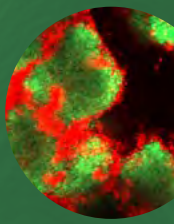
PROGRAMME

Welcome to

Bageco 2023

16th symposium on Bacterial Genetics and Ecology



26 - 30 June
Copenhagen · Denmark

www.bageco2023.org

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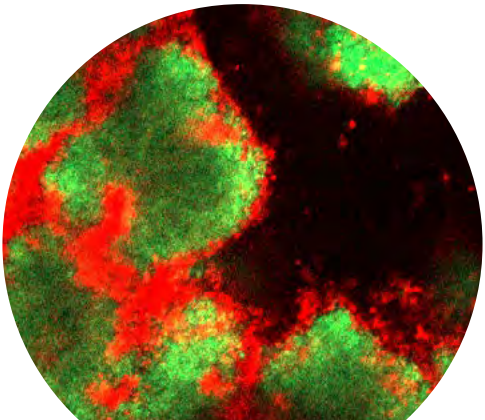

Welcome

Dear attendees,

It gives me great pleasure to welcome you all to the highly anticipated Bageco symposium 2023! This biennial symposium has gained immense recognition as a premier European conference in the realm of Bacterial genetics and ecology. Alas due to the COVID-19 pandemic, the symposium has been postponed for the last two years. Therefore, I am thrilled that we are all finally gathered here together in Copenhagen for BAGECO 2023.

The focus of this year's symposium centres on the captivating theme of "Interactions Across Microbial Ecosystems." Our speaker line-up is comprised of world-class researchers who will present their latest findings to you. BAGECO 2023 also proudly highlights a multitude of innovative oral and poster presentations by talented early-stage researchers representing over 30 countries and five continents.

It is then my hope that BAGECO will once again serve as a platform for fostering connections among leading scientists from both academia and industry. And that together, we will foster insightful discussion around current challenges and prospects so we can contribute to the broader fields of microbiology, microbial ecology, and metagenomics.

BAGECO 2023 encompasses five distinct themes:

1. Host-Microbe Symbioses
2. Microbe-Microbe Interactions
3. Horizontal Gene Transfer and Barriers
4. New Approaches/Technologies in Microbial Ecology
5. Microbes and the Green Transition

The themes are organized into 14 sessions to promote opportunities for interaction and idea exchange between fellow scientists within their own fields of interest.

Despite the packed schedule over the four symposium days, I would also encourage you to try to take the time to immerse yourself in the unique culture and vibrant atmosphere of charming and modern Copenhagen.

Once again, I extend my warmest welcome to Copenhagen and BAGECO 2023!

Sincerely,
Søren Johannes Sørensen

Organisation

Local committee

Prof. Søren J. Sørensen
Head of Section of Microbiology,
Department of Biology,
University of Copenhagen

Prof. Mette Burmølle
Section of Microbiology,
Department of Biology,
University of Copenhagen

Assistant Prof. Jonas S. Madsen
Section of Microbiology,
Department of Biology,
University of Copenhagen

Prof. Mads Albertsen
Section for Bioscience and Engineering,
Environmental Microbiology Department
of Chemistry and Bioscience,
Aalborg University

Prof. Ákos T. Kovács
Section for Microbial and Chemical
Ecology Bacterial Interactions and
Evolution, Department of Biotechnology
and Biomedicine, DTU

Scientific committee

Prof. Dr. Kornelia Smalla
Institute for Epidemiology and Pathogen
Diagnostics, Julius Kühn-Institut, Federal
Research Institute for Cultivated Plants

Prof. Dr. Gabriele Berg
Institute of Environmental Biotechnology,
Graz University of Technology

Prof. Dr. Heike Schmitt
Department of Biotechnology,
Delft University of Technology

Prof. Dr. Rodrigo Costa
Department of Bioengineering,
Instituto Superior Técnico,
University of Lisbon

Prof. Dr. Christoph C. Tebbe
Institute of Biodiversity,
Johann Heinrich von Thünen Institute,
Federal Research Institute for Rural Areas,
Forestry and Fisheries

Prof. Dr. Ines Mandic Mulec
Department of Microbiology,
The Biotechnical Faculty,
University of Ljubljana

Prof. Dr Itzhak Mizrahi
Faculty of Natural Sciences,
Ben-Gurion University of the Negev

Prof. Dr. Tim Vogel
Laboratoire Ampère Ecole Centrale de Lyon,
Université de Lyon

Prof. Dr. Stefan Bertilsson
Department of Aquatic Sciences
and Assessment; Section for Ecology
and Biodiversity, Swedish University of
Agricultural Sciences

General Information

Conference website
www.bageco2023.org

Congress venue
Maersk Tower
Blegdamsvej 3B
2200 Copenhagen
Denmark

Conference Secretariat
CAP Partner
Nordre Fasanvej 113, 2
DK-2000 Frederiksberg
Denmark
Tel.: +45 70 20 03 05
info@cap-partner.eu
www.cap-partner.eu

Badges
The congress name badges must always be worn during the congress. Access to the congress venue will not be granted without the name badge issued by the Conference Secretariat. The badges must also be worn to access the welcome reception as well as the congress dinner.

Cloak room
A cloak room located in the plenary room (Niels Jerne Auditorium) will be available throughout the conference.

Information for Speakers
Bring your presentation to the plenary room (Niels Jerne Auditorium) at the venue. A technician will help you upload the presentation to the computer.

Please make sure to upload your presentation at least 1 hours before your session starts. Please bring your presentation on a USB stick. At the end of the congress, all presentations will be deleted to secure that no copyright issues will arise.

WiFi
Free access to the WIFI at the congress venue is provided.
Network name: KU guest
Please open the browser and create an account, the password will be sent to you via E-mail.

Social events

Welcome Reception

Date 26 June 2023
Time 18.00 - 19.30
Place The conference venue, Mærsk Tower, 15th floor: udsigten

If you are registered, you can see a drinks-icon on your badge. Please remember to bring your badge to get access.

Conference Dinner

Date 29 June 2023
Time 19.00 - 01.00
Place Banegården, Otto Busses Vej 45a, 2450 Copenhagen

How to get to the dinner venue: See Google Maps

NB: The dinner ticket is not included in the registration fee. If you are registered for the dinner, you can see a small "cutlery"-icon on your badge. Please remember to bring your badge to get access.

Boat trip

Date 28 June 2023
Time 18.45 – 20.00
Place Gammel Strand boat stop opposite the parliament (Ved Stranden 26, 1061 Copenhagen)

How to get from the venue to the harbour:
See Google Maps

There are 150 free tickets, you can get these at the congress registration on a first come first served basis.

Programme

Monday, 26 June 2023	
16:00	**Registration opens**
17:00-17.15	**Opening** Søren J. Sørensen
17:15-18:00	**[K1] Opening lecture: Seeing is understanding: Next generation chemical imaging for super-fast functional analyses of microbiomes** Michael Wagner
18:00-19:30	**Welcome Reception (15th floor)**

Tuesday, 27 June 2023

Theme 1: Microbe-microbe interactions
Chairs: Ines M. Mulec and Mette Burmølle

08:00	**Registration**
	Session 1
09:00-09:35	**[K2] Multi-level selection favours the evolution of mutualistic cooperation in microbial communities** Christian Kost
09:35-09:55	**[O1] Cell-cell connection mediated by bacterial stalks facilitates metabolic cross-feeding** Miaoxiao Wang
09:55-10:15	**[O2] Succession of microbial community composition mirrors the secondary metabolite potential during marine biofilm development** Pernille Bech
10:15-10:35	**[O3] Cooperation facilitates bacterial niche expansion** Chunhui Hao
10:35-10:55	**Coffee break (1st floor, stair area)**
	Session 2
10:55-11:30	**[K3] Functional impacts of interspecies interactions in mixed biofilms** Mette Burmølle
11:30-11:50	**[O4] Pseudomonas stutzeri changes the fitness landscape of evolving Bacillus velezensis** Xinli Sun
11:50-12:10	**[O5] Nutrient-dependent interactions between Salmonella enterica serovar Typhimurium and Bacillus subtilis in biofilms** Eli Podnar
12:10-13:10	**Lunch (1st floor, stair area)**
12:20-12:50	**Symposium: Atrandi Biosciences** **Microfluidic platform for single cell metagenomic sequencing** Meilee Ling
13:10-14:10	**Postersession 1 (area around the auditorium on 1st floor)**
	Session 3
14:10-14:45	**[K4] Bacterial biodiversity drives the evolution of CRISPR-based phage resistance** Edze Westra
14:45-15:05	**[O6] Bacterial predators drive the evolution and maintenance of antibiotic resistance in complex microbial communities** Samay Pande
15:05-15:25	**[O7] Characterising active virus infection of nitrifying archaea and bacteria in soil** Graeme Nicol
15:25-15:40	**Coffee break (1st floor, stair area)**

Theme 2A: Horizontal gene transfer and barriers

Chairs: Kornelia Smalla and Jonas S. Madsen

	Session 4
15:40-16:20	**[K5] From micro to macroscales: Challenges to incorporate horizontal gene transfer into risk assessment of AMR** Heike Schmitt
16:20-16:40	**[O8] Pseudomonas plasmids revisited: new insights and comprehensive analysis of their features for horizontal gene transfer** Masaki Shintani
16:40-17:00	**[O9] β-lactamase production by gut commensals rescues sensitive bacteria and sustains microbiome diversity** Asmus Kalckar Olesen
17:00-18:00	**Postersession 2 (area around the auditorium on 1st floor)**

Wednesday, 28 June 2023

Theme 3: Host-microbe symbioses
Chairs: Itzik Mizrahi and Rodrigo Costa

08:00	**Registration**
	Session 5
09:00-09:35	**[K6] Capturing unique inter-individual features of host-microbiome interaction processes through personalization of human gut models** Tom Van de Wiele
09:35-09:55	**[O10] Digestive exophagy of bacterial biofilms by the amoeba predators histolytica and its impact on stress tolerance, antibiotic resistance, and cytotoxicity** Ilana Kolodkin-Gal
09:55-10:15	**[O11] Sex predicts gut microbiota variations in wild yellow baboons (Papio cynocephalus)** Marina Bambi
10:15-10:35	**[O12] Composting in ant-plant nests? Metabolic potential of bacterial communities for degrading chitin- and cellulose-rich substrates in ant-made patches** Verónica Barrajón-Santos
10:35-10:55	**Coffee break (1st floor, stair area)**
	Session 6
10:55-11:30	**[K7] Gut Microbial Synthetic communities to study how microbial ecologic networks collaboratively ferment glycans from diet and mucin** Clara Belzer
11:30-11:50	**[O13] Fragrant or stinky - The nasal microbiome and its role in human olfactory performance** Christina Kumpitsch
11:50-12:10	**[O14] Partners forever! A surprisingly high diversity of Acetobacter is maintained throughout long-term experimental evolution of Drosophila simulans populations.** Bosco Gracia-Alvira
12:10-13:10	**Lunch (1st floor, stair area)**
13:10-14:10	**Postersession 3 (area around the auditorium on 1st floor)**
	Session 7
14:10-14:45	**[K8] Rumen Ecosystem as a Model for Understanding Trophic Networks: A Top-Down Bottom-Up Approach to Deciphering the Microbiome-Metabolome Interplay** Itzik Mizrahi
14:45-15:05	**[O15] Predicting the stability of gut microbial communities using viral-prokaryotic genome-centric analysis machine learning in atopic eczema patients** Die Hu
15:05-15:25	**[O16] How to drive the transmission of seed bacterial communities to seedling** Alain Sarniguet
15:25-15:40	**Coffee break (1st floor, stair area)**

	Session 8: shared session of all themes
15:40-16:00	**Quantification of microbial ecology in natural communities: Pathway selection in anaerobic fermentation** Rebeca Gonzalez-Cabaleiro
16:00-16:20	**[O18] Control of Bacillus subtilis by large prophage elements – physiology, ecology and evolution perspective.** Anna Dragos
16:20-16:40	**[O19] The global ecology of archaea** Alexander Mahnert
16:40-17:00	**[O20] A previously uncharacterised hydrogenase dominates fermentation in the human gastrointestinal tract** Caitlin Welsh
17:00-18:00	**Postersession 4 (area around the auditorium on 1st floor)**
18:45-20.00	**Boat trip** Free tickets at registration on a first-come-first-serve basis

Thursday, 29 June 2023

Theme 2B: Horizontal gene transfer and barriers

Chairs: Kornelia Smalla and Jonas S. Madsen

08:00	**Registration**
	Session 9
09:00-09:35	**[K9] Breaking barriers: exploring novel immune evasion paradigms** Rafael P. Redondo
09:35-09:55	**[O21] CRISPR-Cas immunity hitchhikes with beneficial mobile genetic elements increasing the spread of drug resistance** Iolanda Domingues
09:55-10:15	**[O22] High diversity of the emerging pathogen Acinetobacter baumannii in livestock and human wastewaters** Stefanie Glaeser
10:15-10:35	**[O23] Diverse anti-defense systems in the leading region of plasmids** David Burstein
10:35-10:55	**Coffee break (1st floor, stair area)**
	Session 10
10:55-11:30	**[OK10] Differences in vertical and horizontal transmission dynamics shape plasmid distribution in clinical enterobacteria** Alvaro San Millan
11:30-11:50	**[O24] Carbapenem hyper-resistance mediated by blaNDM-1 gene adaptative amplification** Mario Pulido-Vadillo
11:50-12:10	**[O25] Anti-defense systems in archaeal viruses: predicting novel acrs and acas, redefining AcrIII-1, and perspectives on the defense landscape of archaea** Laura Martinez Alvarez
12:10-13:10	**Lunch (1st floor, stair area)**
13:10-14:10	**Postersession 5 (area around the auditorium on 1st floor)**

Theme 4: New approaches/technologies in microbial ecology
Theme 5: Microbes and the green transition
Chairs: Stefan Bertilsson, Timothy M. Vogel and Christoph C. Tebbe

	Session 11
14:10-14:45	**[K11] Disentangling plant- and environment-mediated drivers of active rhizosphere bacterial community dynamics during short-term drought** Ashley Shade
14:45-15:05	**[O26] Natural product biosynthetic potential reflects macroevolutionary diversification within a widely distributed bacterial taxon** Rodrigo Costa
15:05-15:25	**[O27] Microplastics increase the selective potential of antibiotics at sub-inhibitory concentrations** Concepcion Sanchez-Cid
15:25-15:40	**Coffee break (1st floor, stair area)**
	Session 12
16:00-16:20	**[K12] Towards more sustainable agriculture through managing soil and root-associated microbiomes** Kornelia Smalla
16:20-16:40	**[O28] The fate of pathogens, antibiotics, and resistance genes in treated wastewater irrigated soils and crops** Osnat Gillor
16:40-17:00	**[O29] The hidden effects of liming on microbial community members** Jasper Schierstaedt
17:00-18:00	**Postersession 6 (area around the auditorium on 1st floor)**
19:00-01.00	**Conference Dinner at Banegården/Copenhagen**

Friday, 30 June 2023

Theme 4: New approaches/technologies in microbial ecology
Theme 5: Microbes and the green transition

Chairs: Stefan Bertilsson, Timothy M. Vogel and Christoph C. Tebbe

08:00	**Registration**
	Session 13
09:00-09:35	**[K13] Illuminating Microbial Collectives through Transcriptome Imaging** Daniel Dar
09:35-09:55	**[O30] Improvement of hydrogen production by genetic modification of cyanobacterial strains** Galyna Kufryk
09:55-10:15	**[O31] How do two strains of lactic acid bacteria cooperate to improve soy juice fermentation?** Hélène Falentin
10:15-10:35	**[O32] De novo assembled single-cell transcriptomes from aquatic phytoflagellates reveal metabolically distinct dormant cell types** Aditya Jeevannavar
10:35-10:55	**Coffee break (1st floor, stair area)**
	Session 14
10:55-11:30	**[K14] Microbial Metabolism Under the Microscope: A Cellular View of Host-Microbe Interactions** Manuel Liebeke
11:30-11:50	**[O33] New perspectives on pangenomes, intra-species diversity and ecological and evolutionary dynamics in freshwater bacteria** Stefan Bertilsson
11:50-12:10	**[O34] Analysis of over 100,000 genome-scale metabolic reconstructions indicates a non-random distribution of tyrosine metabolic niches in the prokaryotic phylogenetic tree** Ulisses Nunes da Rocha
12:10-13:10	**Lunch (1st floor, stair area)**
13:10-14:10	**Postersession 7**
	Session 15: Shared session of all themes
14:10-14:30	**T6SS-mediated competition limits seedling transmission of Xanthomonas campestris pv. campestris and drives assembly of seed-associated bacterial communities in vitro** Tiffany Garin
14:30-14:45	**[O36] Gut microbiome functions enriched in C. elegans** Johannes Zimmermann
14:45-15:05	**[O37] Biosynthetic amplicon geneFISH for selective extraction of secondary metabolite producers from environmental microbiomes** Yannick Buijs
15:05	**Closing** Local Committee

Thematic Issue on Interactions across Microbial Ecosystems

Editors:
Søren J. Sørensen, University of Copenhagen, Denmark, sjs@bio.ku.dk
Kornelia Smalla, Julius Kühn Institute, Germany, kornelia.smalla@julius-kuehn.de
Mette Burmølle University of Copenhagen, Denmark, burmolle@bio.ku.dk
Itzhak Mizrahi, Ben-Gurion University of the Negev, Isreal, imizrahi@bgu.ac.il

We are pleased to invite you to submit your manuscripts for consideration in a special issue centered around the overall theme for BAGECO2023: Interactions across Microbial Ecosystems. This special issue aims to bring together innovative research exploring various aspects of microbial ecology and its relevance to broader scientific domains.

We welcome manuscripts encompassing any of symposium subtopics, including:

- ✔ Host-microbe interactions in animal and plant systems.
- ✔ Interactions among bacteria, viruses, and fungi.
- ✔ Horizontal gene transfer and barriers.

Recent breakthroughs in infrastructure, bioinformatic tools, and methodologies applicable to microbial ecology. Aspects of microbiology in agro- and biotechnology, with a special focus on climate change and the green transition. We encourage authors to contribute original research, reviews, or perspectives that shed light on the diverse and dynamic nature of microbial ecosystems and their impact on various scientific fields.

The thematic issue is prepared in connection with the 16th Symposium on Bacterial genetics and ecology (BAGECO) held in Copenhagen, Denmark on 26-30 June 2023. Non-attendees are welcome to submit too.

All submitted papers will be subjected to our standard independent peer-review. FEMS Microbiology Ecology will consider full length Research Papers or Mini-Reviews/ perspectives. There is no maximum length for papers, but the length should be justified by the content. Pre-submission inquiries may be directed to the Editors Veljo Kisand or Martin Hahn.

Authors should specify "Bageco 2023" in the cover letter. Accepted manuscripts will be published in regular issues of the journal and the Thematic Issue will be compiled and made available online in 2024. For instructions for authors please see the FEMS Microbiology Ecology's instructions to authors.

SUBMISSION DEADLINE: 31 October 2023

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Exhibitor directory

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Atrandi Biosciences is an emerging developer and provider of innovative microfluidic and high-throughput screening solutions. Its novel technology platform enables researchers to gain unparalleled insights into the biology of single cells, offering immediate applications in single-cell sequencing, drug and antibody iscovery, func4onal metagenomics, microbial analysis, directed evolution, and synthe4c biology. With systems installed in leading academic and commercial laboratories around the world, Atrandi Biosciences is committed to providing simple-to-adopt and easy-to-use single-cell analysis solutions that accelerate your research.

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FEMS
www.fems-microbiology.org

We are made up of an active and diverse network of around 30,000 professionals who are committed to advancing microbiology for the benefit of society in the areas of health, energy, food, materials, and the environment. Set up in 1974, today we are a growing coalition of 56 Member Societies from 40 countries. Around half of those in our network are early career researchers, and others are business partners, scientists or campaigners. Our commitment is to help support microbiologists do their work, promote the best in microbiology research and knowledge to the world, and bring microbiologists together to share that knowledge. As a not-for-profit organization, we reinvest our revenues into supporting microbiologists throughout their career, publishing and promoting scientific research in our journals and organizing events to bring scientists together. Our Members are vitally important to our work, and we seek to support them do their work to jointly advance microbiology.

ISME
www.isme-microbes.org

ISME is a non-profit association in the field of Microbial Ecology. We connect, sponsor, provide platforms & information, organize events and are the proud owners of The ISME Journal and ISME Communications - our scientific publications.

Industry symposium

Atrandi Biosciences

Microfluidic platform for single cell metagenomic sequencing

Date and time 27th June -12:20-12:50
Room Niels Jerne Auditorium
Speaker Meilee Ling, Postdoctoral researcher, Technical University of Denmark

Meilee Ling[1], Judit Szarvas[1], Baptiste Jacques Philippe Avot[1], Patrick Munk[1], Frank Møller Aarestrup[1]

[1] *National Food Institute, Technical University of Denmark*

Abstract
Single-cell metagenomic sequencing has the potential to advance our understanding of microbial communities and their roles in various biological and environmental processes. Recent advancements in the use of microfluidic devices in single-cell metagenomic has emerged as an approach for high-throughput and high-resolution analysis of individual microbial cells within complex communities. Traditional methods for isolating and sequencing individual cells often rely on laborious techniques and thus limiting the throughput of the sequencing analysis. Microfluidic devices provide a better method for automating and enabling efficient and high-throughput single-cell metagenomic sequencing.

Here I present an overview of the application of microfluidic devices in single-cell metagenomic sequencing and workflow use in high-throughput single-cell metagenomic sequencing from environmental sample. Microfluidic devices can be coupled with various downstream analysis techniques, including DNA amplification methods and sequencing platforms. With further advancements in microfluidic device technology and optimization of protocols, these combinations enable comprehensive genomic profiling of individual cells. Despite the advantages, challenges remain in the development and adoption of microfluidic devices for single-cell metagenomic sequencing. Optimization of cell capture and lysis protocols for environmental samples are areas that require continued research and improvement. Additionally, due to the unique characteristics of single-cell data from complex matrixes, custom bioinformatics pipelines and approaches tailored to specific research questions are essential to address challenges such as sparsity, amplification biases, and data heterogeneity from single cell metagenomic sequencing.

