused by plantation renew could be found in one month after trees cutting. No direct correlation was found between GHG fluxes and the shifts observed in microbial community, suggesting that microbial community structure and function were not the main drivers of GHG fluxes in this system.

P 239

Genomic and physiological analysis of biofilm formation in *Staphylococcus xylosus*

C. Schiffer[1], M. A. Ehrmann[1], R. F. Vogel[1]
[1]*Technische Universität München, Lehrstuhl für technische Mikrobiologie , Freising, Germany*

Introduction: *Staphylococcus xylosus* is a coagulase negative commensal on mammal skin and a widely used starter in raw sausage fermentation. In the sausage matrix, assertiveness of starter strains against autochthonous staphylococci is an important factor in strain selection. *S. xylosus* is known for being a strong biofilm former. This ability may increase its assertiveness against autochthonous staphylococci by concomitant induction of colonization resistance in the fermenting sausage niche.
Objectives: Biofilm formation was characterized in strains of *S. xylosus* with respect to phenotypic variations and their relation to differences in the genome by comparative genomics.
Material and Methods: DNA was isolated and sequence data were obtained from five different *S. xylosus* strains. Genomic data was assembled and annotated using routine bioinformatic tools. Genome comparison followed by identifying group and strain specific genes, characterization of the mobilome, the plasmidome and screening for virulence associated factors. Phenotypic assays on bacterial surface properties and biofilm formation were performed, examining also the influence of the physiochemical characteristics of the medium and type of support used.
Results: Within the investigated *S. xylosus* strains, we identified differences in surface properties, adherence potential to various supports and subsequent biofilm formation. We were able to link some of the phenotypic variations to differences in their genomes. Additionally, we found lactate, glucose and NaCl to influence adhesion behavior in a strain specific way.
Conclusion: Biofilm formation of *S. xylosus* is strain specific with respect to surface properties and environment. This knowledge can be used to explain strain specific differences of assertiveness in sausage fermentation. Screening for a defined set of marker genes derived from comparative genomics can be used to identify assertive biofilm formers among *S. xylosus*.
This work was supported by project AIF 19690N.

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Structural and regulatory genes related to nitrogen fixation in *Paenibacillus brasilensis*

B. do Carmo Dias[1], F. Faria da Mota[2], L. Seldin[1]
[1]*Universidade Federal do Rio de Janeiro, Instituto de Microbiologia Paulo de Góes, Departamento de Microbiologia Geral, Rio de Janeiro, Brazil*
[2]*Instituto Oswaldo Cruz, Fiocruz, Rio de Janeiro, Brazil*

Nitrogen is abundantly present in the atmosphere as its stable form dinitrogen (N_2). However, it is the main constraint in natural and agricultural ecosystems as most organisms are unable to incorporate N_2 into a carbon skeleton, becoming dependent on reactive forms of nitrogen for their growth. A specialized group of prokaryotes utilizes the enzyme nitrogenase encoded by the *nif* genes to catalyze the conversion of atmospheric nitrogen (N_2) to ammonia (NH_3), a process called biological nitrogen fixation (BNF). BNF is regulated at the transcriptional level in response to the amount of fixed N_2 and to the oxygen levels. In Gram-negative bacteria, this regulatory mechanism is well known, but it remains poorly characterized in Gram-positive bacteria. *Paenibacillus brasilensis* PB24 is a facultative anaerobic, diazotrophic Gram-positive bacterium, but the presence and the regulation of *nif* genes in this species is still unknown. In this study, the *nif* operon was identified in *P. brasilensis* PB24 through the functional annotation by SEED from the open read frames (ORF) predicted by the software RAST version 2.0. We found nine genes in the *nif* operon involved in the assembly of nitrogenase. A genomic comparison investigated through BLASTn (visualized using BRIG) and the MAUVE program among different *Paenibacillus* species based on *nif* operon showed an operon with conserved synteny with small variations, mainly in the flanking genes. The possible transcriptional mechanism was investigated searching for patterns of recurrent nucleotide sequences (motifs) preceding the promoter of the *nif* operon, using the MEME tool. The motif with the best alignment was selected and compared to the regulatory sequences available in the TOMTOM software database. The proposed BNF binding-sites regulators of Gram-positive GlnR and TnrA previously described in literature were also found in *P. brasilensis* PB24.