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Keynote abstracts

[K2] MULTI-LEVEL SELECTION FAVOURS THE EVOLUTION OF MUTUALISTIC COOPERATION IN MICROBIAL COMMUNITIES

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Cooperative interactions challenge evolutionary theory: why should one organism invest costly resources to benefit other individuals rather than using them to enhance its own fitness? Despite this conundrum, obligate interactions, in which two or more microorganisms exchange costly metabolites, are common in natural microbial communities. However, the factors facilitating the evolution of metabolic cooperation remain poorly understood.

We addressed this issue by experimentally coevolving two amino acid auxotrophic strains of ***Escherichia coli***, whose growth depended on a reciprocal exchange of essential amino acids. Our results show that coevolved auxotrophic genotypes rapidly evolved cooperative cross-feeding within less than 150 generations.

A more detailed analysis also revealed the ecological mechanism driving the evolution of mutualistic cooperation. Auxotrophic cells formed multicellular clusters, within which an increased cooperative investment of cells was immediately rewarded by enhanced levels of amino acids the newly emerged cooperators received in return. In addition, competition between clusters that differed in their degree of cooperativity favoured mutants that produced more amino acids at a cost to themselves. Finally, a life cycle evolved, in which cells alternated between being part of a multicellular cluster or predominantly occurring as single cells. The resulting population dynamics increased the rate of evolutionary adaptation and molecular evolution.

Together, these results show how simple interactions between two bacterial genotypes can give rise to emergent population dynamics, which in turn have profound consequences for the genotypes involved.

[K3] FUNCTIONAL IMPACTS OF INTERSPECIES INTERACTIONS IN MIXED BIOFILMS

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Biofilms are diverse, harboring multiple, interacting species. Such interspecies interactions lead to emergent properties unique to the community setting and are referred to as community intrinsic properties. We have studied biofilm interspecies interactions, and their structural and functional impacts, in a four species bacterial community composed of the soil isolates Stenotrophomonas rhizophila, Xanthomonas retroflexus, Microbacterium oxydans and Paenibacillus amylolyticus.
We have designed a 3D-printed, degradable leaf model to mimic and simplify bacterial biofilm life in nature. These artificial leaves were printed in various carbon sources, including cellobiose and hemicellulose (xylan), where we observed induced growth and biofilm formation of the mixed biofilm compared to the individual species grown in isolation. This hints towards expanded metabolic capacities of the community.
We also studied the spatial community organization on the leaves using staining and fluorescent in situ hybridization in combination with confocal microscopy.
Moreover, in a semi-solid surface model, we observed swarming behavior by one of the species, ***P. amylolyticus,*** likely due to production of surfactants. In the presence of the other community members, ***X. retroflexus*** in particular, swarming was initiated significantly sooner. Besides ***P. amylolyticus,*** other species were present at the swarm outer edges, suggesting that these get access to new niches by joining the community.
In conclusion, community intrinsic properties were shown to induce several functional capabilities of the mixed biofilm related to access to and utilization of resources, emphasizing their relevance for function and fitness of bacterial ecosystems.

[K4] BACTERIAL BIODIVERSITY DRIVES THE EVOLUTION OF CRISPR-BASED PHAGE RESISTANCE

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About half of all bacteria carry genes for CRISPR-Cas adaptive immune systems, which provide immunological memory by inserting short DNA sequences from phage and other parasitic DNA elements into CRISPR loci on the host genome. Whereas CRISPR loci evolve rapidly in natural environments, bacterial species typically evolve phage resistance by the mutation or loss of phage receptors under laboratory conditions. I will discuss how this discrepancy may in part be explained by differences in the biotic complexity of in vitro and natural environments. Specifically, by using the opportunistic pathogen Pseudomonas aeruginosa and its phage DMS3vir, we show that coexistence with other human pathogens in a synthetic community tips the balance in favor of the evolution of CRISPR-based resistance, with important knock-on effects for the virulence of P. aeruginosa. I will discuss our current understanding of the mechanisms that cause this transition from surface resistance to CRISPR immunity in a microbial community context, and the implications for the community structure.

[K5] FROM MICRO TO MACROSCALES: CHALLENGES TO INCORPORATE HORIZONTAL GENE TRANSFER INTO RISK ASSESSMENT OF AMR

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Horizontal gene transfer (HGT) can enhance the spread of antibiotic resistance genes between bacterial species, which can ultimately lead to public health risks brought about by new combinations of resistance genes with human (or animal) pathogens. The extent of HGT is difficult to quantify, and thus is the assessment of the human health risks related to exposure to antibiotic resistant bacteria which originate from HGT events. Reservoirs in which HGT between gut bacteria of human or animal origin and environmental bacteria can occur include (hospital) sewers and wastewater treatment plants as well as manure-fertilized soils. To understand in how far HGT contributes to the spread of antibiotic resistance, insight in the role of boundary parameters of the respective reservoirs, such as temperature, bacterial densities, and nutrient availability is needed. Furthermore, current HGT test systems often use conditions that are difficult to extrapolate to natural environments. Here, the application of fluorescently labelled strains can ease detection of HGT events of low frequency in conditions more similar to relevant environments. In model systems for manure-amended soils, HGT can indeed be detected. However, the time window for (observable) HGT events is limited by the survival of manure-borne donor bacteria. Both in model systems and in field studies, bacteria and resistance genes introduced to soils with manure decline after manuring events, highlighting these boundaries. Despite new developments in the detection of HGT, these processes at short time- and distance scales are still difficult to quantify in a manner that might inform human risk assessment. This is illustrated by an overview of current approaches to assess human exposure to resistant bacteria through e.g., contact with surface water on the scale of complete river catchments, or through contact with wastewater.

[K6] CAPTURING UNIQUE INTER-INDIVIDUAL FEATURES OF HOST-MICROBIOME INTERACTION PROCESSES THROUGH PERSONALIZATION OF HUMAN GUT MODELS

Tom Van de Wiele[1]

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The last decade of human microbiome research has increasingly shown its clear association with human health status. The development of in vitro technologies mimicking host-microbiome interaction processes in the lab has enabled conducting mechanistic research that complements clinical observations and unravels the putative causal role of the microbiome in health maintenance or disease etiology, progression, and aggravation. This complementary in vitro / in vivo research has also resulted in the development of precision medicine, nutraceuticals and/or live biotherapeutics as preventive or therapeutic strategies.
Yet, a somewhat difficult aspect to grasp is the large degree of interindividual variability in health effects with intervention studies often being confronted with stratification of the study cohort into responders and non-responders. This is mainly related to underlying determinants such as genetic polymorphisms, diet, disease history or, amongst others, also the microbiome.
Here, we describe how existing in vitro technologies for studying the human gut microbiome can be tailored in novel experimental setups to better capture this level of interindividual variability and investigate to what extent differences in human microbiome composition- or even better- functionality are a contributing factor to the variations in disease severity on the one hand or variations in therapeutic success on the other hand. A better understanding of what (microbiome) factors make an individual a responder or non-responder will eventually culminate in the development of more personalized medicine and dietary strategies.

[K7] GUT MICROBIAL SYNTHETIC COMMUNITIES TO STUDY HOW MICROBIAL ECOLOGIC NETWORKS COLLABORATIVELY FERMENT GLYCANS FROM DIET AND MUCIN

Clara Belzer[1]

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Glycans from our diet and gut mucin have an impact on the microbial ecologic interactions in the human gut are. It is a challenge to unravel the metabolic interactions and trophic roles of the highly complex and individual gut microbiomes. Synthetic minimal gut microbiomes are used as a tool to investigate key microbes in microbial cross-feeding and syntrophic interactions that lead to the degradation of mucin and dietary glycans. In my group we designed synthetic gut microbiomes to study adult (MDb-MM), infant (BIGSyC) and mucosal (PMDs), microbial ecologic and metabolic interactions. Our communities range from 6-16 bacterial species covering all tropic niches and cross-feeding potential within the gut environment of interest. Through 16S rRNA gene-based composition analysis, metabolite measurements, metatranscriptomics and metaproteomics we investigate community dynamics, stability, inter-species metabolic interactions and their trophic roles. Our synthetic communities co-exist in a stable state under ***in vitro*** conditions in continuous anaerobic bioreactors. This enables us to tweak ecologic parameters like carbon source and adding or leaving out key microbial species and to study resistance and resilience to such perturbations. Overall, our work provides crucial insights into the co-existence, metabolic niches, and trophic roles of key intestinal microbes in a highly dynamic and competitive in vitro ecosystem. As such we provide a model to test the effect of dietary or other changes on the gut microbiome ecologic structure and functionality.

[K8] RUMEN ECOSYSTEM AS A MODEL FOR UNDERSTANDING TROPHIC NETWORKS: A TOP-DOWN BOTTOM-UP APPROACH TO DECIPHERING THE MICROBIOME-METABOLOME INTERPLAY

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The mammalian gut microbiota is known to be instrumental in shaping the functional attributes of its host. Recent research has highlighted the significant role of gut bacterial communities in maintaining the well-being and proper function of their hosts. The rumen, a compartment in the bovine digestive tract, is an exemplary model for studying host-microbe interactions. The rumen microbiota is essential for the proper physiological development of the rumen and for the conversion of plant mass into basic food products, making it of immense importance to humans. In my lecture, I will present some of our recent findings on the rumen ecosystem and its significance in understanding the interactions between host and microbiome. I will delve into the trophic networks that exist within the rumen, discussing how a top-down bottom-up approach can be used to decipher the interplay between the rumen microbiome and metabolome.

[K9] BREAKING BARRIERS: EXPLORING NOVEL IMMUNE EVASION PARADIGMS

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Mobile genetic elements (MGEs) facilitate horizontal gene transfer among bacteria, but their replicative self-interests have a negative impact on host fitness. Consequently, bacteria have evolved diverse anti-MGE defense mechanisms, including CRISPR-Cas, a family of RNA-guided adaptive immune systems that have revolutionized gene editing. To counteract immunity, MGEs have developed small protein inhibitors called anti-CRISPRs. However, little is known about alternative inhibitory mechanisms. In my talk, I will discuss a novel anti-CRISPR strategy that is widely exploited by phages and other MGEs across bacterial taxa. Since MGEs drive horizontal gene transfer, characterizing the offense and defense mechanisms that arise in MGE-host conflicts is crucial for understanding gene flow dynamics in microbiomes.

[K10] DIFFERENCES IN VERTICAL AND HORIZONTAL TRANSMISSION DYNAMICS SHAPE PLASMID DISTRIBUTION IN CLINICAL ENTEROBACTERIA

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Conjugative plasmids can transfer both vertically and horizontally in bacterial communities, playing a key role in the dissemination of antimicrobial resistance (AMR) genes across bacterial pathogens. AMR plasmids are widespread in clinical settings, but their distribution is not random, and certain associations between plasmids and bacterial clones are particularly successful. However, the contribution of vertical and horizontal transmission dynamics to plasmid distribution and maintenance in clinically relevant bacterial communities remains poorly characterized. In this study, we used a collection of wild type enterobacteria isolated from hospitalized patients to perform a comprehensive analysis of the transmission dynamics of the carbapenem resistance plasmid pOXA-48. We combined ***in vitro*** and ***in vivo*** experimental approaches to quantify key traits responsible for vertical (AMR level) and horizontal (conjugation frequency) plasmid transmission. Our results revealed a significant degree of variability in these traits across different bacterial hosts, with ***Klebsiella*** spp. strains showing both higher pOXA-48-mediated AMR levels and higher conjugation frequencies than ***Escherichia coli*** strains. Using experimentally determined parameters, we developed a simple mathematical model to interrogate the contribution of vertical and horizontal transmission to plasmid distribution in bacterial communities. Simulations revealed that a small subset of clones, combining high vertical and horizontal plasmid transmission ability, played a critical role stabilizing the plasmid in the different polyclonal microbial communities. Taken together, our results indicate that variability in plasmid transmission dynamics dictate successful associations between plasmids and bacterial clones, shaping AMR evolution.

[K11] DISENTANGLING PLANT- AND ENVIRONMENT-MEDIATED DRIVERS OF ACTIVE RHIZOSPHERE BACTERIAL COMMUNITY DYNAMICS DURING SHORT-TERM DROUGHT

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Mitigating the effects of climate-related stress on crops is important for global food security. The microbiome associated with plant roots, the ***rhizobiome***, can harbor beneficial microbes that alleviate the impacts of stress. However, the drivers of rhizobiome recruitment during periods of stress to the plant and the environment are unclear. We hypothesize that selective activation of dormant rhizobiome members contributes to the assembly trajectory during and after stress. To address this hypothesis, we present data from an experiment greenhouse designed to assess the active rhizobiome members and their dynamics in response to short-term drought stress for two very different agricultural crops: the prospective biofuel feedstock switchgrass (***Panicum virgatum)*** and the critical food legume common bean (***Phaseolus vulgaris*** L.). We distinguished the active members of the rhizosphere that were responsive to plant cues from others that were responsive to the direct environmental changes that accompanied the stress. We propose that improving understanding of microbial activation could inform rhizobiome management to promote plant resilience and provide ideas for future research directions to maximize success in plant-microbiome intervention for crop benefit.

[K12] TOWARDS MORE SUSTAINABLE AGRICULTURE THROUGH MANAGING SOIL AND ROOT-ASSOCIATED MICROBIOMES

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Soil microorganisms are key players of soil and plant health. Several recent studies provided insights on the effects of agricultural management on soil physicochemical properties, microbiome composition and plant growth. Harnessing this potential for microbiome-based solutions might contribute to reduce agrochemical inputs and improve agricultural sustainability. At first, this requires a better understanding of factors shaping the soil and rhizosphere microbiome in agroecosystems. By using long-term field experiments located in different climatic zones, we could show that not only the site (e.g., soil type) and plant (e.g., plant species, developmental stage, cultivar) characteristics but also agricultural practices (e.g., tillage, fertilization, crop rotation) affect the structure and functionality of soil and root-associated microbiomes. This highlights the potential of agricultural practices to steer the soil microbiome into a more beneficial state supporting soil and plant health. In field experiments in Uruguay, we demonstrated that soils under conservation practices such as pasture, reduced tillage and organic fertilization exhibited a distinct microbiome, that differed from conventionally managed soils and likely contributed to the improvement of soil structure and plant yields.

Another option to manage microbiomes is to add plant-beneficial microorganisms by inoculation. I will present examples from greenhouse and field trials, where we inoculated microorganisms as single strains or as consortia. The inoculation with plant-beneficial microorganisms resulted in a modulation of the indigenous rhizosphere microbiomes which varied e.g., based on the agricultural history of the soil, and likely contributed to the inoculants' efficacy. These interdisciplinary studies under close-to-practice conditions provided important insights into a better understanding of options and the ecology of microbiome management, paving the way towards microbiome-based agricultural solutions in the future.

[K13] ILLUMINATING MICROBIAL COLLECTIVES THROUGH TRANSCRIPTOME IMAGING

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Clonal bacterial populations contain coexisting, yet phenotypically distinct sub-populations. This plasticity provides resilience to unpredictable environmental changes, such as antibiotic exposure or nutrient depletion and can also facilitate cooperative interactions via specialization in costly activities such as virulence factor production, forming an extended basis for sociality. However, the single-cell phenotypic landscape in any given bacterial species remains largely unexplored due to the technical challenges of profiling individual bacteria, particularly in spatially structured biofilms and host tissues. In my talk, I will introduce a new imaging technique called par-seqFISH (parallel and sequential RNA Fluorescence ***In Situ*** Hybridization), which enables the profiling of the expression of hundreds of genes within individual bacterial cells while preserving their spatial context in the sample. I will present our recent application of this new technology to study the opportunistic pathogen ***Pseudomonas aeruginosa*** in planktonic and biofilm modes of growth. I will also discuss how this new approach could help us reveal new insights into microbial interactions and their regulation.

[K14] MICROBIAL METABOLISM UNDER THE MICROSCOPE: A CELLULAR VIEW OF HOST-MICROBE INTERACTIONS

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Small molecules – metabolites – provide the basis for chemical interactions between hosts and microbes. Most current techniques are unable to spatially link the metabolic phenotype and genotype of microorganisms, in situ, at a scale relevant to microbial interactions.
Mass spectrometry imaging (MSI) and fluorescence microscopy are the powerful tools we use to address two technical challenges: linking metabolite production to specific organisms in mixed communities and studying spatial metabolomes of symbioses in situ. In this talk, I will focus on the potential of correlative MSI on an uncultivable, invertebrate-microbe system at the micrometer level to reveal the secret chemical life of microbes.
In our lab, we integrate chemical imaging with 3D tomography and microscopy. These combined methods provide a culture-independent approach to connect anatomical structure and metabolic function in millimeter-sized symbiotic animals. We developed a correlative imaging workflow to connect the in-situ production of metabolites with the organ-scale and cellular 3D distributions of mutualistic and pathogenic (micro)organisms in the same host animal.

Oral presentations

[O1] CELL-CELL CONNECTION MEDIATED BY BACTERIAL STALKS FACILITATES METABOLIC CROSS-FEEDING

Dr. Miaoxiao Wang[1,2], Ms Na Luo[4], Dr. Giovanni Stefano Ugolini[5], Dr. Yong Nie[3], Professor Xiao-Lei Wu[3], Professor Martin Ackermann[1,2]

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The ability of bacteria to interact and exchange metabolites with one another is crucial for their survival in changing environments. Although many of these bacterial interactions rely on passive diffusion for metabolic exchange, it is often limited by dilution and decreased efficiency over distance. Establishing direct cell-cell connections among interacting cells is thought to be one way to circumvent this problem, but we lack evidence to address how direct cell-cell connections affect metabolic interactions. In this study, we found that cells of a dimorphic prosthecate bacterium (DPB), Glycocaulis alkaliphilus 6B-8T, used their stalks for direct connection to the cells from a variety of other bacterial species, including Corynebacterium glutamicum. Although Glycocaulis cells cannot grow on glucose solely, they reproduce in co-culture using the metabolites (including nine amino acids and twelve organic acids, as revealed by metabolomic analysis) secreted by Corynebacterium, establishing a cross-feeding interaction. Through quantitative single-cell level experiments in microfluidics, we revealed that this stalk-dependent adhesion could facilitate the metabolic cross-feeding by shortening the diffusion distance of the metabolites. Moreover, the metabolites can be directly transferred through the stalks, preventing the nutrient waste occurred in passive diffusion. Our further analysis shows that a variety of DPBs possess the ability to use their stalks to connect with other bacterial species, which facilitates their interactions. These DPBs belong to specific taxa that are different from Caulobacteraceae and Hyphomonadaceae, providing a novel perspective on the lifestyles of DPBs. Together, our findings highlight the importance of direct cell-cell connections in enhancing metabolic interactions and shed light on the mechanisms that support the survival and interactions of bacterial communities in changing environments.

[O2] SUCCESSION OF MICROBIAL COMMUNITY COMPOSITION MIRRORS THE SECONDARY METABOLITE POTENTIAL DURING MARINE BIOFILM DEVELOPMENT

PhD Pernille Bech[1], PhD Scott Alexander Jarmusch[1], PhD Jacob Agerbo Rasmussen[2], Professor Lone Gram[1], PhD Nathalie Nina Suhr Eiris Henriksen[1]

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In nature, secondary metabolites allow microorganisms to interact with other community members, however, the degree to which community dynamics can be linked to secondary metabolite potential remains largely unknown. In this study, we address a fundamental problem in microbial community ecology, namely the interplay between community succession and secondary metabolism variation. We used 16S-18S-rRNA gene and adenylation domain amplicon sequencing, genome-resolved metagenomics, and untargeted metabolomics to track the taxons, biosynthetic gene clusters (BGCs) and metabolome dynamics in situ of microorganisms during marine biofilm succession over 113 days. Two succession phases were identified during the community succession, with a clear successional shift around day 29, where the alkaloid quorum sensing related molecules, pseudanes also were detected. The microbial secondary metabolite potential changed between the succession phases and only a few community members, including Myxococotta spp, were responsible for the majority of the BGC potential in the early succession phase. In the late succession, bryozoans and benthic copepoda arrived, the microbial non-ribosomal peptide (NRP) potential drastically decreased as a result of the elimination of the prolific secondary metabolite producers. Conclusively, this study suggests that the early succession of the marine biofilm community favors prokaryotes with high NRPS potential, while the late succession is dominated by multicellular eukaryotes and a reduction in bacterial NRPS potential.

[O3] COOPERATION FACILITATES BACTERIAL NICHE EXPANSION

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Ecologists are often fascinated by the impressive adaptability of bacteria to different environments. While habitat generalists, which can survive in many different environments, are thought to have unique characteristics when compared to habitat specialists with limited distribution, the exact mechanisms responsible for these distinctions are still unknown. One proposed mechanism for the evolution of generalists is bacterial cooperation, as this behaviour can provide bacteria with additional benefits when adapting to multiple distinct habitats. Despite the prevalence of this hypothesis, no one has yet conducted a thorough investigation. In this study, we utilized comparative genomics approaches across approximately 30,000 bacterial species to investigate the role of bacterial cooperation in the transition from habitat specialists to generalists. Our findings revealed that (1) generalists carry more cooperative genes than specialists and (2) cooperative genes are more likely to be accessory genes in the microbial pangenomes, suggesting that these genes may frequently undergo gain and loss during evolution. We then investigated the causality of the correlation by examining whether cooperation enables the expansion of bacterial niches, or if it's the other way around, with niche expansion promoting the acquisition of cooperative genes. Our use of ancestral state reconstruction provided supportive evidence that the acquisition of cooperative genes precedes the niche expansion of bacteria, further highlighting the facilitating role of cooperation in bacterial adaptation to multiple habitats.

[O4] PSEUDOMONAS STUTZERI CHANGES THE FITNESS LANDSCAPE OF EVOLVING BACILLUS VELEZENSIS

Dr Xinli Sun[1], Zhihui Xu[1], Guohai Hu[2], Jiyu Xie[1], Ruifu Zhang[1], Qirong Shen[1], Ákos Kovács[2]

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Bacterial interaction can alter community structure, yet the mechanisms underpinning this effect remain unknown. Here, we investigate how bacterial interaction influences the evolution of phenotypic and genetic diversity. Bacillus velezensis SQR9 was allowed to evolve alone and in the presence of a partner species Pseudomonas stutzeri XL272 under biofilm condition. B. velezensis rapidly diversified into three ecotypes under both conditions but the frequency varied. These ecotypes displayed phenotypic trade-off among biofilm formation, swarming motility, free-living growing capacity, and exopolysaccharide production. Whole genome re-sequencing correlated these phenotypic changes with certain mutations, including ywcC, comP, degS, swrA and spo0F. In contrast, P. stutzeri displayed no observable morphotype change when co-evolving with B. velezensis, but the parallel evolution in an unknown gene encoding capsular biosynthesis protein increased its exopolysaccharide production in coculture. The evolved P. stutzeri variants altered the fitness landscape of B. velezensis ecotypes, allowing a cheater ecotype to outcompete the matrix-producing ecotypes. These findings are in accordance with the “Black Queen Hypothesis”: adaptive function loss that occurs through the reliance on a partner species.

[O5] NUTRIENT-DEPENDENT INTERACTIONS BETWEEN SALMONELLA ENTERICA SEROVAR TYPHIMURIUM AND BACILLUS SUBTILIS IN BIOFILMS

Eli Podnar[1], Dr. Eva Kovačec[1], Dr. Tjaša Danevčič[1], Dr. Bram Lories[2], prof. dr. Hans Steenackers[2], prof. dr. Ines Mandic-Mulec[1]

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Salmonella enterica serovar Typhimurium is one of the most common foodborne pathogens affecting both humans and animals. Salmonella is ubiquitous and can also be present in nutrient-poor environments outside the host. Due to the spread of antibiotic resistance, new strategies are needed to limit foodborne pathogens. One of the approaches involves the antagonistic properties of probiotic bacteria such as Bacillus subtilis. The vast majority of the literature dealing with B. subtilis-Salmonella interactions is conducted in broilers, and although these studies are essential for the development of probiotics, they do not address the mechanisms mediating pathogen-probiotic interactions not how probiotics affect pathogen's biofilm.

We tested the competition between the two bacteria, B. subtilis PS-216 and S. Typhimurium SL1344, under different environmental conditions focusing on nutrient availability. The results show that under nutrient-rich conditions B. subtilis PS-216 inhibits the growth of S. Typhimurium SL1344 and reduces its biofilm thickness. The antagonistic potential of B. subtilis is greatly impaired in a mutant with an inactive pks operon, which is responsible for the synthesis of the secondary metabolite bacillaene and acts as an antagonist against S. Typhimurium. In addition, the presence of S. Typhimurium in the coculture increased the activity of the PpksC promoter, which controls bacillaene production, suggesting that B. subtilis senses and responds to a Gram-negative competitor. In nutrient-depleted conditions B. subtilis lost its antagonistic effect and entered sporulation, but in coculture S. Typhimurium inhibited spore formation of its competitor. The results show that the two bacteria must be in direct cell-to-cell contact for inhibition to occur and that the sporulation inhibition rate depends on the population density of S. Typhimurium. Moreover, preliminary results show that upon direct contact, Salmonella uses type 6 secretion system and triggers sigma B-dependent stress response in B. subtilis.

The work reveals molecular determinants of the competition and its tight dependence on cell-cell contact and environmental conditions, highlighting the importance of evaluating probiotic strains against pathogens under conditions relevant to the intended use.

[O6] BACTERIAL PREDATORS DRIVE THE EVOLUTION AND MAINTENANCE OF ANTIBIOTIC RESISTANCE IN COMPLEX MICROBIAL COMMUNITIES

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Bacteria living in natural complex microbial communities use a variety of contact-dependent and contact-independent mechanisms of antagonism. Survival in such communities, therefore, requires resistance to the antimicrobial mechanisms expressed by the antagonists. Since microbial predators such as Myxococcus xanthus use a variety of antimicrobial mechanisms, we hypothesized that the arms-race between antimicrobial mechanisms (expressed by M. xanthus) and resistance to such mechanisms (in non-myxobacterial species) might be prevalent in nature. In line with our hypothesis, we demonstrate that the presence of M. xanthus in soil samples is responsible for the abundance of antibiotic resistance. Interestingly, only M. xanthus isolates that can invade natural social communities can drive the enrichment of antibiotic resistance. Further, we demonstrate that the observed effect of M. xanthus on the prevalence of antibiotic-resistant isolates is driven by the release of a growth-inhibitory cocktail by the rapidly dying population of M. xanthus. Together, we demonstrate that the presence of a generalist microbial predator M. xanthus has an influence on the maintenance of antibiotic resistance in natural microbial communities.

[O7] CHARACTERIZING ACTIVE VIRUS INFECTION OF NITRIFYING ARCHAEA AND BACTERIA IN SOIL

Dr Sungeun Lee[1], Dr Christina Hazard[1], **Professor Graeme Nicol[1]**

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While the complexity of prokaryotic communities in soil is relatively well understood, we are currently ignorant of the role of viruses in influencing their ecology. In marine systems, viruses play a major role in killing cells and controlling population sizes, augmenting function through the transfer of auxiliary metabolic genes (AMGs) and influencing fluxes of carbon and nitrogen from organic to dissolved inorganic pools. In comparison, there remains a paucity of studies that characterize the impact of viruses on hosts in soil and impacts on biogeochemistry and rates of ecosystem processes. Microbially-mediated nitrification is central to nitrogen losses and emissions of nitrous oxide from soil, contributing to global warming and stratospheric ozone depletion. As nitrification is performed by taxonomically and functionally restricted groups of autotrophic organisms, nitrifiers have become an established model group for linking diversity, evolution, ecophysiology, and function in soil. However, the diversity and impact of virus infection on these organisms are unknown. To identify active interactions between individual host and viruses, a first approach followed the transfer of assimilated carbon from autotrophic prokaryotes to viruses. Specifically, microcosms were amended with urea and $^{13}CO_2$ followed by DNA stable isotope probing (DNA-SIP) and metagenomic analysis. Hybrid analysis of GC mol% fractionation and DNA-SIP resulted in the identification of viruses of active ammonia oxidizing archaea (AOA) only, but which represented a novel virus lineage distinct from previously described families. To enable characterization of viruses also infecting ammonia- and nitrite oxidizing bacteria (AOB, NOB), a second approach used incubations followed by filtration of virus-like particles to increase the recovery of virus metagenomes. This included differential inhibition of specific nitrifier groups with DMPP, 1-octyne or acetylene (as a control) to alleviate competition and increase the abundance of viruses infecting non-inhibited groups. This approach dramatically increased the recovery of high-quality virus genome sequences, with 225 virus contigs associated with those specifically infecting AOA, AOB or NOB, including 69 complete or near-complete virus genomes ranging in size between 34 and 212 kb, all representing novel virus families. Soil AOA viruses did not contain AMGs associated with ammonia oxidation, as found typically in marine systems, but were enriched in multicopper oxidase genes potentially involved in copper sequestration. In summary, these data demonstrate that virus infection of nitrifiers is a dynamic process during soil nitrification, and that targeting a specific process is a useful approach for characterizing novel soil viruses with high resolution.

[O8] PSEUDOMONAS PLASMIDS REVISITED: NEW INSIGHTS AND COMPREHENSIVE ANALYSIS OF THEIR FEATURES FOR HORIZONTAL GENE TRANSFER

Ph.D. Masaki Shintani[1,2], Ph.D. Haruo Suzuki[3], Ph.D. Hideaki Nojiri[4], Ph.D. Masato Suzuki[5]

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Plasmid-mediated dissemination of antimicrobial resistance genes (ARGs) among gram-negative bacterial pathogens, including Pseudomonas aeruginosa, poses a global public health threat and plasmid classification in the past needs to be clarified for the future. Plasmid incompatibility (Inc) groups IncP-1 to IncP-14 were proposed for R factors carrying ARGs identified in Pseudomonas in 1970-80s. However, a little sequence-based analysis has been performed for IncP-2, P-5, P-11, P-12, and P-13 plasmids. Here, the complete nucleotide sequences of the archetype plasmids, Rms139 (IncP-2), Rms163 (IncP-5), RP1-1 (IncP-11), R716 (IncP-12), and pMG26 (IncP-13), were determined. In addition, several plasmids in novel subgroups of IncP-1 were collected from different environments, and their sequences were also determined. The gene encoding replication initiation protein (RIP) of each plasmid other than IncP-1 plasmids was predicted based on BLAST search using the amino acid sequences or AlphaFold 2-mediated protein structure models, and then origin of vegetative replication (oriV) sequences were manually predicted. Mini-plasmids with each IncP-2, P-5, P-11, and P-12 RIP and oriV sequence were successfully replicated in P. aeruginosa. On the other hand, pMG26 (IncP-13) was integrated in the chromosome and no RIP gene was found within the region. PCR analyses and conjugation assays showed that pMG26 is not an extrachromosomal element (plasmid) but an integrative and conjugative element (ICE). Different plasmids carrying ARGs belonging to some novel subgroups of IncP-1 group were found, whose host ranges were different from those of known plasmids, showing wide diversity of IncP/P-1 plasmids1. All these elements were shown to be conjugative and important for horizontal gene transfer of ARGs. Plasmids carrying the RIP gene sequences specific to each Inc group were collected from the public plasmid database, and a pangenome analysis of each element was performed to reveal core and non-core genes, including ARGs. This study provides an updated understanding of Pseudomonas plasmids for the clinical and environmental isolates, including multidrug-resistant of Pseudomonas species.

1. Hayakawa et al., 2022, Appl. Environ. Microbiol. 88, e0111422

[O9] B-LACTAMASE PRODUCTION BY GUT COMMENSALS RESCUES SENSITIVE BACTERIA AND SUSTAINS MICROBIOME DIVERSITY

Phd Asmus Kalckar Olesen[1,3], Qinqin Wang[1,3], Sandra Sanchez[2], Shaodong Wei[2], Rafael Pinilla-Redondo[1], Lorrie Maccario[1], Qiqi Fu[1], Martin Ian Bahl[2], Jonas Stenløkke Madsen[1], Søren Johannes Sørensen[1]

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It is well established that antibiotic treatment disturbs the micro-ecological balance of the gut microbiome. However, little is known about microbial responses to antibiotic stress and cooperative resistance among bacteria in the gut. Here, we used Wistar rat models to investigate the effects of β-lactamase expression by a commensal strain, Escherichia coli Nissle 1917, on the gut microbiome, during antibiotic treatment with amoxicillin. We found that the presence of β-lactamase producers, during amoxicillin treatment, significantly decreased the reduction of phylogenetic and observed diversity, in the microbial gut community. Amoxicillin treatment strongly affected the phylogenetic composition of the gut community; however, the β-lactamase producers reduced the amoxicillin disruption of the phylogenetic composition in the gut microbial community. Notably, we report that the copy number of antibiotic resistance genes was positively correlated with the rescue of diversity in the microbiome, revealing that β-lactamases facilitate cooperative resistance within the microbiome in vivo. Moreover, our experimental results show that cooperative resistance aids in the restoration of microbiomes post-antibiotic treatment.

[O10] DIGESTIVE EXOPHAGY OF BACTERIAL BIOFILMS BY THE AMOEBA PREDATORS HISTOLYTICA AND ITS IMPACT ON STRESS TOLERANCE, ANTIBIOTIC RESISTANCE, AND CYTOTOXICITY

Eva Zanditenas[1], **Dr. Ilana Kolodkin-Gal[2]**, Prof. Serge Ankri[1]

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The human protozoan parasite Entamoeba histolytica is responsible for amebiasis, a disease endemic to developing countries. E. histolytica trophozoites colonize the large intestine, primarily feeding on bacterial cells. However, bacterial cells can form aggregates or structured communities that are too large for phagocytosis. Mapping the transcriptome of trophozoites interacting with probiotic Bacillus subtilis biofilms or their planktonic counterparts revealed differences in trophozoite gene expression. Biofilm-induced genes allow the trophozoite to invade and degrade bacterial biofilms. Biofilm-parasite interactions enhance Reactive Oxygen Species (ROS) tolerance and regulate the parasites' virulence while altering the biofilm cells' antibiotic tolerance. The specific activation of matrix-degrading enzymes in response to the microbial biofilm strongly suggests that amoeba is adapted to biofilm prey and may serve as a new unexplored reservoir of novel therapeutic approaches to treat biofilms. Furthermore, our findings here show that B. subtilis biofilm may serve as a protective shield for mammalian cells, hindering the progression of the parasite toward them. Biofilm-amoeba interactions are revealed as significant regulators of the parasite's stress tolerance and pathogenicity.

[O11] SEX PREDICTS GUT MICROBIOTA VARIATIONS IN WILD YELLOW BABOONS (PAPIO CYNOCEPHALUS)

Master's degree Marina Bambi[1], PhD Giulio Galla[2], PhD Claudio Donati[2], PhD Francesco Rovero[1], PhD Heidi Christine Hauffe[2], PhD Claudia Barelli[1], PhD Matthias Uwe Scholz[3]

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The role of gut microbiota diversity in animal ecology and conservation has become a key topic, especially since the contribution of these bacterial and fungal communities to host growth and health has been recently recognized. Most investigations in wildlife have focused on the study of extrinsic (e.g., diet, habitat) rather than intrinsic factors (e.g., sex, genetic background) affecting variation in animal gut communities. However, since male and female mammals often differ in biological traits and functional needs, sex is likely to play a major role in gut microbiota variation. Here, we evaluated if and how sex is associated with the gut microbiota richness and composition of wild yellow baboons (Papio cynocephalus) living in two habitat types, protected and unprotected forests of the Udzungwa Mountains in Tanzania. To understand whether sex and habitat type affect gut microbiota variation, we determined the sex of 34 yellow baboons (19 females and 15 males) from fecal pellets collected non-invasively using two marker genes (SRY and DDX3X). We then combined these results with amplicon sequencing datasets focusing on bacterial (V3-V4 region of the 16S rRNA gene) and fungal (ITS1-ITS2) communities of the same pellets. We found that females had gut microbiotas with a higher bacterial richness [Kruskal test; Shannon (alpha diversity): P = 0.010] and different composition [ANOVA; weighted Unifrac (beta diversity): P = 0.030] compared to males, in agreement with the strong morphological and behavioral dimorphisms shown between sexes of this species. Furthermore, forest type had a greater impact on females than males, such that the gut microbiotas of females from the two forests differed significantly in fungal composition [pairwise adonis test; Bray-Curtis: P = 0.02] and bacterial richness [pairwise Wilcoxon Rank Sum test; Shannon: P = 0.023], while those of males did not. These results indicated that the impact of habitat disturbance varied with sex, suggesting that intrinsic biological factors should be carefully considered when investigating wild animal biodiversity at any scale, and that such intraspecific variation could impact the outcome of conservation actions. However, research on the metabolic pathways, through shotgun sequencing, are encouraged to verify whether greater gut bacterial richness, such as those observed in baboon females, may translate into a greater diversity of metabolic functions.

[O12] COMPOSTING IN ANT-PLANT NESTS? METABOLIC POTENTIAL OF BACTERIAL COMMUNITIES FOR DEGRADING CHITIN- AND CELLULOSE-RICH SUBSTRATES IN ANT-MADE PATCHES

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Ant-plant associations have recently emerged from bipartite relationships to multi-species networks involving many different organisms. One of the most widespread associations in the Neotropics is the pioneer tree Cecropia spp. and its partner Azteca spp. ants. Like in other tropical ant-plant mutualisms, fungi, bacteria, and nematodes are typically found in domatia of Azteca ants, and especially abundant in ant-made specialized structures named as “patches”. Since Azteca ant workers continuously deposit cellulose- and chitin-rich substrates to the patches, we hypothesize that these structures serve as nutrient recycling spots like an in-situ farming compost. In fact, cellulose and chitin degradation activity was recently detected in patches from the Azteca-Cecropia complex. However, which specific microbial taxa are contributing to the decomposition of these compounds remains unknown. To investigate the metabolic potential of patch microbial communities for degrading such biopolymers, we analyzed short- and long-reads metagenomics data from patches of 10 Azteca ant colonies. After combining different assembly methods, we obtained 200 bacterial MAGs (>80% completeness, <5% contamination) belonging to 21 different taxonomic classes out of the 34 classes that were detected as relative-read abundant (> 0.1% relative abundance) in the unassembled metagenomes. By identifying genes involved in the degradation process of each biopolymer in these MAGs, we were able to determine which bacterial taxa are potentially involved in nutrient transformation in the ant-made patches. This study provides the baseline for future investigations elucidating the potential roles and ecological relationships of bacterial communities in this complex multipartite association, which will increase our understanding of the potential nutrient recycling functions of patches in ant-plant nests.

[O13] FRAGRANT OR STINKY - THE NASAL MICROBIOME AND ITS ROLE IN HUMAN OLFACTORY PERFORMANCE

PhD Christina Kumpitsch[1], Dr. Florian Ph. S. Fischmeister[2], Dr. Sonja Lackner[3], Dr. Sandra Holasek[3], Dr. Tobias Madl[4], Dr. Hansjörg Habisch[4], Dr. Axel Wolf[5], Dr. Veronika Schöpf[6], Dr. Christine Moissl-Eichinger[1]

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Background:
The ability to smell is mediated by olfactory neurons in the ceiling of the nose, which is inhabited by numerous microorganisms. In general, microbes are intertwined with human health and disease by communicating with human body cells (1,2).
The disability to smell (dysosmia) is an incisive event, caused by mechanical impact, infection, or disease, or occurs subtly during ageing. It affects psychological, social, and behavioral performance and impacts the quality of life tremendously (3,4).
The microbiome of people suffering from dysosmia have higher levels of gut-associate butyrate producers (e.g. Faecalibacterium) adjacent to the olfactory area indicating a negative effect of the strong and unpleasant smelling butyrate on olfactory function (5).

Objectives:
We assume that the nasal microbiome contributes to the olfactory capability in humans. Therefore, our aim in this study was to identify and understand the role of the microbiome in olfaction to increase therapeutic opportunities and by that reduce the burden of olfactory dysfunction and increase life quality again.

Methods:
This study compared the nasal and stool microbiome of normosmics (normal olfaction) and dysosmics (impaired olfaction) via 16S rRNA gene sequencing. Specific samples were selected for metatranscriptomic and metabolomic analyses. Additionally, these microbiome measures were correlated with dietary parameters and inflammatory biomarkers to further investigate the interaction of the nasal microbiome with olfactory performance.

Results:
Participants with reduced olfactory performance (dysosmics) had a higher microbial load adjacent to the olfactory mucosa (nose) compared to normosmics. This excess microbial material appears to consist of dead biomaterial.
Contrary to the assumptions of our pilot study (5), the dead material did not originate from the intestine but from oralization (e.g., Fusobacterium, Porphyromonas) of the nasal cavity or from the external environment. Also, the Methanobrevibacter signatures found in the nose did not belong to the same clade as those observed in the feces.
In general, the normosmics tended to have a “healthier” diet compared to dysosmics,

including less meat and more omega-3 fatty acids.

Conclusion:
The higher amount of dead microbial material with more microbial signatures associated with oral locations suggests an impaired mucociliary clearance. Additionally, olfaction is connected to the sense of taste, hence the dietary behavior is affected by olfactory dysfunction and influences the gut microbiome.
Future studies should therefore consider the naso-oral axis as well as potential effects of olfactory dysfunction on the stool microbiome.

[1] Sahin-Yilmaz et al.,2011; DOI:10.1513/pats.201007-050RN
[2] Tizard et al.,2021; DOI:10.1017/S1466252320000262
[3] Boesveldt et al.,2017; DOI: 10.1093/chemse/bjx025
[4] Croy et al.,2014; DOI:10.1093/chemse/bjt072
[5] Koskinen et al.,2018; DOI:10.1038/s41598-018-19438-3

[O14] PARTNERS FOREVER! A SURPRISINGLY HIGH DIVERSITY OF ACETOBACTER IS MAINTAINED THROUGHOUT LONG-TERM EXPERIMENTAL EVOLUTION OF DROSOPHILA SIMULANS POPULATIONS.

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The microbiome has a strong impact on the physiology and development of many animals, including Drosophila flies. In Drosophilids, the host and bacterial symbionts participate in a complex interplay: bacteria modulate host nutrition and behaviour, but at the same time their growth is regulated by the host's immune system. The microbial community associated with Drosophila is composed mainly of species from the families Lactobacillaceae, Acetobacteraceae and Enterobacteriaceae. Most of the research has focused on the relative abundance of higher taxonomy levels, but little is known about the inter- and intra-species dynamics between the co-occurring taxa of the community. To highlight the importance of intra-family diversity, we make use of 20 Drosophila simulans populations that have adapted independently to two novel laboratory temperature regimes for more than 100 generations. In order to study how the microbial community in each fly population has evolved throughout the experiment, we combine time-series 16S rRNA amplicon sequencing, metagenomic data and whole genome sequencing of individually cultured strains. We report that at least five Acetobacter species co-occur in the populations since the beginning of the experimental evolution. For one of the species, Acetobacter indonesiensis, we found two diverged clades in the same fly population. The long-term maintenance of this unexpected high intra- and inter-specific diversity of Acetic Acid Bacteria species in laboratory-raised Drosophila suggests niche partitioning in the host. We will show how genome-resolved metagenomics and in vivo experiments with gnobiotic flies can disentangle the functional differences between the Acetobacter taxa, the mechanisms by which this diversity is maintained in the host.