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Reproducibility of bacterial MALDI-TOF mass spectra

M. Strejcek[1], T. Pankova[1], T. Šmrhová[1], O. Uhlík[1]
[1]*University of Chemistry and Technology, Prague, Dept. of Biochemistry and Microbiology, Prague, Czech Republic*

Matrix Assisted Laser Desorption/Ionization Mass Spectrometry with Time-of-Flight detector (MALDI-TOF MS) has been proposed as a promising tool for bacterial identification and classification mainly due to its high throughput, cost-effectiveness and rapid performance. MALDI-TOF mass spectrum of directly measured microbial intact cells or their protein extracts is populated with the mass signals of the most abundant cell proteins. Since the majority of such proteins are from ribosomal protein family, the mass spectrum represents a culture-specific profile. This fingerprint can be compared against a referential database for microbial identification. Alternatively, computation of spectrum-to-spectrum similarities between multiple microorganisms can be used for isolate dereplication, i.e. reducing isolate number for subsequent analyses in ecological studies. While MALDI-TOF MS measurements of technical replicates showed high reproducibility, our previous work identified high biological variation in some of the environmental bacterial isolates. This variation included changes in mass signal intensity and even presence/absence of certain signals. We hypothesized that the sample preparation and the bacterial culture age were the main sources of mass spectra variability. In the presented work, multiple bacterial strains of environmental relevance were cultivated in the same nutrient broth while different sample preparation and cultivation times were applied. The differences in mass spectra were detected using number of univariate indices such as number of peaks detected, average intensity or average signal-to-noise ratio. Individual peaks that were differentially expressed under varying experimental conditions were identified by shrinkage discriminant analysis. We concluded that while some mass signal changes were detected over the culture cultivation time, the largest biological variation was bound to certain organisms, specifically Gram-positive bacteria, suggesting cell wall composition might play important role in bacterial MALDI-TOF MS analysis. Funding is acknowledged of the Czech Science Foundation grant no. 17-00227S.

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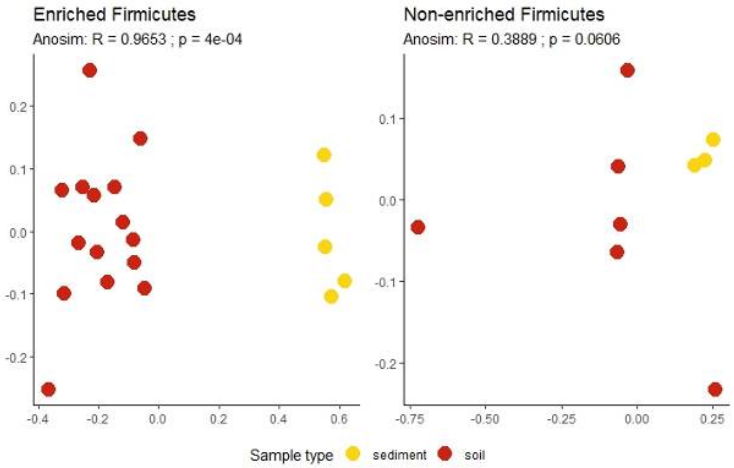
Comparison of dormant thermophilic endospores in Arctic sediments and soils suggests different dispersal patterns and sources

G. Vitali[1], B. B. Jørgensen[1], C. Hubert[1], C. Hanson[1]
[1]*Queen Mary University of London, School of Biological and Chemical Sciences, London, United Kingdom*

Endospores of thermophilic bacteria in the phylum Firmicutes ("thermospores") are dispersed from warm sources to habitats that are not suitable for thermophilic growth - most notably, Arctic marine sediments. Although the diversity of thermospores in the sediments of Svalbard (Norway) has been extensively characterised, the source environment(s) from which they originate remain unknown.
We hypothesise that thermospores in Svalbard marine sediments originate from marine, warm deep biosphere habitat(s), such as mid-ocean ridge venting systems or off-shore petroleum reservoirs, and are dispersed long-distances as dormant spores via ocean currents. If so, thermospores will be rare in terrestrial environments of Svalbard, or, if present, will be different from those in marine sediments. To test this hypothesis, we compared the 16S rRNA gene composition of all Firmicutes and of thermophilic Firmicutes in soils and fjord sediments from Kongsfjorden, Svalbard. To target dormant thermophilic Firmicutes, samples were first pasteurised and incubated in brackish medium under anoxic conditions at 50 °C for 48 – 92 hours.
In concordance with our hypothesis, we found that the community composition of enriched thermophilic Firmicutes was significantly different between soils and sediments (ANOSIM: R = 0.97; p = 0.0001). In contrast, the community composition of all Firmicutes (those present prior to heated enrichment) in soils and sediments did not differ (ANOSIM: R = 0.39; p = 0.060), as shown in fig.1. Further, of the 596 OTUs Firmicutes OTUs identified, only 4 are found in both soil and sediment samples. None of these lineages belong to genus *Desulfotomaculum*, which is prominently observed in Svalbard marine sediments and responsible for thermophilic sulphated reduction.
These findings suggest that thermospore communities in Svalbard sediments are unlikely to be derived from atmosphere or soil and implying distinct sources for sediment and soil communities and the existence of dispersal barriers.