[O15] PREDICTING THE STABILITY OF GUT MICROBIAL COMMUNITIES USING VIRAL-PROKARYOTIC GENOME-CENTRIC ANALYSIS MACHINE LEARNING IN ATOPIC ECZEMA PATIENTS

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Aims: Beta-diversity dispersion based on Bray-Curtis distances of a set of samples calculated using 16S amplicon sequencing data can indicate the stability in microbial communities. We hypothesize that a genome-centric analysis of the biodiversity of viruses and prokaryotes can predict the stability in the gut microbiome of children with and without atopic eczema (AE). Methods: Bray-Curtis distances to centroids of two 10-year-old children's groups (AE: 17 children with AE; non-AE: 13 healthy children) were calculated. We also sequenced metagenomes from the gut DNA of the two groups to test our hypothesis. After, we recovered metagenome-assembled genomes (MAGs) and uncultivated virus genomes (UViGs) using MuDoGeR. We generated 33 new samples to balance our dataset using Synthetic Minority Oversampling Technique (SMOTE). We predicted the stability of the community using a random forest regression model (RF) based on BrayCurtis distances to centroids (target) and MAG and UViG coverages (features). We evaluated our RF model's accuracy using the root mean squared errors (RMSE) and R2 from a linear curve of the observed and predicted values. Results: The stability of the gut microbial community in non-AE was higher than that observed for the AE children based on the average higher interquartile ranges of Bray-Curtis's distance of each group's samples to their centroids (t test, $p < 0.05$). 2255 MAGs and 2024 UViGs recovered from our metagenome dataset. The MAGs were affiliated with 11 phyla. UViG Taxonomic analysis indicated 1633 UViGs affiliated with the Phylum Uroviricota; however, 391 UViGs were not affiliated with any known taxa. The 0.075 RMSE and 0.799 R2 of our RF showed that our bioindicators were good predictors of stability in the gut microbial communities. We identified 24 MAGs and 59 UViGs as AE bioindicators of community stability using the RF's mean decrease Gini coefficient analysis. These 24 MAGs belonged to Firmicutes A (17), Bacteroidota (4), Actinobacteriota (3). The 59 UViGs were distributed to 10 families, the most dominant being Peduoviridae (26). Most of these UViGs (46) were temperate viruses, and we could assign hosts for 47 UViGs, from which 5 are also bioindicators (4 Clostridia, 1 Bacteroidia). Conclusions: Our study demonstrated that the gut microbiome of children with AE is a good model for exploring analysis stability in human gut microbiomes. Our bioindicators may be used to define omics-based diagnostic tools for AE, and an in-depth analysis of their genetic potential may open doors for novel microbiome-based treatments for AE.

[O16] HOW TO DRIVE THE TRANSMISSION OF SEED BACTERIAL COMMUNITIES TO SEEDLING

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Seeds represent the starting point of plant microbiota assembly, which includes commensals, pathogens and beneficial microbes. The seed is therefore a key vector organ for plant health and protection. However, harnessing beneficial plant-microbiota interactions requires exploring and understanding the processes involved in microbiota assembly on seeds and its modifications during germination and plant emergence.
A prerequisite was to describe the importance of seed microbiota in a large-scale meta-analysis across 50 plant species that shows a core microbiota composed of 30 microbial taxa and a very diverse flexible microbiota (Simonin et al. 2022). However, our results suggest that the most abundant seed microorganisms are not necessarily the ones that have the highest transmission success to seedlings. Our studies on bean, radish and rapeseed show that plants first recruit rare bacterial taxa from soil and seed (Rochefort et al 2019, Chesneau 2022). To better understand the transmission and effects of seed-borne taxa, we then performed inoculations of seed-borne bacterial synthetic communities (SynComs) on seeds. These experiments demonstrated a phylogenetic signal associated with the transmission success of strains from seeds to seedlings and the important role of mass effects. Additionally, we observed a strong influence of the composition of the initial SynCom on transmission success due to biotic interactions. SynCom inoculations also led to a modulation of various plant phenotypes, including emergence rates, seedling development and impact of a soil-borne pathogenic fungus. Altogether, we present evidence of the feasibility and potential of plant microbiota engineering using seed borne bacterial SynComs inoculated on seeds.

- Chesneau et al. 2022 https://doi.org/10.1128/mbio.01648-22
- Rochefort et al. 2019 https://doi.org/10.1128/mSystems.00446-21
- Simonin et al. 2022 https://doi.org/10.1111/nph.18037

[O17] QUANTIFICATION OF MICROBIAL ECOLOGY IN NATURAL COMMUNITIES: PATHWAY SELECTION IN ANAEROBIC FERMENTATION

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Under anaerobic fermentative conditions, environmental limitations enforce strong selective pressures that constrain microbial growth. This obligates microorganisms to be highly efficient energy scavengers. Although carbon influx is generally high in natural or engineered fermentative systems, the possibilities for energy harvesting (ATP production) are limited in absence of external electron acceptor. Under these conditions, the energy remains in the products formed, which can be seen as an opportunity for sustainable production of valuable chemicals. Moreover, under these strong selective pressures, microbial communities seem to increase their metabolic heterogeneity, selecting short pathways that fulfil the available thermodynamic niches. In theory, this creates an opportunity for engineering, directing product selection by controlling the operational variables. But it can also help fundamental quantification of microbial ecology, as this selection returns metabolic stoichiometries that are consistently observed. Quantification of ecology and inter/intra- species interactions remain as fundamental challenges in microbiology. Explaining the strong selection of the metabolic activities observed for anaerobic fermentative microbial populations, might help to unveil the ecological rules that govern the assembly of communities. In previous work, I demonstrated that fermentative pathways selection is driven by maximization of the energy available per electron transferred in the involved redox reactions that form a specific metabolic activity. Considering that higher driving forces (dissipated energy) reduce the overall enzymatic cost of a catabolic activity, and with the objective to deepen these preliminary analyses, I have developed a mathematical model for quantification of driving forces and ATP production of anaerobic fermentative pathways. This optimization tool, by definition of electron carrier ratios and conserved moieties, predicts the intracellular metabolite concentrations required to maintain cellular survival while running a specific metabolic activity. I have used this tool to analyze the catabolic process of glucose fermentation to acetic and butyric acids. This is the most common metabolic step in the acidogenic process of anaerobic fermentation of carboxylates. Considering the ubiquity of electron bi- and co-furcation in fermentation metabolisms, I have analyzed all theoretically possible stoichiometries, ranging from solely producing acetic or butyric acid to all possible combinations that enable a closed electron balance. The results show that the experimentally observed stoichiometry, equimolar production of the acids per 1.5 moles of glucose, corresponds to the stoichiometry that ensures the higher driving force meanwhile maintaining the levels of ATP production. These results suggest that observed catabolic stoichiometries are strongly selected by maximization of energy harvested and minimization of enzymatic costs.

[O18] CONTROL OF BACILLUS SUBTILIS BY LARGE PROPHAGE ELEMENTS – PHYSIOLOGY, ECOLOGY, AND EVOLUTION PERSPECTIVE

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Aim: Phages are viruses that infect and kill bacteria, but some of them also integrate into bacterial chromosome and establish long-term reproductive alliance with their host, when they can profoundly impact bacterial physiology, ecology, and evolution. Certain bacterial species, like Bacillus subtilis, are exceptionally rich in prophage elements. Some of these phages, so called SPbetaviruses, characterize with regulatory switch lifestyle and rather large genomes (~130 kB) thereby with high potential for host control. Our aim is to understand how SPbetaviruses control bacterial physiology, interactions and evolution.
Methods: We screen publicly available, complete genomes of B. subtilis for presence of SPbetaviruses using prophage-prediction tools. We isolate diverse representatives of SPbetaviruses from their native hosts and use them to lysogenize model host strain to further access phage impact on B. subtilis. We work with available transcriptomics data to extract growth conditions where host control by phage may manifest, or phage genes which are the most relevant for such control. We also use experimental evolution, phenotypic assays, interbacterial competition assays and genetic engineering, to address mechanisms of host control by phage.
Results: Genomic analysis reveals that SPbetaviruses are abundant, as they are present in roughly 40% of B. subtilis isolates. Transcriptomics data suggest that certain phage genes are silent during lytic cycle and activate only under specific growth conditions, pointing out towards physiological states of the host that could be controlled by the prophage. We also showed that evolution under sporulation selection regime promotes recombination of SPβ with a low-copy number phi3Ts (also SPbetavirus) 'hitchhiking' in certain B. subtilis lab isolates. This recombination results in spontaneous induction of lytic cycle, allowing the chimera phages to prey on B. subtilis ancestral strain. Further research with isogenic bacterial host lysogenized with different SPbetaviruses, demonstrates strong impact of these phages on physiology and ecology of their host species, modifying features such as growth rate, cell shape, sporulation dynamics or bacteriocin production.
Conclusions: We believe that SPbetaviruses play an important role in physiology, ecology, and evolution of its host species, affecting traits that can be relevant for applications of B. subtilis. By reshuffling their genetic modules, SPbetaviruses may additionally accelerate evolution of the host.

[O19] THE GLOBAL ECOLOGY OF ARCHAEA

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Aim: Earth has diverse environmental niches that support various microorganisms such as bacteria, archaea, viruses, and small eukaryotes. These microorganisms have adapted to different physical and chemical conditions over time, forming stable microbial networks (Shaffer et al., 2022). However, the global diversity and distribution of archaea, known as the archaeome, have not been studied comprehensively using advanced multi-omic techniques. Thus, our study aims to investigate the archaeome in a genome-centric approach to understand how it adapts to various environmental habitats and to reveal its co-occurrence patterns with other microorganisms like bacteria, fungi, and viruses.

Methods: We analyzed a multi-omic dataset from the Earth Microbiome Project consisting of 880 diverse microbial community samples. The dataset included shotgun metagenomics, targeted amplicon sequencing of the 16S, 18S rRNA gene and ITS gene region, liquid chromatography-tandem mass spectrometry, and gas chromatography mass spectrometry. We focused specifically on the archaeome that involved complementing metagenome-assembled genomes (MAGs) with a reference alignment-based approach to determine operational genomic units.
To classify the archaeome based on different biomes, we identified the differential abundance of distinct archaeal lineages. Additionally, we used linear regressions to determine significant associations between metabolites and archaeal species and employed sparCC to detect significant co-occurrences of specific archaeal lineages with bacteria, fungi, or viruses.

Results: The original EMP500 publication (Shaffer et al., 2022) revealed that environmental conditions, particularly salinity, were the primary driver for microbiome and metabolite compositions. Our study, which specifically focused on the global archaeome and 51 archaeal and 1214 bacterial MAGs, confirmed the significance of physical and chemical conditions in distinguishing microbiomes. We found that the composition of the archaeome could serve as an excellent predictor of specific habitats, with the highest accuracy observed in samples from plants, non-saline sediments, saline soils, and freshwater (accuracy ranged from 78% to 93%). According to differential abundance analysis halophiles like Haloquadratum (q-value = 4.18E-16) and Halomicrobium (q-value = 6.32E-14) were significantly associated to saline soils and surfaces, while Nitrosopumilus (q-value = 4.44E-2) seemed to prefer saline soils and water resources. On the other hand, methanogens like Methanomassilicoccales (q-value = 1.88E-6) and Methanocorpusculaceae (q-value = 3.34E-2) were significantly associated to samples from animals. However, on the contrary Methanosaeta (q-value = 6.22E-4) was specifically associated to freshwater.

Conclusion: To our knowledge, our study presents the first-ever multi-omics data resource of the global archaeome.

Shaffer, J.P. et al. Nat Microbiol 7, 2128–2150 (202

[O20] A PREVIOUSLY UNCHARACTERISED HYDROGENASE DOMINATES FERMENTATION IN THE HUMAN GASTROINTESTINAL TRACT

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Molecular hydrogen (H_2) is an important metabolite cycled by microorganisms within the human gastrointestinal tract (GIT) with key roles in human nutrition and health. H_2 is produced during fermentation by various bacteria and consumed as an energy source by other bacteria, including acetogens, methanogens, and sulfate reducers. H_2 has traditionally been used as an indicator of gut dysbiosis through breath tests and the disruption of H_2 cycling is associated with colorectal cancer, IBD and other GI tract disorders. Despite strong links to human health, the microorganisms, enzymes, and pathways responsible for gastrointestinal H_2 production remain unresolved. Here we show that a previously uncharacterized enzyme, the group B [FeFe]-hydrogenases encoded by all four dominant gut phyla, primarily mediates fermentative H_2 production in gut microbiota. Leveraging a vast dataset of 300 stool and biopsy metagenomes, 78 metatranscriptomes, and 801 gut bacterial isolate genomes, we show that the genes for this enzyme are abundant, highly expressed, and widely distributed in the human GIT. Based on transcriptomic and gas chromatography analysis of 16 taxonomically diverse gut isolates, the group B [FeFe]-hydrogenases mediates rapid H_2 production during fermentative growth. Furthermore, Bacteroides, a genus previously unknown to be hydrogenogenic, are dominant H_2 producers in this environment. Furthermore, biochemical characterisation confirmed that the group B [FeFe]-hydrogenase is catalytically active and binds a diiron center. This combination of culture-dependent and culture-independent analysis provides new insights into how H_2 is produced within the human GI tract, and identifies the key groups involved, enhancing our ever-growing understanding of the impacts of the gut microbiota on human health.

[O21] CRISPR-CAS IMMUNITY HITCHHIKES WITH BENEFICIAL MOBILE GENETIC ELEMENTS INCREASING THE SPREAD OF DRUG RESISTANCE

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CRISPR-Cas are sequence-specific prokaryotic immune systems that can inhibit horizontal gene transfer. Previous studies have shown that CRISPR immunity is positively selected when it confers resistance against parasitic mobile genetic elements (MGEs), such as virulent phages, but is selected against when it interferes with the acquisition of beneficial genes, for example when they target a plasmid that encodes an antibiotic resistance gene that is critical for survival. Nonetheless, bioinformatic analyses reveal that antimicrobial resistance (AMR) plasmids are a common target of CRISPR-Cas systems in natural environments. Given the experimental evidence for selection against CRISPR-mediated targeting of beneficial DNA elements, it is unclear why CRISPR-Cas systems that target AMR elements persist in natural environments.

We hypothesized that there could be direct benefits associated with CRISPR-mediated targeting of MGEs that confer a fitness advantage. Specifically, we reasoned that when bacteria are exposed to a diverse pool of beneficial MGEs, the sequence-specific nature of CRISPR could allow bacteria to target beneficial, but relatively mediocre MGEs (e.g., plasmids carrying only a single AMR gene) while enabling bacteria to be infected with the best (e.g., multi-drug resistant) MGEs available.

To explore this, we developed an epidemiological model and experimental system. Our results show that, under ecologically more complex scenarios, where bacteria are exposed to more than one MGE, CRISPR-mediated immunity against beneficial MGEs can provide a selective advantage by enabling bacteria to associate with the most advantageous MGEs in the population. The CRISPR-immune strain is then favored because it hitchhikes with the most beneficial MGE available, which creates epidemiological feedback also resulting in the more efficient spread of that more advantageous MGE.

This work provides novel and important insights into the reason why CRISPR-Cas systems frequently target beneficial plasmids and shows that CRISPR-Cas can both inhibit and promote the spread of MGEs and antimicrobial resistance genes, depending on MGE diversity in the population.

[O22] HIGH DIVERSITY OF THE EMERGING PATHOGEN ACINETOBACTER BAUMANNII IN LIVESTOCK AND HUMAN WASTEWATERS

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Carbapenem-resistant Acinetobacter baumannii are causing tremendous treatment problems in hospitals. There is still a knowledge gap on the abundance and stability of acquired resistances and the diversity of resistant Acinetobacter in the environment. In this study the diversity and antimicrobial resistances of Acinetobacter spp. released from livestock and human wastewater into the environment was studied. Fifty-two A. baumannii isolates were cultured from raw and digestated manure of different biogas plants and most stages of the rural wastewater treatment plants (WWTP) (no hospital wastewater receiving) and the two studied urban WWTPs receiving veterinarian and human hospital wastewater. Multi-locus sequence typing (MLST) identified 23 novel and 12 known sequence types (STs) of A. baumannii. Most novel ST were cultured from livestock samples and the rural WWTP. A. baumannii isolates from livestock and the rural WWTP were susceptible to carbapenems, colistin, ciprofloxacin, ceftazidime, and piperacillin. In contrast, A. baumannii isolates from the two urban WWTP showed a clinical linkage with respect to MLST and were multi-drug resistant (MDR). The presence of viable A. baumannii in digested manure and sewage sludge confirmed the survival of the strict aerobic bacteria during anoxic conditions. The study showed the spread of diverse Acinetobacter strains into the environment with a strong association of clinically MDR A. baumannii strains from the inflow of hospital wastewater to WWTPs. A more frequent detection of Acinetobacter in sewage sludge than effluent waters indicated that particle-attachment of Acinetobacter cells must be considered by the risk assessment of those bacteria.

[O23] DIVERSE ANTI-DEFENSE SYSTEMS IN THE LEADING REGION OF PLASMIDS

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Plasmids and other conjugative elements facilitate the horizontal transfer of genes encoding various functions, including antibiotic resistance and virulence factors. In order to successfully establish in recipient bacteria, the conjugative elements must overcome various defense mechanisms, such as CRISPR-Cas systems, restriction enzymes, and SOS response. Here, we demonstrate that a diverse repertoire of anti-defense genes is encoded on plasmids' leading region, which is the first to transfer to recipient cells. This feature holds for different conjugative elements and types of anti-defense genes. We show that these genes are located in an orientation and downstream to regulatory elements that could allow early expression while the plasmids are still in ssDNA form. Our results could lead to the discovery of novel anti-defense systems among the numerous uncharacterized gene family encoded in the leading region. Further, our findings reveal a new facet of plasmid dissemination and provide theoretical foundations for developing conjugative delivery systems for natural microbial communities.

[O24] CARBAPENEM HYPER-RESISTANCE MEDIATED BY BLANDM-1 GENE ADAPTATIVE AMPLIFICATION

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Antimicrobial resistance (AMR) poses a major concern to public health worldwide. Carbapenemases represent one of the most worrisome AMR mechanisms, since they confer resistance to most β-lactams, including carbapenems and cephalosporins, which are considered critically important for the treatment of life-threatening infections caused by multi-drug resistant bacteria. blaNDM-1 is a widespread carbapenemase gene frequently associated with several mobile genetic elements (MGEs) as insertion sequences (ISs) or plasmids (1). In previous studies, we have found blaNDM-1 related to a common genetic structure in different Enterobacteriaceae species (2). This structure was located in an IncC-type plasmid and formed tandem repeated units of about 9 kb, carrying one copy of the blaNDM-1 gene flanked by ISCR1 elements. The aim of this work was to understand the genetic dynamics behind this ISCR1_blaNDM-1 element in terms of adaptation and modulation of carbapenem resistance phenotype and its possible relation to the putative replicative transposition of ISCR1 sequences. For this purpose, the original IncC-type plasmid (pCW-NDM-1, hereafter) was transferred from a wild-type ST1196 Escherichia coli isolate into a laboratory K12 MG1655 E. coli strain by conjugation. This strain was used to perform different evolutionary assays in liquid culture, under different carbapenem selective pressures. The Minimal Inhibitory Concentration (MIC) to meropenem of evolved strains was evaluated in order to assess their phenotypic resistance profiles. Likewise, the ISCR1_blaNDM-1 element copy number was determined by Real-Time quantitative PCR (RT-qPCR) for each evolved strain.

In general, the evolution of different populations under carbapenem pressure was observed to induce an increase of up to 16 times the meropenem MIC of the original populations after 48 hours. This phenomenon of hyper-resistance to carbapenems was due to an up to 20-fold increase in the blaNDM-1 copy number with respect to the pCW-NDM-1 plasmid carrying the structure. Furthermore, a direct linear correlation was observed between the number of blaNDM-1 gene copies per bacterium and the MIC of the population evolved.

These observations revealed the role of this MGE as a mechanism of adaptative gene amplification in response to a stress condition. In this sense, the amplification of the blaNDM-1 gene poses a threat to the effectiveness of carbapenems since it can lead to a hyper-resistance phenotype in response to clinical treatment.

References:

1 Acman et al., 2022. Nature Communications.
2 Delgado-Blas et al., 2022. mSystems

[O25] ANTI-DEFENSE SYSTEMS IN ARCHAEAL VIRUSES: PREDICTING NOVEL ACRS AND ACAS, REDEFINING ACRIII-1, AND PERSPECTIVES ON THE DEFENSE LANDSCAPE OF ARCHAEA

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Around one hundred anti-CRISPR (acr) families have been identified in bacterial viruses, adjacent to regulatory Acr-associated (aca) genes acting as transcriptional repressors of the acr locus, with twelve known families. This contrasts with the 3-4 acr families and a single aca reported in archaeal viruses, despite the widespread presence of CRISPR-Cas systems in archaea (85% of archaeal genomes encode a CRISPR-Cas system vs. 42% of bacterial genomes). While acrs have been largely discovered using the guilt-by-association approach, this strategy has limited impact in archaeal viruses because know acrs have a narrow distribution and are not encoded in operons. Here, we exploit the requirement of an early and strong expression for acrs to predict novel anti-defense genes in several archaeal virus families. We determined the consensus sequence of the promoter regulating acr-expression and used it to screen for anti-defense genes in the genomes of archaeal viruses, identifying 25 novel families of anti-defense genes whose transcription is regulated by the same strong promoter, some of which have anti-CRISPR activity, while others inhibit toxin-antitoxin systems.

Moreover, we provide the first experimental evidence for aca8 as the acr-associated transcriptional regulator of anti-defense genes in rudiviruses. This protein encodes a helix-turn-helix (HTH) DNA binding domain, similarly to all known aca in bacteriophages, and specifically binds to the promoter of anti-defense genes. Additionally, we identify a novel aca family (tentatively aca14) regulating the transcription of anti-defense genes in lipothrixviruses. Remarkably, this protein encodes a ribbon-helix-helix (RHH) domain instead of an HTH motif. We show that aca proteins regulate anti-defense genes beyond anti-CRISPRs, and that RHH-motifs can act as regulators of anti-defense gene transcription.

We also investigated the role of AcrIII-1 in archaeal viruses. AcrIII-1 is a RING nuclease protein that degrades cyclic tetra-adenylate (cA4), a nucleotide second messenger produced after the activation of several type III CRISPR-Cas systems. We demonstrate that AcrIII-1 has no anti-CRISPR activity during infection of S. islandicus LAL14/1 with SIRV2 due to the protein´s late expression, making it unable to inhibit CRISPR-Cas immunity at the onset of infection. We also evaluate the possibility that AcrIII-1 exerts acr-activity in other viruses, concluding that its regulatory sequence and genomic neighborhood do not support a general role as acr in archaeal viruses. However, we find additional roles for this protein during infection that result in a mild replicative advantage for SIRV2. Furthermore, our results uncover a role of cA4 unrelated to type III CRISPR-Cas systems.

[O26] NATURAL PRODUCT BIOSYNTHETIC POTENTIAL REFLECTS MACROEVOLUTIONARY DIVERSIFICATION WITHIN A WIDELY DISTRIBUTED BACTERIAL TAXON

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Flavobacteriaceae spp. are key players in global biogeochemical cycling and known for their versatile carbohydrate and peptide degradation capacities. However, knowledge of their secondary metabolic traits as possible adaptive features underlying their broad range occurrence in terrestrial and marine ecosystems is still narrow. Here, we analysed 2680 genomes to determine whether natural product biosynthesis potential reflects the taxonomic diversity of Flavobacteriaceae species. We uncovered 12493 secondary metabolite biosynthetic gene clusters (BGCs), with 9330 BGCs detected from 1923 Flavobacteriaceae genomes, and 3163 BGCs retrieved from 757 genomes of the closely related Weeksellaceae family. Noticeably, 88.6% of the observed BGCs were inferred to lead the biosynthesis of likely novel natural products. We found an unanticipated, large diversity of BGCs encoding carotenoid (n = 2,225) and flexirubin pigments (n = 1,256), the vast majority awaiting formal description. A previously unknown phylogenetic signal reflecting natural product biosynthesis diversification within the studied taxa was unveiled, as the distribution of closely related BGCs across genomes usually followed family- and genus-specific patterns. Aquimarina, Kordia and Tenacibaculum spp. possessed large genomes and a wide repertoire of BGCs and peptidases, likely underpinning their broad host range and opportunistic-to-pathogenic behaviour. Using a machine learning approach (Feature Selection), we reveal that marine and non-marine Flavobacteriaceae genomes are differentially enriched in CAZymes and peptidases with distinct functionalities and molecular targets. Our findings reveal tightly intertwined taxonomic and natural product biosynthesis diversification in the Flavobacteriaceae family. We posit that the carbohydrate, peptide, and secondary metabolism triad synergistically shape the evolution of this keystone bacterial taxon and break new ground for the study of Flavobacteriaceae spp. as sources of novel drug leads.

[O27] MICROPLASTICS INCREASE THE SELECTIVE POTENTIAL OF ANTIBIOTICS AT SUB-INHIBITORY CONCENTRATIONS

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Microplastics (MPs) are major pollutants that are massively released into the environment. In urban waters, biofilms can form on plastic surfaces and thus MP particles might affect environmental bacteria and their associated resistome. In addition, antibiotics may adsorb onto plastic surfaces and have greater effect on plastic-associated bacteria than on planktonic bacteria. Therefore, the presence of MPs in the environment could pose a risk for antibiotic resistance development and dissemination in environmental settings. The goal of this study was to determine the impact of microplastics in combination with antibiotics on environmental bacterial growth and antibiotic resistance. We hypothesized that the presence of MPs could increase the selective potential of antibiotics at sub-inhibitory concentrations on environmental bacteria and their associated antibiotic resistance genes (ARGs). DNA was extracted from urban river bacteria enriched in TSB medium or sterile water over 72 hours, with and without microplastics and gentamicin and ciprofloxacin at sub-inhibitory concentrations. The 16S rRNA and gentamicin resistance genes were amplified by qPCR to determine pollutant effects on growth and ARG selection. In addition, the influence of MPs and antibiotics on river water communities was evaluated by sequencing the 16S rRNA gene. Bacterial abundance was lower in the MP fraction than in the liquid fraction (both with or without MPs). Antibiotics had no effect on bacterial abundance in any fraction and were thus overall sub-inhibitory. Bacterial exposure to both gentamicin and ciprofloxacin at sub-inhibitory concentrations induced a larger shift in baterial community composition in the MP fraction than in the liquid fraction (with or without MPs), with an increase in the relative abundance of Citrobacter, Klebsiella and Pseudomonas. Gentamicin and ciprofloxacin selected for gentamicin resistance genes in the MP fraction, both in TSB medium and in sterile water. Therefore, our results are consistent with antibiotics at sub-inhibitory concentrations having a larger impact on environmental antibiotic resistance in the presence of MPs. This study adds to the concerns related to the role of microplastic pollution on the emergence of antibiotic resistance in the environment.

[028] THE FATE OF PATHOGENS, ANTIBIOTICS, AND RESISTANCE GENES IN TREATED WASTEWATER IRRIGATED SOILS AND CROPS

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Climate change improves freshwater scarcity, thus endangering communities living in arid and semi-arid environments. The use of treated wastewater (TWW) for irrigation presents a viable alternative to freshwater and could ensure food security. However, TWW irrigation could present a considerable health risk, especially for freshly eaten produce. The general goal of our project was to challenge the restrictions imposed on the use of TWW for the irrigation of vegetables. We hypothesized that the soil could present a barrier to TWW associated pathogens, antibiotics, and resistance genes (ARGs) en route to the crops. To test our hypothesis, we carried out field experiments for four years using TWW and freshwater to irrigate tomatoes, cucumbers, and melon crops. We tested different water qualities, irrigation practices, soil, and crop types (>400 samples) monitoring human pathogens (bacteria, viruses, and protists), antibiotics, ARGs, soil and plant properties, as well as agronomic parameters. The abundance, diversity and source of microbial pathogens were monitored throughout the field trials using a combination of cultivation-based and molecular techniques. Furthermore, the dissemination of nine antibiotics and their corresponding ARGs was evaluated using analytical and molecular methods, respectively. The results show that contaminants in the soil and crops could not be related to water quality or agricultural practice. In fact, the main modulators of contaminants were soil types, mediating abundance, and diversity of pathogens and ARGs. There was only one exception; the plastic mulch-covered soil irrigated with TWW retained and accumulated certain antibiotics. Overall, our results indicate that secondary TWW does not enrich soils or crops with human pathogens, antibiotics, or ARGs. We thus suggest that the pathogens introduced by effluent serve as prey to micropredators such as soil-free living amoeba (FLA). To test our hypothesis, we first demonstrate that effluent bacteria are preyed on by soil FLA, and then we isolated FLA from agricultural soil and followed their predation dynamics. Our results demonstrate the importance of understanding soil trophic interactions and their potential benefits to sustainable agricultural practices.

[O29] THE HIDDEN EFFECTS OF LIMING ON MICROBIAL COMMUNITY MEMBERS

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Lime ($CaCO_3$) is a common soil amendment used to increase the pH in acidified soils. This improves the soil fertility and increases plant's growth and biomass. A plethora of publications discussed these effects from a rather economic point of view, whereby the plant and its yield were in focus. However, liming can have a significant impact on bacterial soil community, both in terms of their composition and diversity. Although soil pH is reported as key factor in shaping bacterial community, varied effects of liming on soil bacteria was observed in several studies. It appears, that mechanisms by which liming influences the soil microbial community are rather complex. The increase of soil pH can change nutrient availability and thus may influences bacterial growth and activity. Interestingly enough, the human pathogen Salmonella enterica is heavily influenced by lime application, which can determine its fate in agricultural soils. Our microcosm-scaled studies showed immense improvement of S. enterica's persistence in soil after liming, determined by colony forming units (CFU). The effect was reproducible in several soil types obtained from various agricultural fields in Germany, where different liming strategies were used. The persistence of S. enterica was enhanced regardless of the application strategy. In soils that have been limed every year over several decades, the persistence was just as stable as in samples that have only been treated with lime once, during the experiment.

A global effect of liming on microbiota was observed in a 16S amplicon sequencing approach. The community composition changed over the course of the experiment. The major difference was observed between soils that have been limed for decades compared to not limed soil samples, while an application of lime during our experiments had only minor effects on soil microbiota. The most interesting observation was a dependency of the shift on fertilization (P/Mg), which was applied to the soils every year over several decades.

In summary, our results indicate that liming can cause a severe shift of abundances of single bacterial taxa in complex, hard-to-estimate processes over time. Other influencing factors like fertilization may interfere with those processes. In consequence, pathogens like S. enterica benefits unintentionally from particular soil management practices.

[O30] IMPROVEMENT OF HYDROGEN PRODUCTION BY GENETIC MODIFICATION OF CYANOBACTERIAL STRAINS

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Cyanobacteria are a diverse group of photoautotrophic prokaryotes that are found in a variety of environments and can be grown in the laboratory. With genomic information available for more than 130 cyanobacterial strains, many varieties of cyanobacteria can be genetically engineered to produce hydrogen. Cyanobacteria are ideal cell factories for hydrogen production because they have low nutrient requirements and are capable of using light to generate biomass from water and carbon dioxide.

Cyanobacteria produce hydrogen through two key enzymes, nitrogenase and bidirectional hydrogenase, and oxidize molecular hydrogen by uptake hydrogenase. The main role of uptake hydrogenase is to protect nitrogenase from oxidative damage and prevent feedback inhibition of both nitrogenase and bidirectional hydrogenase, which reduces the net gain of molecular hydrogen. Inhibition of the hydrogen-oxidizing activity is important in order to maximize hydrogen yield from cyanobacterial cultures. Uptake hydrogenase has two subunits; the small subunit directs electron transport to the large subunit, which has an active site that binds molecular hydrogen. These subunits are encoded by hupS and hupL genes, respectively, that can be genetically modified to reduce activity of the uptake hydrogenase and increase hydrogen production.

HupS and hupL genes were inactivated in Anabaena sp. PCC 7120 and Anabaena variabilis ATCC 29413 by insertional mutagenesis, and the knockout mutants were segregated and verified by sequencing. Hydrogen production in the mutant and wild type strains was measured by gas chromatography.

Genetic inactivation of uptake hydrogenase in cyanobacteria affects strains in various ways. In Anabaena sp. PCC 7120 it increased hydrogen production by 4-7-fold while in Anabaena variabilis ATCC 29413 it reached a 5-fold improvement, compared to the wild type strain. Uptake hydrogenase knockout strains can be the starting point for further genetic modifications for the purpose of enhancement of their hydrogen-producing capacity.

Hydrogen can be used as an alternative biofuel, and cyanobacteria can generate it using the renewable energy of the sunlight. Additionally, photosynthetic growth of cyanobacteria is consuming carbon dioxide and reduces the carbon footprint by producing polymers of carbon. The ability to reduce carbon dioxide pollution as well as produce energy dense hydrogen makes cyanobacteria significant in biofuel research.

[O31] HOW DO TWO STRAINS OF LACTIC ACID BACTERIA COOPERATE TO IMPROVE SOY JUICE FERMENTATION?

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Green transition incites to food innovation, notably to favor the acceptability of plant proteins in a context of diet including replacement of animal proteins. Lactic acid bacteria (LAB) are largely used for food fermentations. The use of different LAB strains as starter with different properties can bring complementary functionalities participating to the overall quality of fermented food (acidification, texture, aroma) [1]. Fermentation can also benefit from interactions between LAB, resulting in a more efficient fermentation process. In soy yogurt production, few is known about positive LAB interactions beneficial to fermentation process. We investigated the metabolism of Lactobacillus delbrueckii CIRM-BIA 865 (Ld865) and Lactiplantibacillus plantarum CIRM-BIA 777 (Lp777) two strains efficient to ferment soy juice (SJ) [2]. We aimed to reveal how Lp777 and Ld865 interact to improve SJ fermentation. When these strains were co-cultivated in SJ, the fermentation time to obtain a yogurt-like texture was reduced by 30% compared to monocultures. Furthermore, Ld865 cells were four times shorter and ten times more numerous in cocultures than in monocultures [3]. Thanks to transcriptomic and metabolic analyses carried out during fermentation, we compared adaptations of both bacterial metabolisms in coculture versus in monoculture. Transcriptomics data showed that Lp777 induced the expression of genes involved in the regulation of redox potential at a higher level in coculture than in monoculture. The redox potential in the co-cultured SJ was intermediate of monocultured SJ. A better regulation of the redox potential in coculture seems to involve metabolism of sulfur compounds (methionine, cystine) and the degradation of hydrogen peroxide by Lp777. Moreover, Ld865 and Lp777 metabolized diverse carbon and nitrogen sources. Ld865 has an homofermentative metabolism, only consumed sucrose and produced lactic acid [3]. Lp777 consumed sucrose, galactose and amino acids released by proteolytic activities of Ld865. Its metabolism is homofermentative at the beginning of acidification and further heterofermentative, producing lactic and acetic acids. Finally, LAB interactions, i.e., cooperation for redox management, cross-feeding for nitrogen and competition for carbon, in soy yogurt can be compared to previously described milk ones. In conclusion, associating LAB strains with distinct and complementary metabolic profiles can be useful to improve SJ fermentation. Identifying the molecular nature of bacterial interactions opens new avenues for the selection of cooperating strains in plant-based fermentation, thus facilitating green transition of food production.

[1] Derozier et al. 2023 PlOsOne, [2] Harlé et al. 2020. IJFM, [3] Harlé et al. 2023 AEM submitted

[O32] DE NOVO ASSEMBLED SINGLE-CELL TRANSCRIPTOMES FROM AQUATIC PHYTOFLAGELLATES REVEAL METABOLICALLY DISTINCT DORMANT CELL TYPES

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Novel single-cell transcriptomics techniques have rapidly become a standard approach to decode cell identity, development, and interactions in mammalian model organisms. The potential of these techniques to uncover metabolic dynamics in aquatic single-celled organisms is huge, but evidence of their applicability to non-model, poorly understood microeukaryotes remains limited. Living Ochromonas triangulata cells from early and late growth phases were FACS-sorted based on food vacuole staining and chlorophyll signal, and single-cell transcriptomic libraries were prepared following the Smart-seq2 protocol. Transcriptomes of 768 single cells were sequenced using the Illumina NovaSeq 6000 instrument. Lacking a reference genome, transcriptomes were assembled de novo using Trinity. Open reading frames, identified using TransDecoder, were annotated by BLASTing against the Swiss-Prot database. Following read mapping against the assembly, differential expression analysis was done using DESeq2, gene set enrichment using fgsea against MSigDB, and metabolic mapping using pathview against pathways from the KEGG Orthology database. Additionally, ribosomal RNA was detected using RiboDetector and taxonomy assignment, to both the single cell eukaryote and the associated prokaryotes (whether ingested or in metabolic crosstalk with), and downstream phylogenetic analysis was performed. Clustering the read counts revealed the presence of two distinct transcriptional states corresponding to the growth phase, log and lag, as well as a third distinct cluster of cells made up of both. Cells in this third cluster expressed less than 10% of the housekeeping genes. We hypothesize that this cluster represents encysted cells in dormancy justifying their presence in both early and late groups. Most differentially expressed genes were downregulated in the mixed dormant group and enriched for GO ontologies associated with chloroplast thylakoid membrane. Pathways associated with core carbon metabolism like glycolysis, tricarboxylic acid cycle, and carbon fixation, and ribosome-functioning were the most downregulated in the mixed dormant group. Phylogenetic analysis correctly identified ~90% of the cells to the species taxonomic level. In conclusion, this study demonstrates the power of single cell transcriptomics for environmental applications where reference and annotation sources might be scarce and the potential for sequencing and deciphering unculturable microeukaryotes.

[O33] NEW PERSPECTIVES ON PANGENOMES, INTRA-SPECIES DIVERSITY AND ECOLOGICAL AND EVOLUTIONARY DYNAMICS IN FRESHWATER BACTERIA

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Closely related bacteria can be highly variable at the genomic level. Such heritable traits emerge as adaptations to different environmental niches and have a profound influence on microbial community dynamics and their ecological function. Accordingly, individual genomes or clonal populations will merely contain a fraction of the total genetic diversity seen in operationally defined species, i.e., the pangenome. To enable rational and large-scale exploration of such intra-species diversity also for uncultured microbial populations, we have developed a set of new tools and bioinformatic approaches to partition core and flexible genomes in metagenomic datasets while at the same time providing a modular view of the pangenome where we can identify operons and genomic islands and track their prevalence across different bacterial populations. In this way we make population genetics of the uncultured prokaryotic majority tractable and pave the way for in depth studies of factors and processes that underpin evolution and govern biodiversity. To demonstrate the potential of these approaches, we exploited freshwater metagenomes and available genomes of abundant freshwater bacteria to study the impacts of geographic separation on population structure. Focusing on broadly distributed freshwater taxa such as Actinobacteria acI, Candidatus Fonsibacter, Polynucleobacter and Candidatus Methylopumilus, we found that population differentiation increased significantly with spatial distance in all species. Different species showed contrasting rates of geographic divergence and dramatically different temporal population dynamics within ecosystems. While certain populations hardly diverged over several years, others displayed high divergence after merely a few months, matching the genome-level divergence seen in populations separated by thousands of kilometers. Based on our findings, we hypothesize that populations with higher strain diversity evolve more monotonously, while low strain diversity enables more drastic clonal expansion of genotypes.

[O34] ANALYSIS OF OVER 100,000 GENOME-SCALE METABOLIC RECONSTRUCTIONS INDICATES A NON-RANDOM DISTRIBUTION OF TYROSINE METABOLIC NICHES IN THE PROKARYOTIC PHYLOGENETIC TREE

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A key factor in our response to caused rapid changes in biodiversity on Earth is understanding how individual microbes' metabolic niches affect microbial community structure, stability, and functional potential [Tsoi et al. Biotechnology Advances, 2019]. In our study, we use genome-scale metabolic reconstructions of over 100,000 metagenome-assembled genomes (MAGs) to challenge the ecological hypothesis that "everything is everywhere: but the environment selects", using the metabolism of the aromatic amino acid tyrosine as a test case. The use of metabolic reconstructions captures a holistic view of the microbe's metabolism by comparing the presence of functional genes, and recent studies have shown the applicability of genome-scale metabolic reconstructions for studying metabolic niche theory [Régimbeau et al. Ecology Letters, 2021].

We combined two large-scale MAGs databases, GEM [Mayfach et al. Nature Biotechnology, 2021] and CLUETERRA [Nunes da Rocha et al. https://www.ufz.de/index.php?en=47300]. MAGs from the two databases have been assembled from metagenomic samples taken around the globe, resulting in 104,433 MAGs. We used the CarveMe metabolic reconstruction tool [Machado et al. Nucleic Acids Research, 2018] to create genome-scale metabolic reconstructions from all the MAGs and assessed the prevalence of three tyrosine-related KEGG metabolic modules among the reconstructions.

We created 104,401 genome-scale metabolic reconstructions from the two MAG databases, representing 144 phyla belonging to both Archaea (20) and Bacteria (124). We found that the Shikimate module and the subsequent tyrosine biosynthesis from chorismite are, on average, present in nearly 70% of all MAG-derived metabolic reconstructions in each of the 144 phyla. On the other hand, tyrosine degradation was, on average, present in less than 9% of the MAGs in each phylum. Two phyla, Bdellovibrionota (356 MAGs) and Eremiobacterota (140 MAGs), were enriched with tyrosine degradation in 60% of the metabolic reconstructions representing these phyla.

Our high-throughput analysis of prokaryotic metabolism shows that genome-scale metabolic reconstructions can be used to identify non-random distributions of metabolic niches within the prokaryotic tree of life. We found that the distribution of tyrosine degradation was non-randomly distributed among our microbes, whereas the central function of tyrosine biosynthesis was highly prevalent. These data support the view that some metabolic pathways are general, and others are enriched in a few taxonomic groups. Additional analysis of tyrosine degradation among closely related species may provide information on whether the distribution of tyrosine degradation is determined by evolutionary history. Furthermore, our analysis can assess non-random distributions of all metabolic modules within the prokaryotic tree of life.

[O35] T6SS-MEDIATED COMPETITION LIMITS SEEDLING TRANSMISSION OF XANTHOMONAS CAMPESTRIS PV. CAMPESTRIS AND DRIVES ASSEMBLY OF SEED-ASSOCIATED BACTERIAL COMMUNITIES IN VITRO

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Aim: Seeds harbor diverse microbial communities that are essential for plant growth and health. As seeds are resource-limited and have limited space, microbial competition likely plays a significant role in shaping the seed microbiota. The objective of this study was to examine the role of interference competition mediated by the type VI secretion system (T6SS) in the transmission of the seed microbiota to seedlings.

Methods: We focused on a radish seed-borne strain of Stenotrophomonas rhizophila that is highly competitive against the phytopathogenic Xanthomonas campestris pv. campestris (Xcc) (Torres-Cortés et al., 2019) and has a high seedling transmission capacity (Simonin et al., 2023). We characterized the T6SS genetic cluster and its putative effectors in S. rhizophila and generated T6SS-deficient mutants. We then evaluated the impact of T6SS-mediated competition on bacterial community composition by first conducting pairwise confrontations between S. rhizophila and Xcc, and then by confronting S. rhizophila to synthetic communities (SynComs) of seed-borne bacterial strains. SynCom compositions were estimated using gyrB amplicon sequencing.

Results: Our results demonstrate that the T6SS of S. rhizophila not only reduces the population size of Xcc in vitro, but also in planta during seed-to-seedling transition. Moreover, T6SS strongly modulates SynComs composition, negatively impacting the relative abundance of more than 25 bacterial strains belonging to four different bacterial orders.

Conclusion: These results suggest that the T6SS of S. rhizophila could have significant implications for controlling plant diseases by reducing the transmission of the primary inoculum. To fully understand its ecological significance, further evaluations of this system will be conducted in planta inoculated with complex bacterial communities. This study provides new insights into the mechanisms that underlie the assembly of the seed microbiota and highlights the importance of intermicrobial competition via T6SS in shaping microbial communities.

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Simonin, M., Préveaux, A., Marais, C., Garin, T., Arnault, G., Sarniguet, A., Barret, M., 2023. Transmission of synthetic seed bacterial communities to radish seedlings: impact on microbiota assembly and plant phenotype (preprint). Microbiology.