Fig. 1



P 243

Does ecology drive turnover of genes in prokaryotes?

A. K. Watson[1]
[1]*Sorbonne University, Paris, France*

Prokaryote genomes include gene families with diverse evolutionary histories. While a relatively small "core" set of gene families are broadly conserved across prokaryotes and resistant to lateral gene transfer, it is also broadly accepted that their genomes are chimeric. Prokaryotic genomes include genes acquired by lateral gene transfer from other cellular genomes and from mobile genetic elements, and it has been suggested that a majority of prokaryotic gene families have been subject to lateral gene transfer at some point in their evolutionary history. We are interested in understanding how ecology may drive these processes. Here, we explore patterns of gene sharing and the functions of gene families in thousands of bacterial and archaeal reference genomes, as well as mobile genetic elements. Notably, many gene families are relatively sparsely distributed, and/or are unique to particular lineages. This pattern is indicative of a strong turnover of genes; new genes are invented while old genes are lost. We tested whether the turnover of genes correlates with the species ecology, i.e. whether new genes code for functions that may contribute to adjusting to a changing environment or concern broader cellular functions. If case 1, ecology may be a strong driver of a major process of genome evolution (aka turnover). If case 2, prokaryotes reinvent themselves more extensively, possibly to cheat death and the mueller ratchet that is still too strong on prokaryotes for them to keep on using an old genetic recipe. Otherwise, the turnover of genes may be a broad and neutral process.

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Insights into culturomics of the rumen microbiome

T. Zehavi[1], M. Probst[1], I. Mizrahi[1]
[1]*Ben-Gurion University, Life Sciences, Be'er Scheva, Israel*

Cultivation of undescribed rumen microorganisms is one of the most important tasks in rumen microbiology. In this study, we aimed to discover the potential of culturomics for characterizing the rumen microbiome and for identifying factors, specifically sample dilution and media type, which affect microbial richness on agar plates. Our cultivation experiment captured 23% of all operational taxonomic units (OTUs) found in the rumen microbiome in this study. The use of different media increased the number of cultured OTUs by up to 40%. Sample dilution had the strongest effect on increasing richness on the plates, while abundance and phylogeny were the main factors determining cultivability of rumen microbes. Our findings from phylogenetic analysis of cultured OTUs on the lower branches of the phylogenetic tree suggest that multifactorial traits govern cultivability. Interestingly, most of our cultured OTUs belonged to the rare rumen biosphere. These cultured OTUs could not be detected in the rumen microbiome, even when we surveyed it across a 38 rumen microbiome samples. These findings add another unique dimension to the complexity of the rumen microbiome and suggest that a large number of different organisms can be cultured in a single cultivation effort.

Figure 1. Potential of culturomics for the rumen microbiome. **(A)** Venn diagram illustrating the OTUs detected in the defined medium (orange), the undefined medium (green), and the original rumen sample and its dilutions (gray). **(B)** Pie chart illustrating the cultivable and uncultivable fractions of the detected rumen microbiome. **(C)** Stacked bar plots describing the phylogenetic composition of available cultures from public collections, the cultured OTUs detected on the plates, the rumen sample used for culturing in this study, and rumen microbiomes as reported previously by Henderson et al. (2015) .

Figure 2. Comparison of the phylogenetic distances between OTUs growing on the plates and OTUs detected in the original rumen sample. The mean phylogenetic distances between OTUs that grew on plates was calculated and compared to the distribution of phylogenetic distances between the OTUs of the original rumen sample. The distribution of distances from the rumen OTUs is as a box plot. The average phylogenetic distance between OTUs of the cultured group being compared is as an orange oval.