[O36] GUT MICROBIOME FUNCTIONS ENRICHED IN C. ELEGANS

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The microbes living in the native habitat of the nematode Caenorhabditis elegans usually serve as food. However, certain bacteria can accumulate in worms and show beneficial or protective effects. We studied the microbiome composition across the host life cycle and compared the host microbial communities to substrate samples and controls without worms. Interestingly, we uncovered gene clusters involved in gut microbiome functioning to be differentially abundant exclusively for host microbes. In addition, we evaluated the impact of stochastic effects. We found that deterministic effects were more prominent in microbial samples from the host, highlighting the importance of functional traits for microbiome assembly.
Furthermore, we investigated the role of adaptive strategies in shaping the microbiome. Life history traits are generally less studied for microorganisms. We developed a framework based on genome screening and metabolic modeling to classify bacteria into competitors, stress-tolerators, and regenerators. Finally, we identified microbial strategies relevant to host colonization.

[O37] BIOSYNTHETIC AMPLICON GENEFISH FOR SELECTIVE EXTRACTION OF SECONDARY METABOLITE PRODUCERS FROM ENVIRONMENTAL MICROBIOMES

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Microbial secondary metabolites (SMs) exert significant effects on both micro-scale processes such as microbial community assembly, as well as macro-scale processes like pathogen treatment and antimicrobial resistance. To facilitate better understanding of the understudied role of SMs in microbiomes and simultaneously avert the antimicrobial resistance crisis, it is important to increase our catalogue of isolated microbial compounds. As has been identified by metagenomic and functional domain amplicon sequencing studies, there is still a large untapped genetic potential for biosynthesis of novel SMs in various natural microbiomes. However, most of these microbes are unfortunately recalcitrant to cultivation, complicating the discovery of their natural products. Here we present a fluorescence in situ hybridization (FISH)-based method that selectively extracts, irrespective of culturability, microbial community members with a high biosynthetic gene content. First, biosynthetic geneFISH probes are synthesized by PCR amplification of the conserved ketosynthase (KS) and adenylation (AD) biosynthetic domains using degenerate primers, followed by fluorescent labeling of the amplified polynucleotides. The resulting biosynthesis-geneFISH probes are then hybridized to target cells using the direct geneFISH protocol, which we modified to be applicable to suspended cells in solution for compatibility with fluorescence activated cell sorting (FACS). For development and optimization of this method we used two biosynthetically gifted bacterial model strains, the Gram-negative marine bacterium Pseudoalteromonas rubra and the Gram-positive soil dwelling Streptomyces coelicolor. Sequence analysis of AD and KS amplicons from the two model strains revealed that only ≈ 10% of these biosynthetic domains present in the genomes were amplified using the most broadly applicable primers. Nevertheless, we managed to selectively label both S. coelicolor and P. rubra with the biosynthetic amplicon geneFISH probes, as observed by fluorescence microscopy. To further demonstrate this proof of principle, flow cytometry measurements revealed that labeled P. rubra and S. coelicolor cells could be effectively distinguished from negative controls (using a non-binding polynucleotide probe) with labeling efficiencies of 84.0 and 69.9 %, and false positive rates of 1.7 and 2.9 %, respectively. Furthermore, we applied the method to seawater samples spiked with P. rubra cells at various relative abundances. This experiment will demonstrate the sensitivity of the method towards relative abundance of the target cells, as well as indicate which seawater bacterial taxa are promising secondary metabolite producers.

Poster presentations

[P1] AEROBIC ANOXYGENIC PHOTOTROPHS PLAY AN IMPORTANT ROLE IN NUTRIENT CYCLING WITHIN CYANOBACTERIAL MICROCYSTIS BLOOM AGGREGATES

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During the bloom season, the cyanobacterium Microcystis forms aggregates which include a complex assemblage of other bacteria within an exopolymer matrix. Bacterial degradation of organic compounds within this matrix is known to release nutrients to support Microcystis growth. However, little is known about the nutrient regeneration pathways and the major players. During a seven-month study of bacterial communities comparing free-living and aggregate-associated bacteria in Lake Taihu (China), we show that aerobic anoxygenic phototrophic (AAP) bacteria were significantly more abundant within aggregates than in free-living samples, suggesting a role for them in aggregate community function. We then used metagenomes from cyanobacterial aggregates in ten lakes spanning four continents to recover 102 high-quality metagenome-assembled genomes (MAGs), including 49 AAP bacteria MAGs and 53 non-AAP bacteria MAGs. This revealed that AAP bacteria likely play major roles in C, N, S, and P cycling within bloom aggregates. AAP bacteria functions include organic matter decomposition, dissimilatory nitrate reduction to ammonium, sulfur oxidation, and organic P mineralization. Publicly available transcripts from Microcystis blooms in Lake Erie (US) and Taihu mapped onto a comprehensive MAG database of both AAP and non-AAP bacteria (224 MAGs) constructed by combining the MAGs from the ten lakes with high quality MAGs from Lakes Erie, Taihu, Champlain, and Pampulha Reservoir, revealed high levels of expression of nutrient cycling pathways in AAP bacteria. Results show that AAP bacteria recycle organic nitrogen, organic phosphorus, and oxidize and detoxify reduced sulfur compounds providing recycled NH4, and inorganic P to Microcystis. Our results suggest that AAP bacteria and Microcystis coexist symbiotically as a microbial interactome.

[P2] REGULATION AND DEGRADATION OF SECONDARY METABOLITES IN PSEUDOMONAS PROTEGENS BY SOIL- AND PLANT ASSOCIATED BACTERIA

Morten Hansen, Mario Wibowo, Scott Jarmusch, Thomas Larsen, Zsófia Dènes, Akos Kovacs, Carlos Lozano, Adele Kaltenyte, Sabrina Pittroff, Aaron Andersen, Mikael Strube, **Professor Lars Jelsbak**[1]

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Pseudomonas spp. and their secondary metabolites are frequently studied model organisms for biocontrol and have been experimentally or commercially applied as such. We use P. protegens to determine how chemical interaction with soil- and plant microbiomes modulate the effective concentration of bioactive secondary metabolites produced by this well-characterized plant-beneficial species. Specifically, we focus on mapping chemical interaction networks and how these interactions modulate both production as well as degradation of biocontrol-relevant secondary metabolites:
We identify and characterize secondary metabolite degradation processes using combinations of LC-MS, GNPS molecular networking, and Imaging Mass spectrometry to track the “fate” of selected metabolites within synthetic communities of soil bacteria. Here, we show that the cyclic lipopeptide orfamide is subjected to complex degradation processes in which multiple bacteria contribute to its complete degradation in a sequential manner. This degradation protects orfamide-sensitive organisms in the community. In relation to regulation, we show that secondary metabolites from both P. protegens and soil and plant bacteria can be part of intricate interaction networks in which sequential “back-and-forth” metabolite exchange result in a multitude of changes in levels of secondary metabolites and ultimately modulate biocontrol activities.
Overall, our work demonstrates the importance of identifying chemical interactions between potential biocontrol strains and soil- and plant microbiomes and that strategies to control or mitigate these interactions can determine the activity and efficiency of biocontrol strains.

[P3] BURKHOLDERIA CONTAMINANS INDUCES THE BACILLIBACTIN PRODUCTION OF BACILLUS VELEZENSIS AND INITIATES A POSITIVE METABOLIC CYCLE IN THE TRI-SPECIES BIOFILMS

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Biofilms are ubiquitous in nature and have different effects, such as causes disease , corrosion, and plant growth promotion. Its complex structure provides space and opportunities for interaction between different microorganisms. Siderophores also play a key role in this complex interaction. Here, we used transcriptome, mutant construction, metabolome analysis to uncover the syntrophic positive metabolic cooperation between Acinetobacter baumannii XL380, Burkholderia contaminans XL73 and Bacillus velezensis SQR9. We firstly discovered the B. contaminans XL73 could induce the Bacillibactin expression of B. velezensis SQR9 while reducing its own siderophore production. At the same time, B. velezensis SQR9 could use the BCAAs produced by B. contaminnas XL37. In the tri-species coculture, the A. baumannii XL380 promotes the positive interaction. This study provides a different perspective on siderophore for microbial interaction in complex biofilms

[P4] 3D-PRINTED ARTIFICIAL LEAVES FOR INVESTIGATING MICROBIAL INTERACTIONS WITHIN MULTISPECIES BIOFILM COMMUNITIES

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Aim: In nature, up to 80% of microorganisms live in biofilms that are composed of numerous interacting species[1]. Biofilm formation on decaying biomass in soil and marine ecosystems is crucial for efficient decomposition of complex molecules and global nutrient cycling. While microbial interactions, such as metabolic cross-feeding, are believed to play a key role in these processes, such interactions remain largely unexplored due to the complexity of natural ecosystems[2]. This study aims to develop synthetic biofilm ecosystems to unravel microbial interactions involved of utilizing different carbon sources.
Methods: We established a protocol to fabricate 3D printed degradable, artificial leaves containing defined carbon sources to mimic and simplify the conditions in natural environments. A four-species bacterial community[3] was used as the model system to form biofilms on artificial leaves, followed by combined approaches of live/dead staining and fluorescence in situ hybridization (FISH) microscopic imaging.3
We further built genome-scale metabolic models to explore potential metabolite exchanges between community members[4,5].
Results: We screened various carbon sources to be utilized by the synthetic biofilm community, including cellobiose and hemicellulose (xylan) of which several led to significantly higher biofilm formation ability by the four-species community than monocultures. These carbon sources were successfully tested in 3D printed artificial leaves to facilitate biofilm growth. Biofilms imaged by Confocal Laser Scanning Microscopy (CLSM) showed distinct growth patterns and spatial structures on different carbon sources. Simulation by genome-scale metabolic models further indicated the potential metabolic cross-feeding (e.g., amino acids) within the community members.
Conclusions: Both of our experimental and simulating results suggest that interspecies interactions result in improved utilization of different carbon sources by the community compared to monocultures. The use of 3D-printed artificial leaf substrates is a promising approach for building ecologically relevant model systems with defined conditions. Coupled with genomic analysis and metabolic network modelling, this method could find broad applications in studies of microbial interactions in multispecies communities and will help explaining how such interactions are modulated by local microenvironments under defined compositional complexity.

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[P5] THE IMPORTANCE OF A MULTISPECIES COMMUNITY AND SPATIAL ORGANIZATION DURING BACTERIOPHAGE EXPOSURE

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Bacteriophages (phages) are omnipresent, and bacteria are in constant risk of being infected and killed. Thus, ranges of defense mechanisms are required for bacteria to survive, as highlighted by the vast amount of intracellular anti-viral defense mechanisms recently identified. Survival is however not only a matter of intracellular protection, exemplified by the expression of curli fibers in biofilms, which are able to prevent phages from accessing the cell surface. Since phages generally are very specific and only infect a narrow range of bacterial strains, we speculated how the presence of multiple species would influence the infection dynamics. We hypothesized that non-susceptible species would be able to shield susceptible cells when growing together in biofilms.
To test this hypothesis, and thus investigate the bacterial survival in multispecies biofilms, we used a three-species community of Vibrio anguillarum, Kluyvera cryocrescens and Escherichia coli, where E. coli was the sensitive target of coliphage T7 infection. With the use of flow cytometry and confocal laser scanning microscopy, we identified significantly increased survival of E. coli when growing with a neighbor rather than a when growing in biofilms alone. We were able to manipulate the spatial organization in biofilm community structure by sequential addition of species. Hereby, a layered biofilm was established, with one species forming a biofilm layer on top of E. coli, which colonized the bottom biofilm layer. With this model, we observed that V. anguillarum was able to secure a high level of E. coli survival in the presence of T7 phages, whereas E. coli growing either alone or in co-culture with K. cryocrescens were not protected as well.
These observations show that bacteria from living in multispecies biofilm communities may be protected from phages. It also highlights the importance of considering interspecies interactions microbial eco-evolutionary dynamics. Future project activities include testing of different mutants of V. anguillarum to identify potential matrix components important for phage exclusion. We also plan to test how the community dynamics changes when all species are in risk of infection. For that we have isolated and characterized three new phages.

[P6] MOTILITY MEDIATES SATELLITE FORMATION IN CONFINED BACTERIAL COLONIES

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Biofilms are commonly thought of as 2D habitats for microbial life, but there are niches where bacteria grow as 3D conformations. So, what are the mechanisms controlling bacterial growth in these environments? Especially, how does cell motility drive spatial organization?
We use confocal laser-scanning microscopy to image mono-clonal spherical colonies of the model organism E. coli K-12. We find satellite colonies emerge around E. coli colonies embedded in semi-soft agarose (0.3%) [1]. We identify the key structures controlling colony morphology, by studying mutants lacking surface structures involved in adhesion or motility processes and required to form biofilms. By linking the experimental observations with the results of classical motility assays, we conclude that the emergence of satellite colonies is a direct consequence of flagella-based motility.
We also find that satellites appear in different nutritional media, but its manifestation is highly sensitive to changes in the extracellular matrix elasticity.
Complementing these experiments, we build a mathematical model of how cell motility drives the spatial organization of spherical colonies. When paralleled with computational simulations, our study suggests that satellite formation allows bacterial communities to spread faster, while retaining the close interactions and protection supplied by the community.
Overall, our research provides fundamental insights into how bacteria grow and invade three-dimensional settings and has potential implications to find strategies to combat bacterial spread.

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[P7] TROPODITHIETIC ACID PRODUCTION INFLUENCES ACTIVATION OF A GENE TRANSFER AGENT IN PHAEOBACTER PISCINAE

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Horizontal gene transfer (HGT) is an important mediator of bacterial evolution, and in Rhodobacteraceae, gene transfer agents (GTAs) are believed to be a major driver of horizontal gene transfer. GTAs are virus-like particles, which transfer pieces of the bacterial host DNA through a mechanism combining aspects of natural transformation and transduction. However, GTA release happens through cell lysis and thus at the expense of the donor cell, necessitating a tight control of this process. In Phaeobacter piscinae S26, we found that the production of the antibiotic compound tropodithietic acid (TDA) represses the activation of an uncharacterized GTA. In this study, we characterize this novel GTA and investigate the possible regulatory mechanisms behind this repression. Through bioinformatic analysis, we identified homologs of 38 out of 42 genes of the multi-locus GTA genome, including a homologue of GafA, a direct activator of GTA release, which is repressed by TDA production. In Dinoroseobacter shibae, GafA is repressed by the LuxIR2 quorum sensing system, and we found that this system is homologous to the PgaIR system of Phaeobacter, which TDA is proposed to act as an autoinducer of. Based on these data, we propose a regulatory system where TDA interacts with PgaR to repress GafA, leading to a repression of GTA activation. Experimental verification of this pathway is currently on-going. These data contribute to the growing understanding of how GTA regulation is achieved. Although the role of GTAs in Nature remains under debate, they have been proposed to accelerate bacterial adaptation and evolution. GTAs may also facilitate the dissemination of antibiotic resistance, a major threat to human health, providing another important incentive for studying them. Underpinning the regulatory mechanisms governing GTA release is thus an important part understanding of what role GTAs play in Nature.

[P8] THE EFFECT OF PROBIOTIC SUPERNATANT ON IN VITRO BIOFILM GROWTH OF PATHOGENS ISOLATED FROM URINARY CATHETERS

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The effects of probiotic lactic acid bacteria (LAB) on in vitro biofilm growth of pathogens, isolated from urinary catheter infections, were evaluated in this study. Two LAB, Lactobacillus plantarum (SP1340), and Lactobacillus rhamnosus (SP1341) were grown used to obtain cell-free supernatant (CFS) in Artificial Urine Medium (AUM) supplemented with different concentrations of MRS (10% and 50%) for 24 hours and 48 hours. Four common urinary tract pathogens isolated from indwelling catheters, Escherichia coli, Pseudomonas aeruginosa, Klebsiella oxytoca, and Proteus mirabilis were cultured as mono-, and four-species biofilms in LB with different concentrations of CFS (10%, 25%, 50% and 75%).

Both L. plantarum and L. rhamnosus cultures grew well and lowered pH in 50%MRS. The pH of 24-h L. plantarum, 48-h L. plantarum, and 48-h L. rhamnosus cultures decreased from 6.5 to approximately 4. The pH of the 24-h L. rhamnosus culture didn't decrease significantly, indicative of a lack of substantial growth. The biovolumes of pathogen mono-species biofilms in CFS, obtained from LAB growth in 50%MRS, were assessed through crystal violet quantification.

Mono-species biofilm formation of all pathogens was inhibited and only 10%CFS led to biofilm formation. Higher concentrations of CFS completely inhibited biofilm formation, except when biofilms were grown in 24-h L. rhamnosus CFS, where biofilm formation was higher than others, relatively. Furthermore, the biomass of both mono-species biofilm and four-species biofilm in CFS obtained from the culture at 50%MRS for 48 hours was assessed. It was found that the absolute biomass of the four-species biofilm was higher than that of all mono-species biofilms.

Thus, the in vitro growth of mono- and four-species biofilms were inhibited in LAB supernatant, which may be pH dependent. The biovolume of the four-species biofilm was higher than that formed by mono-species biofilms.

[P9] ENVIRONMENTAL SHAPING OF THE BACTERIAL COMMUNITY IN INFANT GUT

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From early life, children are exposed to a multitude of environmental exposures that may be crucial of crucial for healthy development. As infants' gastrointestinal tract is highly vulnerable during this time and is still in a developmental phase, establishing a healthy microbiome and proper immune function. This stage is characterized by a diverse and delicate microbiota, which can be easily influenced by external factors such as diet, medication, and environmental exposures. We hypothesize that infants' early postnatal experiences might be an important factor for the child and the early colonization of the gut microbiome. To explore this hypothesis, we analyzed the influence of various environmental exposures on 2686 fecal samples from 695 children at ages 1 week, 1 month, 1 year, 4 years, 5 years and 6 years from the Copenhagen Prospective Studies on Asthma in Childhood2010 (COPSAC2010) cohort. V4 region was amplified and sequenced. We focused on gut microbiome development after birth and considered that early postnatal experiences could have a significant impact on the gut microflora.
Results
As children grow up, their gut microbiome develops over time. This was evidenced by the increase in phylogenetic diversity and Shannon index, with the most rapid rise occurring from 1 week to 4 years old, and continuing to develop until 6 years old. The centroid of beta diversity showed the gut microbiome became more concentrated over time. Moreover, more and more taxa became part of the core microbiome in the gut. Our study found that the gut microbiome of children was influenced by various environmental factors, including hospitalization, antibiotics treatment, delivery mode, and having older siblings. We further observed that hospitalization and antibiotic treatment had a short-term effect on the one month gut microbiota, mainly reducing bacterial biodiversity, and resulted in a remarkable reduction in the richness or abundance of Bifidobacteria.
Conclusions
Current study explores the gut microbiome development in children after birth and their exposure to environmental factors. Our findings demonstrate that children's gut microbiota develop over time and are influenced by hospitalization, antibiotic treatment, and cesarean delivery, which decrease their abundance.

[P10] PHAGE-HOST CO-EVOLUTION IN E. COLI: INSIGHTS INTO DEFENSE AND IMMUNE EVASION STRATEGIES

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Bacteria and their viruses (phages) are recognized as the most abundant biological agents on Earth. These microorganisms have engaged in the ongoing evolutionary battle, resulting in the development of sophisticated mechanisms to overcome each other's defense and counter-defense strategies. To protect themselves against phage infections, bacteria have evolved various anti-phage defense systems, such as CRISPR-Cas and restriction-modification systems, which defense systems, both known and unknown, are often found clustered in specific regions of microbial genomes called "defense islands". Here, we explore the defensive phenotype of several defense systems against a panel of phages in the model bacteria Escherichia coli. Our current analysis revolves identifying phage escaper mutants to pinpoint the specific phage traits, which will allow the verification of phage determinants that confer sensitivity to bacterial immunity. By unraveling the intricate mechanisms underlaying phage-host interactions, we aim to gain insights into the evolution of microbial communities and the potential use of phages in biotechnological applications.

[P11] METAGENOMIC EXPLORATION OF BACTERIOPHAGES-BACTERIA INTERACTIONS IN DRINKING WATER TREATMENT PLANTS

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Biofilters are widely used to produce potable water. Their performance relies on the activity of biofilms formed by diverse bacteria which are increasingly well described. However, the presence, diversity, and ecological role of bacteriophages in these systems have not been studied in depth. Here, we used metagenomics to study the phageome composition and its distribution across 26 drinking water treatment plants. We infer the interactions between phages and the resident microbial communities by leveraging multiple approaches. First, by performing virus–host prediction, which we confronted to the bacterial composition; second, via correlation analysis of phage abundance relative to that of bacteria MAGs; and lastly, by searching for auxiliary metabolic genes.
We found that the virome composition of biofilters is primary influenced by the water source, biogeography, and filter material. We also identified a core virome shared in most of the biofilters, which we linked to persistently dominant bacterial hosts. Additionally, based on the high number of specific links detected between phages and bacterial species and the high proportion of lytic phages, we propose that phages impose strong control on the bacterial populations in these systems. They would prevent the proliferation of single dominant species and contribute to the functional redundancy observed in the dominant microbial groups.

[P12] THE INPUT OF TERRESTRIAL ORGANIC MATTER SHAPES THE SUCCESSION OF MICROBIAL COMMUNITY IN LAKE WATER

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The fall of terrestrial leaves is known to stimulate microbial activity and biomass production in lakes; however, it is unknown whether aquatic or terrestrial microbes decompose leaves after their fall in freshwater systems. Therefore, we studied bacterial (16S rRNA) and fungal (ITS) community succession, respiration (CO2 and CH4 production), and biomass production (phospholipid fatty acid and sterol analyses) during the early-stage decomposition process of leaf litter (Alnus sp., Birch sp., and Populus tremula) in lake water with and without aquatic microbiota in vivo to examine the influence of two microbiomes on microbial succession and activity. Moreover, we explored the role of epiphytic and endophytic microbes in this decomposition process, which has rarely been studied simultaneously. Results showed that the terrestrial leaf addition increased microbial respiration and production of greenhouse gases, and both bacterial and fungal biomass production was strongly accelerated by terrestrial organic matter input. Bacteria contributed 93-99 % to total microbial biomass, whereas fungi formed only 1-7 % of microbial biomass after 21 days incubation time. Terrestrial microbes, especially bacteria, were shown to play a key role in initializing and shaping microbial succession in lentic freshwater. The most important classes structuring bacterial communities were Alpha- and Gammaproteobacteria, Bacteroidetes, and Epsilonbacteraeota. Epiphytic and endophytic bacteria contributed equally to microbial succession, whereas the fungal community was determined by epiphytic fungi. Moreover, fungal communities were more tree-specific than bacteria, whose community succession was similar in all treatments. Results suggest that terrestrial bacteria shape the microbiome succession in lake ecosystems and play a key role in linking terrestrial carbon to a part of an aquatic food web, whereas terrestrial fungi and aquatic microbes play a minor role. Moreover, by controlling fungal growth, bacteria sustain the relatively poor nutritional value of leaf litter and its biofilm for aquatic consumers, The study emphasizes that microbes from different niches, origins, and phylogenetic groups (aquatic vs. terrestrial, epiphytic vs. endophytic, bacteria vs. fungi) all impact differently on microbial processes. Therefore, the roles of these microbial groups should not be neglected in further studies: they all have the potential to differently shape microbial processes, leading to diverging ecological consequences.

[P13] IMPACTS OF DECAYING ROOT ON THE STRUCTURE AND DIVERSITY OF THE SOIL MICROBIAL COMMUNITIES

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The composition of soil microbiomes emerges from previous and existing physico-chemical soil conditions which shape the microbial habitat and the supply of growth factors. Thus, it is important to understand the influence of decaying root material from the previous cultivation season on the composition of the prokaryotic and fungal communities, and to what extent such impacts will depend on soil textures or root genotypes.

To address this question, we collected the soil samples from a Field Soil Column Experiment located in Bad Lauchstädt, Germany, where a maize cultivar wild type (WT) and its root hairless mutant (rth3) were grown on loam and sand (with and without corresponding decaying root of WT and rth3) with 4 independent replicate columns for each treatment. We extracted DNA from 500 mg sized soil samples collected as a composite sample of a depth of 5 to 30 cm from each column at maize growth stage BBCH19. Microbial community composition was analysed by Illumina MiSeq sequencing of 16S rRNA gene and ITS sequence PCR amplicons.

The preliminary data analysis showed that the presence of decaying roots can change the composition of the soil microbiome from rhizosheath to rhizosphere and bulk soil. The relative abundance of Proteobacteria and Myxococcota were significantly increased in the rhizosheath and rhizosphere with the presence of decaying root independent of maize root genotype and soil texture. Bulk soil with decaying root was depleted in Actinobacteriota, Gemmatimonadota but had significantly more Verrucomicrobiota, Myxococcota, Planctomycetota, Nitrospirota and Glomeromycota. Compared to maize root genotype, soil texture had more influences on both prokaryotic and fungal communities in the rhizosheath of living and/or decaying roots.

Different phyla had apparently specific preferences for decaying roots as compared to living roots, suggesting niche differentiation between decaying-root decomposer and living-root exudates assimilator. Further analysis on microbial community changes along the gradient of rhizosheath to rhizosphere and bulk soil can improve our understanding on the microbial community succession as affected by decaying roots.

[P14] SEASONAL DYNAMICS OF PROKARYOTES, FUNGI AND PROTISTS IN CROPLAND SOILS

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Cropland soils are dynamic microbial habitats subjected to strong physical and chemical changes, as a result of agricultural practice including the cultivation of different crops and soil management. In this study, we aim to depict the dynamic changes of soil microbes including bacteria, archaea, fungi, and protists (Cercozoa). Soils were collected at two-week intervals over two years at three neighboring field sites with different soil types (clay vs. loam) and tillage practices (conservative vs. conventional) in Northern Germany. The abundances and compositions of prokaryotic and eukaryotic communities were assessed by real-time PCR and by PCR-amplicon sequencing from soil DNA, respectively. Prokaryotic diversity was assessed by 16S rRNA gene sequencing, fungal diversity by ITS2 and protists by 18S rRNA genes.
Fungal abundance was higher in winter as confirmed by annual replication. In contrast, bacterial abundance peaked in the first in spring and second year in autumn, while archaeal abundance was higher in autumn and winter in the first year but decreased during winter in the second year. Thus, the three domains exhibited different seasonal dynamics. For the community composition, time-decay relationships indicated strong seasonal changes of protists (Cercozoa) followed by fungi and prokaryotes.
For protists, the relative abundance of Endomyxa-Proteomyxidea was peaked in spring, while Filosa-Sarcomonadea showed lowest abundance. For overall community composition, soil texture was the main driver of microbial community structure for all three domains. Beyond that, soil tillage altered the fungal community structure, whereas bacteria and protists were less affected. The particular impact of the cropping sequences and other agriculture practices is currently still evaluated.
In conclusion, bacteria, archaea, fungi, and protists (Cercozoa) followed different seasonal patterns, and protists appeared to be the most susceptible microbiome component to the seasonal dynamics, while fungi were more sensitive to agriculture management.

[P15] EXPLORING THE POTENTIAL INTERACTIONS OF TEMPERATE PHAGE AND THEIR PROKARYOTIC HOSTS IN BROMOXYNIL-TREATED AND CONTROL SOILS

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Aim: Bromoxynil is a selective herbicide, and its continued use may affect virus-microbe interactions in soil. Here, we explored the lifestyle of phages and their potential roles in modulating microbial communities. Our study may help to understand how viruses affect host cells in soils.
Methods: We sequenced 54 metagenomes (36 Bromoxynil and 18 Control) from soils at 0, 4, 8, 16, 29, and 64 days with triplicates. MuDoGeR (Nunes da Rocha U., bioRxiv, 2022) was used to recover metagenome-assembled genomes (MAGs). The quality of MAGs was determined by CheckM and their taxonomy by GTDB-tk. The MAGs were clustered into prokaryotic operational taxonomic units (pOTUs) at an average nucleotide identity of 0.95. VirSorter2, VirFinder, and VIBRANT were used to recover putative viral contigs, and their quality was determined by CheckV. We consider complete and high-quality contigs as uncultivated virus genomes (UViGs). PhaGCN2.0 was used to assign UViG taxonomy. Hosts were predicted using WiSH, and lifestyles using DeePhage.
Results: We recovered 307 MAGs that were clustered into 193 pOTUs (Bacteria 175 and Archaea 18). The phyla with more recovered pOTUs were Actinobacteriota (n=97) and Proteobacteria (n=36). All Archaea were affiliated with the phylum Thaumarchaeota (n=18), which encoded the genetic potential to fix carbon dioxide. We recovered 88 UViGs (temperate phages, n=77, lytic phages, n=11), and the most abundant viral families were Vilmaviridae (n=17) and Mesyanzhinovviridae (n=17). We could predict hosts for ≈61% of the temperate and ≈45% of the lytic phages. We also identified tRNAs (42.9%), head and packaging (17.9%), and DNA, RNA and nucleotide metabolism (13.5%) from 724 coding sequences with predicted function while 3,153 these coding sequences were assigned as hypothetical proteins. We identified four phages that could carry auxiliary metabolic genes (AMGs), which are involved in carbohydrate metabolism and the metabolism of cofactors.
Conclusions: The study recovered a diverse set of Prokaryotic genomes and phages from the analyzed metagenomes, with Actinobacteriota and Proteobacteria bacterial phyla and Thaumarchaeota archaeal phylum being the most recovered MAGs. The identified phages were primarily temperate and belonged to the Vilmaviridae and Mesyanzhinovviridae families, and they encoded AMGs involved in modifying host cell metabolism for their replication. The identification of tRNAs suggests that these viruses may aid the genetic potential of their hosts to produce specific amino acids or to use rare codons, which can impact the hosts' fitness and change the community's functional capacity.

[P16] MULTISPECIES BIOFILM ON DESALINATION MEMBRANE DICTATE THE FUNCTION OF THE BACTERIAL COMMUNITIES RATHER THAN THEIR COMPOSITION

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Wastewater is widely recognized as a significant and reliable water source to supplement the dwindling freshwater supply. Reclaimed WW desalination is environmentally advantageous compared to seawater desalination, as it is more economical in water recovery and energy consumption. These advanced treatment systems are increasingly becoming a strategic water management option that allows unrestricted reuse of reclaimed effluent. The main reason for the deterioration of membrane operation during water purification processes is biofouling, which has therefore been extensively studied. Biofouling was shown to reduce membrane performance reflected by permeate flux decline, reduced selectivity, membrane biodegradation, and consequently, an increase in energy consumption. Biofouling studies focused on the identification of the assembled microbial communities, in an attempt to estimate their role in biofilm development, excretion of extracellular polymeric substances (EPS), and their combined role in reduced membrane performance and lifetime. In our study, we provide a novel insight, suggesting that bacterial functions rather than composition control biofouling traits on reverse osmosis (RO) desalination membranes. We studied the potential activity of RO biofilms at metatranscriptome resolution, accompanied by the morphology and function of the biofouling layer over time, including microscopy and EPS composition, adhesion, and viscoelastic properties. To this end, we cultivated natural multispecies biofilms on RO membranes under treated wastewater flow and extracted mRNA to study their taxonomies and gene expression profiles. Concomitantly, the biofilm structure was visualized using both scanning electron microscopy and laser scanning confocal microscopy. We also used quartz crystal microbalance with dissipation (QCM-D) to characterize the affinity of EPS to membrane-mimetic sensors and evaluated the viscoelasticity of the ex-situ EPS layer formed on the sensor. Our results showed that different active bacterial taxa in five taxonomic classes were assembled on the RO membrane, and the composition changed between 48 and 96 h. However, regardless of the composition, the maturation of the biofilm resulted in the expression of similar gene families tightly associated with the temporal kinetics of the EPS composition, adhesion, and viscoelasticity. Our findings highlight the temporal selection of specific microbial functions rather than composition, featuring the adhesion kinetics and viscoelastic properties of RO biofilm. We suggest that there might be convergent solutions to the rigors of membrane biofouling that pose well-defined functional challenges that would result in effective cleaning protocols for desalinating membranes.

[P17] STRUCTURAL AND FUNCTIONAL MICROBIAL DIVERSITY IN DEADWOOD RESPOND TO DECOMPOSITION DYNAMICS

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Background: Deadwood is a key component in forests. Wood decomposition affects the accumulation and turnover of nutrients, influencing the overall forest productivity. Deadwood is primarily decomposed by fungi through a wide range of ligninolytic extracellular enzymes. Recent findings pointed out the importance of bacteria at the early stages of wood decay, while fewer data are available on the occurrence and role of archaea.
Aim: we aimed to identify changes the diversity of bacteria, fungi, and archaea in natural logs in relation to decay stage and wood properties. We also wanted to determine the main metabolic pathways involved in the decomposition process. We investigated the intra and inter-kingdom species co-occurrences across decay classes. Hypotheses were: 1) fungi are more influenced by changes in wood decay stage and C/N content; 2) the abundance of deadwood degrading genes varies according to decay stage; 3) co-occurrences reflect ecological interactions among species of the same/different kingdom.
Methods: The study was conducted in a partially mixed sessile oak forest in the Autonomous Province of Bolzano (Italy) at 550 m, with average T = 11.4 °C, and 800 mm of average annual precipitation. Plots have been established for long-term research. We sampled deadwood at different decay stages. Studies were performed through metagenomics analysis and in-vitro analysis.
Results: Bacterial alfa-diversity was affected by the decay stage and log characteristics. Log diameter influenced bacterial, fungal, and archaeal beta-diversity. We found higher abundances of cellulose and pectin degrading enzymes in bacteria, while in fungi the enzymes targeting cellulose and hemicellulose were more abundant. The decay class affected the abundance of single enzymes, revealing a shift in complex hydrocarbons degradation pathways along the decay process. Genes related to Coenzyme M biosynthesis were the most abundant, especially at early stages of wood decomposition. The overall methanogenesis did not seem to be influenced by the decay stage. Intra and inter-kingdom interactions revealed complex pattern of community structure in response to decay stage possibly reflecting both direct and indirect interactions.
Conclusions: the log should be considered as an unique and independent environment where the interaction between abiotic factors and biotic communities determines the patterns of microbial diversity. The role of bacteria in wood decomposition should be re-evaluated, especially in relation to the more labile fractions. Co-occurrences networks can indicate contrasting interactions and differential niche preferences and hence genomic studies coupled with in vitro co-culturing are recommended.

[P18] B. SUBTILIS KIN DISCRIMINATION ON MACROSCALE AND SINGLE-CELL LEVEL

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B. subtilis is a widespread bacterium and occupies diverse ecological niches and ecosystems, which leads to frequent contact with other organisms. Most studies have focused on studying genetically homogeneous biofilms, which leaves gaps in knowledge about genetically heterogeneous biofilms and their social interactions. Studies show that Bacillus subtilis can distinguish between kin and non-kin strains, and thus can express different types of social interactions. However, it is not clear how the relatedness between strains affects the formation and development of pellicles, the mechanical properties of the resulting pellicles, and how the interactions between genetically more or less related strains are reflected at single-cell level.

In this study, Bacillus subtilis strains were grown as mixtures of kin and non-kin strains under static conditions, which allowed them to form pellicles. The development of B. subtilis mixed pellicles in a batch growth system were examined with real-time interfacial rheology and confocal laser scanning microscopy. During the lifecycle of the pellicle, bacterial density at the water-air interface, water column, and solid-water interface was continuously monitored. We also investigate the interactions with mixtures of kin and non-kin strains of B. subtilis on a single-cell level, using the microfluidic approach.

We showed that microbial interactions between less related bacterial strains determine the outcome of the competition already in the water column, which affects the dynamics of the formation and disintegration of the floating biofilm and the mechanical properties of the resulting pellicle. The outcome of the competition between non-kin strains can be predictable, in the case where the initial ratio of strains is 1:1 the dominant strain always prevails, eliminating the less related strain, meanwhile in the case of more related strains, both strains coexist. At the single-cell level in the microfluidic system, spatially dispersed aggregates of less related strains were formed until the space in the micro chamber was filled. Subsequently, the dominant strain slowly and steadily reduced the density of the cell population of the less related strain and completely filled the space. In the development of biofilms of non-kin strains, there is a critical initial relationship that affects the outcome of the competition. By changing the initial fraction of strains, we have shown that the outcome of the interaction is not easily predictable, either of the two strains can prevail. In kin strains, there was a homogeneous mixing of cells and their long-term coexistence.

[P19] LIKE CLOCKWORK – THE REGULATION OF PHAGE TAIL-LIKE PARTICLE PRODUCTION IN PSEUDOMONAS

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Pseudomonas protegens CHA0 is a highly competitive plant-beneficial root-colonizing bacterium. To establish itself in an environmental niche, CHA0 relies on an arsenal of weapons to compete against different contenders, including phylogenetically closely related Pseudomonas. To target and kill kin Pseudomonas, CHA0 releases contractile phage tail-like particles, also called R-tailocins. The release of these particles involves the lysis of the producing cell. Thus, a meticulous regulation is required. Although the regulation of tailocin production has been investigated in some detail in the opportunistic pathogen Pseudomonas aeruginosa, little is known about the regulation in environmental Pseudomonas. We first looked at the expression dynamic of the gene cluster encoding R-tailocins in CHA0 following a stress by exposing a transcriptional reporter of tailocin gene expression to either mitomycin C or H2O2, two DNA damaging agents known to lead to the induction of phage tail-like particles. Then, using an RNA-sequencing approach, we found that following a DNA-damaging stress, genes involved in cell division as well as primary metabolism are downregulated, while genes involved in DNA repair such as the SOS system are upregulated. Accordingly, the R-tailocins and prophage clusters were also upregulated in response to the DNA damaging agents as their induction depends on the SOS system. Furthermore, we identified that the hns-like master regulator genes mvaT and mvaV were downregulated. We created transcriptional reporters of the expression of (i) tailocins, (ii) the negative regulator PrtR and (iii) the SOS system, that we transformed into deletion mutants of the two master regulators to better understand their roles in the control of tailocin production. We corroborated that both MvaT and MvaV contribute to the regulation of the R-tailocins gene cluster of CHA0. These findings shed light on how phage tail-like particle assembly is regulated in plant root-colonizing Pseudomonas and help us better understand how these weapons could be deployed in complex microbiomes such as those surrounding plant roots.

[P20] INTERPLAY BETWEEN BIOFILM MATRIX COMPONENTS AND SPECIES COMPOSITION IN MULTISPECIES BIOFILMS

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The biofilm matrix contributes to the settlement of microbial cells on very diverse surfaces, stabilizing biofilms and providing cells with protection against multiple hostile conditions. Moreover, the biofilm matrix can also retain nutrients, enzymes or quorum sensing molecules, facilitating social interactions among biofilm cells. Multiple studies have characterized matrix determinants and their regulation in single species biofilms, while these remain scarcely understood in multispecies biofilms. We have previously isolated and characterized a soil-derived consortium composed of Xanthomonas retroflexus, Stenotrophomonas rhizophila, Microbacterium oxydans and Paenibacillus amylolyticus showing enhanced biofilm biomass and other emergent properties compared to monospecies biofilms. Thus, we used this consortium as a model to explore the effect of interspecies interactions in biofilm matrix production and the impact of mutations in genes encoding matrix components matrix mutants in community stability. Specifically, we investigated a X. retroflexus mutant lacking the Fap amyloid, involved in adhesion, cohesion, and hydrophobicity in other species.
We combined bioimaging using fluorescent markers and fluorescent lectins and matrix proteomics, in order to quantify i) the impact of matrix mutants on species abundance and intermixing, and ii) the expression of extracellular matrix components in mono- vs. multispecies biofilms in different bacterial multispecies consortia.
Fluorescent lectin staining of mono-, dual- and multispecies biofilms evidenced differential binding to matrix glyconjugates. A galactose/ N-galactosamine polymer was enriched in dual and multispecies biofilms. Interestingly, such polymer seemed to be produced by the least abundant species, M. oxydans, but co-localized with the most abundant strain, X. retroflexus, suggesting a close interaction between these species. Additionally, comparative matrix proteomics of mono- and multispecies biofilms evidenced differential expression of secreted and surface-associated proteins, such as enzymes and flagella, respectively. Moreover, a X. retroflexus mutant lacking the functional amyloid Fap altered community composition and biofilm structure compared to a wild-type consortium.
Our data confirms that complex interspecies interactions can influence community stability and spatial organization while mutations in matrix determinants can destabilize such interactions in multispecies biofilms. Such knowledge is relevant for understanding bacterial community dynamics and stability in clinical settings and beneficial applications in microbial biotechnology.

[P21] KIN DISCRIMINATION DRIVES COOPERATIVE AND ANTAGONISTIC INTERACTIONS DURING BACILLUS SUBTILIS PELLICLE FORMATION

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B. subtilis is a soil dwelling bacterium with a diverse social life. Recently we discovered kin discrimination among highly related kin strains of B. subtilis during swarming, where less related non-kin strains showed antagonistic behaviour towards each other in the form of killing and a strong boundary formation between swarms. Because the lifestyle of B. subtilis involves biofilms and it is capable of forming biofilms on almost any surface, it is not surprising that it is the best-known model system for studying biofilm formation. However, little is known about the dynamics of genetically heterogeneous air-water biofilms (pellicles) in the context of kin discrimination. Here, we test the hypothesis that biofilms composed of kin strains exhibit similar dynamics (development of floating biofilms and mechanical properties) to genetically homogeneous biofilms, whereas biofilms composed of non-kin strains exhibit different dynamics of floating biofilm formation and disintegration and mechanical properties of the resulting pellicle.
Bacillus subtilis strains were grown in cocultures of kin and non-kin strains under static conditions, which allowed pellicle formation. The development of B. subtilis pellicles was studied using real-time interfacial rheology and confocal laser scanning microscopy. During the life cycle of the pellicle, bacterial density at the water-air interface, water column, and solid-water interface was continuously monitored. In addition, we investigated kin and non-kin interactions at the single-cell level using a microfluidic approach.
We show that kin interactions during pellicle formation led to cooperation, measured as higher biomass of kin biofilms compared to genetically homogeneous biofilms.
As expected, non-kin interactions resulted in killing of one strain and consequently to biofilm, which exhibited mechanical properties similar to those of biofilms of the dominant strain in monoculture. At the single-cell level, the dominant strain slowly and steadily reduced the presence of the less related strains, but homogeneous mixing of both populations and their long-term coexistence was observed in kin cocultures. In both systems (pellicle and single-cell level) we showed that the outcome of the interaction is not easily predictable, because either strain can gain the upper hand if inoculated at specific critical concentrations.
Our study highlights the importance of kin discrimination in cooperative interactions as well as confirms the previously discovered antagonism of non-kin strain. Moreover, our discoveries imply a broader role for kin discrimination, and our findings could potentially be implemented in the development of multi-strain probiotics, biofertilizers, and in improved fermentation practices.