Fig. 1

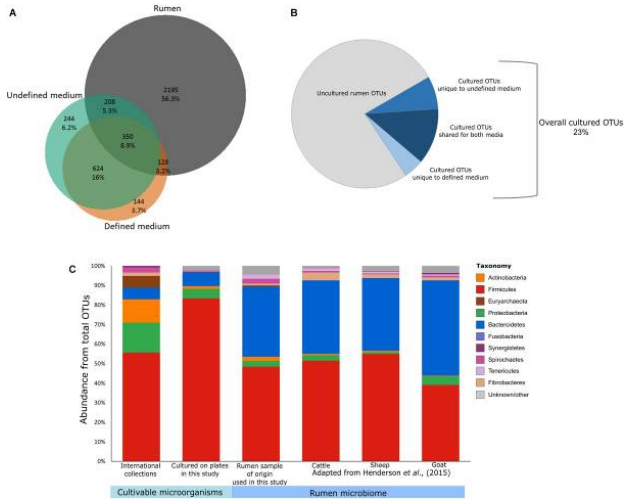
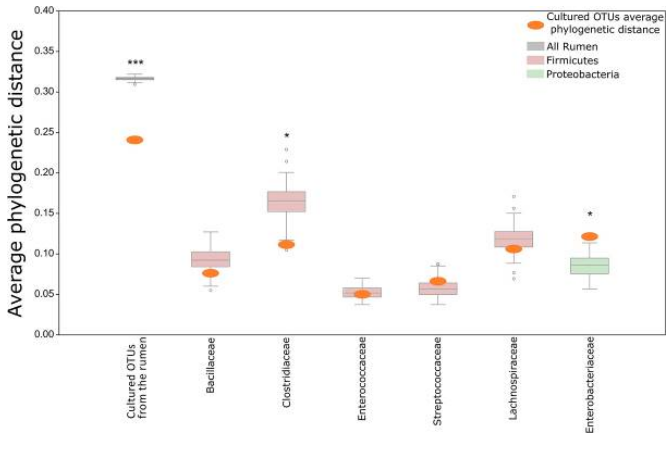


Fig. 2



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Disentangling inter-kingdom and functional traits associated with pulse thermal resistance in corals

E. Santoro Peçanha[1], H. Villela[1], G. Duarte[1,2], C. Vilela[1], A. Michelato[1], C. Rachid[1], R. Toscan[3], F. Côrrea[3], J. P. Saraiva[3], R. Kallies[3], A. Macrae[1], U. Rocha[3], R. Peixoto[1,2,4]

[1]*Federal University of Rio de Janeiro, Microbiology, Rio de Janeiro, Brazil*

[2]*Instituto Museu Aquario Marinho do Rio de Janeiro-AquaRio, Rio de Janeiro, Brazil*

[3]*Helmholtz Centre for Environmental Research, Department of Environmental Microbiology, Leipzig, Germany*

[4]*University of California, Genome Center, Davis, CA, United States*

Thermal-resistant corals have been reported in the Brazilian coast over the last few years. We hypothesized that Beneficial Microorganism for Corals (BMCs) play a role on conferring thermal resistance to their host. Fragments of the coral *Mussismilia hispida* were sampled from Maraú, Brazil after recovering from a massive coral bleaching event in 2016. Both affected (sensitive) and unaffected (resistant) colonies of the same coral species were collected. Coral fragments were exposed to high temperatures in two microcosm experiments, the first with resistant and the second with sensitive organisms, which started at 26ºC, moving on to high temperatures (32ºC/30,5°C, respectively) and going back to 26ºC. Coral fragments from each treatment were sampled before, during the peak of temperature and after the recovery period; and the DNA was extracted and sequencing to disentangling microbiome roles in thermal resistance. Among all physicochemical factors measured, thermal stress was the main factor in NMDS analysis promoting significant changes in the bacterial structure of both resistant and sensitive communities. No significant difference on the maximum quantum yield of the algae-associated Photosystem II (*Fv/Fm*) values and no visible signs of tissue damage were observed for resistant corals after the recovery period, while all sensitive corals bleached at 30,5 ºC. Amplicon sequencing analysis of the 16S rRNA gene showed that, even though both resistant and sensitive coral types are classified as being from the same species, the microbial community was distinct since the beginning of experiment and some bacterial taxa were identified as putative bioindicators of resistance. Alphaproteobacteria was the most abundant class among resistant indicators, sheltering ASVs from Rhizobiales, Parvularculales and Rhodospirillales orders. Metagenomic data from the beginning of the experiment and the peak of temperature, suggested proteins that are associated with thermal-resistant corals, such as cytochrome c and b complexes, zinc transport and Photosystem I and II, which may indicate the harboring of resistant species of *Symbiodinium* and functional traits related with resistance for oxidative stress. Also, 932 viral contigs were found in the metagenome, such as Caudovirales, virus order known to harbor Vibrio phages, which were more related with resistant corals and may play an important role in controlling the pathobiome.

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