[P22] DECIPHERING INTERACTIONS OF BENEFICIAL BACTERIA INOCULANTS WITH THE PLANT HOLOBIONT USING IN VITRO MICROPROPAGATED GRAPEVINES

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Engineering the plant microbiome with beneficial bacteria can improve the growth, health, and productivity of the holobiont. Such plant growth promotion (PGP) effects can be played directly by plant probiotics or can represent the result of their interactions with the endophytic community. These aspects are related to bacterial invasion, an ecological process that in plant microbiology is mostly studied in terms of pathology.
This study aimed at providing insights into the response of the plant holobiont to the delivery of beneficial bacteria, using micropropagated grapevine plants obtained via somatic embryogenesis. The adopted simplified system (i.e., virus-free specimens with an identical genetic background) allows to assess the inoculants' invasion, the effect on the plant under specific growth conditions and to decipher their complex biotic interactions with the pre-existing endophytic microbiome.
We characterized for PGP activities a collection of endophytic strains established from grapevine and lettuce collected in the field. Among the most promising strains, Rhizobium sp. GR12 and Kosakonia sp. VR04 were tagged with genes coding for fluorescent proteins and used to inoculate micropropagated grapevine cuttings. Plantlets were grown in vitro for three weeks under optimal growth conditions or on a diluted medium to mimic nutritional deficit. Afterwards the plant biomass was measured to evaluate the PGP activity of the strains, and the colonization of the plant tissues was assessed through qPCR amplification of the marker genes from the DNA extracted from plant tissues. Both the bacterial strains successfully colonized the endosphere of roots and leaves, but only Rhizobium sp. GR12 boosted the development of the root system of plantlets grown under nutritional deficit, compared to the non-inoculated ones.
We described the endophytic community of micropropagated grapevine plants by integrating high throughput 16S rRNA gene sequencing and cultivation based analyses. Bacterial endophytic community of grapevine cuttings differed from those generally associated to this plant species in the field. Moreover, the composition of the endophytic community was differently modulated by Rhizobium sp. GR12 and Kosakonia sp. VR04 and specific taxa were enriched or depleted in response to the invasion by these bacteria, reflecting the different plant response in terms of growth promotion.
Our results confirmed the importance of interplays between the plant microbiome members and their dependence upon the plant growth conditions, shedding a light on the previously hidden diversity of endophytic community in micropropagated grapevine plants and generating new fascinating questions about their ability to recruit or retain an inseparable minimal microbiome.

[P23] EFFECTS OF PESTICIDES ON SOIL MICROBIAL COMMUNITIES WITH DIFFERENT LEVELS OF PREDATOR-PREY INTERACTIONS

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The toxicity of pesticides to natural ecosystems has been a constant concern, however, the study of the ecotoxicological effects on soil microorganisms and microbe-microbe interactions has lagged behind[1]. Little is known about the effects of pesticides on soil microbial networks including microbial predators (i.e., protists). This is particularly important, as predator-prey interactions affect bacterial diversity, productivity, and the distribution of nutrients to different trophic levels[2], [3]. This study aims to assess the toxicity of etridiazole and acetamiprid in the context of a microbial food-web with varying levels of interaction complexity. Microbial communities were assembled in gamma-sterilised soils with increasing complexity of predator-prey interactions: i. bacteria only (extracted from natural soil), ii. bacteria + selected bacterivorous flagellate protists and iii. bacteria + bacterivorous protists with different feeding modes and prey preferences. Each system was prepared in quintuplicate and sampled on days 0, 7 and 21 after pesticide addition. Functional aspects were addressed by substrate-induced respiration (SIR), microbial biomass measurements, total N and C, NH_4+, NO_3-, NO_2- and PO_4^{3-} analyses. Changes in microbial community composition and abundances of functional groups are currently being assessed by Illumina 16S rRNA gene sequencing and qPCR of bacterial and archaeal nitrifiers. Our results show that at day 7 the presence of pesticides had no significant effect on the functional aspects evaluated in microcosms without protists (i.e., systems with bacteria only), while in the presence of protists a significant effect on respiration was observed. In contrast, at day 21, pesticides significantly affected half of the functional parameters tested in microcosms without protists. In the presence of protists, only respiration was affected. In addition, our preliminary analysis of the 16S rRNA gene sequencing data showed that the dynamics of the microbial communities are also influenced by the level of trophic complexity in the microcosms. We believe that our results, following a food-web assessment approach, shed light on the microbial response to pesticide exposure in soil and the role of microbial trophic complexity in this response.

Keywords: predator-prey interactions, bacterivorous protist, soil bacteria, microbial ecology

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[P24] ELUCIDATING PAIRWISE INTERACTIONS IN A SYNTHETIC HUMAN GUT COMMUNITY BY COMBINING FLOW CYTOMETRY AND UMAP

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The human gut microbiome is a complex system where hundreds of species interact with one another and with the human host. However, the interactions between many gut bacterial species still remain unknown. For this reason, we explored pairwise interactions in a synthetic human gut community. The community consists of three commensal gut bacterial species from the phylum Firmicutes namely Roseburia intestinalis (RI), Blautia hydrogenotrophica (BH) and Faecalibacterium duncaniae (FD) as well as Bacteroides thetaiotaomicron (BT) from the phylum Bacteroidota. These two phyla are known to contain the majority of the most dominant human gut microbial species. We selected these four species because they are prevalent and abundant in fecal samples and occupy different metabolic niches (BT is a primary fermenter, RI and FD are butyrate producers and BH is an acetogen). In addition, BT is differentially abundant across alternative human gut microbial community types known as enterotypes. Co-cultures were grown, without pH control, in a high-throughput fermentation robot in chemostat mode , with fresh medium pumped at a flow rate of 6.9 µL/min after 16 hours of inoculation.. Flow cytometry (FC) data was collected and analyzed using UMAP for six different pairs for time series data, which distinguished between live and dead cell populations based on propidium iodide (PI) staining and separated them from non-cell particles (gating). Gated live cells were then classified according to species. The live/dead stained cell counts showed that the dead cell count (DCC) was between 1.5 and 24.6% of the total cell counts (TCC) for all the pairs except for BH/BT which displayed a significantly higher DCC (ranging from 1.96 to 57.7% of the TCC) and a steep drop in the live cell counts (LCC) after 120 hours since the inoculation. The time series data showed that the LCC for the pairs BH/BT and BT/FD diminished significantly after 120 hours whereas LCC in the other co-cultures remained stable over 120 hours. The TCC calculated using UMAP were significantly correlated with the optical densities (OD) for all the pairs except for BT/FD, which showed that the results obtained from these two methods were comparable for most pairs. In summary, we have shown that co-culture LCC and their variability over time depend on the species combination. In the next step, we plan to collect metabolite data for this system to resolve interaction mechanisms.

[P25] DIVERSITY, CO-OCCURRENCE AND NETWORK ANALYSIS OF THE RHIZOSPHERE MICROBIOTA IN RESPONSE TO INOCULATION OF WINTER WHEAT WITH HARTMANNIBACTER DIAZOTROPHICUS E19

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Winter wheat (Triticum aestivum) is used for bread production and is one of the most important economic crops, along with barley, rice, and maize. Due to the massive environmental impact of fertilisers, like their accumulation in groundwater and eutrophication of surface waters, environmentally friendly solutions are needed to reduce fertiliser use in crop production. Inoculation with plant growth promoting rhizobacteria, like Hartmannibacter diazotrophicus E19 that reduces the stress responses of the plant, is one more sustainable way of promoting plant growth. To analyse the impact of added bacteria on the surrounding soil microbiome of a plant, seeds of winter wheat (Aristaro) were inoculated with the plant growth promoting rhizobacterium H. diazotrophicus E19. The seeds were sown on fields of the organic farm Gladbacher Hof (Hess, Germany), where different plots were prepared with E19 inoculated seeds, and with and without manure fertilisation (4 replicates per treatment). Bulk soil samples were taken before sowing and after harvest. Bulk soil, rhizosphere soil and root samples of the winter wheat were taken at flowering and ripening. After DNA extraction and amplification with bacterial specific primers (16S rRNA gene), fungal specific primers (ITS2 region) and cercozoan specific primers (18S rRNA gene) the amplicons were sequenced with high-throughput Ion Torrent metabarcoding. The analysis of the influence of H. diazotrophicus E19 on the bacterial, fungal and cercozoan microbiome of winter wheat without and with an organic fertiliser will show which effect the plant-growth promoting bacteria has on diversity, co-occurrence, and network of the soil microbiome of the plant rhizosphere.

[P26] MICROBIOME-FUNGICIDE TEBUCONAZOLE INTERACTIONS ALTERS THE STRUCTURE AND DIVERSITY OF FUNGI AND BACTERIA IN A BIOFILM/WATER SYSTEM

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Fungal-bacterial biofilms dominate microbial life in streams and small rivers and constitute an important component for the global biogeochemical cycling, including pesticides. They are also an important source of food for many consumers, yet fluvial biofilms remain poorly explored. Experimental validation of current environmental pollution scenarios is urgently needed to accurately predict the responses of fluvial biofilms to pesticides. The fungicide tebuconazole (TBZ) restricts fungal growth by inhibiting ergosterol biosynthesis. Thereby, pollution of TBZ into agricultural rivers is a threat for freshwater ecosystems. Our study aims to assess the impact of TBZ on fungi-bacteria-algae biomass, diversity, and interactions in stream biofilms, as well as the role of contaminant pre-exposure history on microbial dissipation of TBZ. Natural biofilm communities were harvested from a Swedish stream and exposed to environmental concentrations of TBZ (10 and 100 µg/L) for 24 days under laboratory conditions. Diversity and community structure of fungi, bacteria, and algae were assessed using ITS and 16S amplicon sequencing, pigments and ergosterol were quantified by HPLC/LC-MS, and fatty acids composition was determined by GC-MS. The dissipation capacity was studied using 96h TBZ kinetics tests, where LC-MS analysis of TBZ and its main metabolite hydroxy-tebuconazole (TBZ-OH) in the water column revealed that TBZ exposure did not affect the microbial biotransformation of biofilm, which accounts for approximately 10% of tebuconazole removal from the water column. Fungi represented the most sensitive microbial group, where a 40% inhibition of the biomass was seen at 10 µg/L TBZ. Cyanobacteria were more sensitive than algae and bacteria to TBZ, with a 50% reduction of total biomass at 100 µg/L TBZ. The 16S rRNA sequencing revealed changes in the relative abundance of the main prokaryotic phyla in response to TBZ exposure. The observed increase in prokaryotic diversity under TBZ exposure was associated with TBZ effects on dominant prokaryotic taxa. Results from ITS2 amplicon sequencing will reveal the impact of TBZ on fungal diversity, identify tolerant taxa to the pesticide, and changes in fungi-bacteria interactions. Overall, this study demonstrates that TBZ poses risk for freshwater systems due to its limited microbial biodegradation and high toxicity to fungi.

[P27] ENHANCED MOTILITY BEHAVIOR IN A MULTISPECIES COMMUNITY

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Aim:
Bacterial motility is a way bacteria can conquer new environments and decrease their competition when scoping for resources. There are many ways bacteria can move around; one such mechanism, swarming, is the locomotion of a population of flagellated bacteria on a semi-solid surface. There are many unknowns about bacterial swarming, especially in a multispecies community. While past studies have analyzed bacterial interactions in multispecies biofilms and determined their overall effects, there is a knowledge gap in the mechanistic drivers which underpin the biofilm's community intrinsic properties. In this study, we focused on understanding the swarming behavior of a four species community, which has known synergistic biofilm formation capabilities in vitro. By studying the motility patterns of this community using swarming assays, microscopy techniques, and ratio assessments, we aimed to gain insights into the mechanistic drivers of this behavior.

Methods:
Previously, four strains were co-isolated from the soil residuesphere- Stenotrophomonas rhizophila, Xanthomonas retroflexus, Microbacterium oxydans and Paenibacillus amylolyticus. Swarming assays were conducted with mono, 2-species, 3-species, and 4-species combinations. The effect of adding surfactin, a known swarming compound excreted by Bacillus subtilis, to the non-swarming community members was assessed. Also, the presence of flagella was confirmed in all four strains using transmission electron microscopy. Finally, species ratios were assessed at different positions of the swarms to understand the temporospatial dynamics of swarm development.

Results:
When alone, P. amylolyticus had swarming abilities. The other community members, M. oxydans, S. rhizophila, and X. retroflexus, were non-swarmers when assessed individually. However, X. retroflexus was a swarming enhancer; combinations with both P. amylolyticus and X. retroflexus showed significantly faster swarming initiation. Nonetheless, when the 4-species community was inoculated together, swarming was initiated fastest. Also, the addition of surfactin allowed the typically non-swarming bacteria to demonstrate increased motility capabilities.

Conclusions:
By monitoring the locomotion of the 4-mix community in swarms, we identified key players of this emergent property. Deeping our understanding of bacterial motility patterns gives us an insight into the establishment of hierarchal head-starts in the race for resources.

[P28] DROUGHT AS A MODULATOR OF GENE TRANSFER BETWEEN BACTERIA IN THE RHIZOSPHERE

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Aim: The rhizosphere is widely recognized as a hotspot of diversity and social interaction between bacteria and plants. Bacteria cells are important carriers of mobile genetic elements, such as plasmid, which can be transferred to the bacterial community allowing the acquisition of new genetic traits that can help the adaptation of bacteria under harsh conditions. In an environment such as the rhizosphere, where the plant activity and the plant physiologic state modulate the bacteria community composition, the role of the plasmids could be an important insight of the evolutionary change of the community. Here, we evaluated how Arabidopsis plant under drought conditions can modulate the gene transfer in the rhizosphere.

Material and Methods: Arabidopsis seeds were sowed and then grown in pots until the plants were mature (4 weeks). Pseudomona putida carrying the plasmid pKJK5 (KanR, TetR) tagged with green fluorescence protein (gfp) was used as the donor, and the rhizosphere bacterial community as the recipient. The donor was spread into the rhizosphere, and samples were taken from plants under drought treatment (no water added) and regular watering (control) after 3 and 7 days. Quantification by flow cytometry, sorting and 16S rRNA metabarcoding analysis was used for transconjugant detection and identification.

Results: Transconjugants cells were observed and sorted after 3 days post-donor inoculation under both (regular watering and drought) treatment. However, after 7 days, no transconjugant cells were observed in drought conditions. The 16S rRNA metabarcoding analysis showed changes in the abundance and richness bacteria communities under drought compared to regular watering on 3 and 7 days. The genera Glutamicibacter was found as the most abundant transconjugant under drought whereas Pseudomonas was under regular watering.

Conclusions: This study reveals that drought changed the bacterial community composition in the plant rhizosphere, modulating the plasmid transfer, particularly in transconjugant taxa.

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[P29] INFLUENCE OF MICROBIAL SECONDARY METABOLITES ON THE INTERACTION BETWEEN THE TWO MARINE BACTERIA, PSEUDOALTEROMONAS, AND PHAEOBACTER

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Secondary metabolites play a significant role in shaping microbial communities and confer important physiological and ecological functions for the producing organism. They are mostly known for their antimicrobial activities and a common assumption is that they also provide the producer with a competitive advantage in natural environments. Marine bacteria from the Phaeobacter and Pseudoalteromonas genera have been isolated from Danish coastal areas and both contain species that produce potent secondary metabolites with antimicrobial effects. Several Phaeobacter species can produce the antibiotic metabolite, tropodithietic acid (TDA), which has a broad antibacterial activity, and several Pseudoalteromonas species produce bromoalterochromides (BACs) with antimicrobial properties. Despite these activities, Phaeobacter and Pseudoalteromonas species co-exist in the same environmental niche.

The purpose of the present study was to investigate the interaction in co-cultures of these two antibiotic-producing species. Biosynthetic gene clusters were detected by antiSMASH in both P. piscicida and Phaeobacter sp. and we hypothesized that BACs and TDA were the most influential antibacterial compounds in the interspecies interaction. We established a liquid co-cultivation setup of the two bacteria and using mass spectrometry-based metabolomics we detected significant changes in the global metabolome due to interactions between the two species during liquid co-cultivation. Pseudoalteromonas features dominated in terms of abundance and production of metabolites whereas TDA could not be detected in the liquid co-culture system.

Based on mass spectrometry imaging of interacting cultures, we hypothesized that BACs were important for the interactions and caused a decrease in TDA production. We, therefore, examined the metabolome of Phaeobacter grown with sub-inhibitor concentrations of BACs. This caused a significant change in the global metabolome but did not affect TDA production.

In conclusion, we have established model systems allowing the co-existence of two antibiotic producing bacteria and demonstrated that when co-existing, their metabolome is changed as compared to pure culture growth. Also, the secondary metabolites of one culture are likely responsible for the metabolome change of the other.

[P30] SOIL BACTERIA INTERACTIONS REGULATE PYOLUTEORIN PRODUCTION IN PSEUDOMONAS PROTEGENS DTU9.1

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Bacterial communities are involved in intricate interactions, which can play an important role in the plant rhizosphere. Some members of the soil microbiome are beneficial to plants as they produce antifungal and antimicrobial agents that kill some of the rhizosphere-colonizing parasites. Our model strain- Pseudomonas protegens DTU9.1 (DTU9.1) – has the potential to be a biocontrol agent due to the production of a comprehensive array of secondary metabolites (SMs). One of the prominent SMs in DTU9.1 is an aromatic PKS-NRPS hybrid antibiotic Pyoluteorin (Plt). Albeit this antibiotic is a well-studied and described molecule there have been but a few studies that look into the social perspective that Plt plays in bacterial communities. In this study, we are placing Plt in an ecological context and describing the working mechanism in Plt regarding bacterial interaction. Our findings show a significant increase of pyoluteorin production upon interaction with various bacterial strains, which adds another layer of regulatory complexity to the Plt production pathway. Bacterial interactions illustrate another angle to investigate the regulation and production of Plt and give us a more complete picture of what happens in the soil. Overall, this study aims to shed a light on yet uncovered microbial mechanisms that govern Pyoluteorin biosynthetic gene cluster regulation. This will further broaden our understanding of SM induction, production, and overall natural roles in the soil community.

[P31] UNDERSTANDING ENVIRONMENTAL TRIGGERS FOR ANTIBIOTIC RESISTANCE DUE TO EFFLUX PUMPS.

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The control and function of antibiotic resistance due to efflux pumps is dependent on a range of environmental conditions. In order to develop a clearer and more detailed picture of the triggers for these efflux pumps, we studied the response of Stenotrophomonas maltophilia, a highly versatile environmental bacterium that colonizes various niches (Chauviat et al., 2023). S. maltophilia can survive phagocytosis by amoeba and even multiply within them (Denet et al., 2018). Among the genetic determinants, the presence of numerous efflux pumps including the Resistance Nodulation Division (RND) family in the S. maltophilia core genome (Youenou et al., 2015) could explain this adaptation to amoeba. Very little is known about the natural conditions under which these pumps are expressed, and the few known inducers are associated with plant and amoeba interactions. Previous work has demonstrated that some S. maltophilia RND pumps were expressed when the bacterium was exposed to the supernatant from the S. maltophilia- Acanthamoeba castellanii co-cultivation (Denet et al., 2020). Thus, the pumps could participate in the bacterial adaptation to various niches. This raises questions regarding the environmental conditions under which the pumps are expressed and the molecules that can trigger their expression.
Our work focused on studying the expression of bacterial efflux pumps in amoeba and identifying the determinant(s) involved in the induction of these systems. For this purpose, transcriptional reporters of the expression of RND pumps were constructed to track bacteria during their interaction within amoeba and to simultaneously monitor pump expression using time-lapse confocal microscopy. We were able to determine that specific efflux pumps are expressed in amoeba. To determine which molecule(s) produced during the bacteria-amoeba interaction could trigger RND pump expression, bioguided fractionation combined with feature-based molecular networking were used to identify active fractions and candidate trigger(s). All these data provide a better understanding of the conditions of pump expression and their contribution to the emergence and dissemination of antibiotic resistance in the environment.

[P32] EFFECT OF CLAY (ILLITE) ON THE GROWTH OF PROKARYOTIC NITRIFIERS IN SOIL AGGREGATES

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Studying the interactions of microbes with their edaphic surroundings is vital for understanding the role they play in biogeochemical cycles, e.g., nitrification. Evidently, soil texture has shown to be one of those factors that control microbial diversity and contribute to their community assembly. Clay minerals in particular have higher electrostatic charge and larger surface area compared to other soil particles. As a result, increasing clay content may enhance soil aggregates stability and therefore decrease the rate of water and nutrients diffusion. We hypothesize that such challenging conditions may favor organisms that are better adapted to slow nutrient release (i.e., oligotrophs versus copiotrophs) especially in the isolated nutrient-depleted soil aggregates.

In a microcosm preliminary experiment, we preincubated 30 g of unfertilized soil with sterile illite particles (200% of the original clay content) for 10 d along with a no-illite control. Chitin and chitin + NH_4+ were used as substrates, and soils were incubated for 8 d including no-substrate controls. DNA was extracted from individual soil aggregates (2-4 mg, n = 6 x 6). Bacterial and archaeal 16S rRNA, as well as amoA gene for ammonia oxidizing bacteria (AOB) were quantified at the day of substrate addition, day 4 and 8 from all soils.

We observed no significant change in the bacterial and archaeal communities in both the illite amended soil and the control, except for a decline in the no-substrate control over time ($P<0.05$). However, we observed a significant decline in the amoA gene copy numbers in the illite amended soil compared to the unamended ($P<0.05$) only in the no-substrate control.

These initial results are calling for more in-depth analyses of the prokaryotic nitrifiers including taxa with putative oligotrophic lifestyle, e.g., Comammox. Ultimately, we aim to better understand the effect of clay minerals on microbial community composition and growth which may help predict activities of these bacterial taxa across different soil textures. Studies on the aggregate scale, moreover, provide a clearer perspective of soil biogeochemical processes and their major biotic and abiotic influencers.

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[P33] EXPLORING TEMPORAL PATTERNS OF PROKARYOTIC FUNCTIONAL GENE ABUNDANCES RELATED TO N-CYCLE UNDER DIFFERENT AGRICULTURAL MANAGEMENTS

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Due to the frequent application of nitrogen (N) fertilizers in cropland soils there is a strong selective pressure and changeable conditions for microbial communities mediating the biogeochemical cycling of nitrogen. Here we analysed the effect agricultural management on the abundance of prokaryotic genes mediating the transformation of nitrogenous compounds in order to elucidate their seasonal patterns and indicate functional interactions between them.
A total of seven functional genes were analysed from directly extracted soil DNA by means of quantitative real-time PCR. Soil samples were collected over a period of two years at two-week intervals from three neighbouring fields of a farm in Northern Germany, to quantify the following genes: nifH, archaeal/bacterial amoA, nirK, nirS, nosZI and nosZII). The three fields differed in soil textures (one clay vs. two loam) and by tillage managements on the loam field (conservative vs. conventional).
Copy numbers ranged from 5.62 x 10^6 to 5.4 x 10^{10} genes per gram dry soil. Across both years nosZI was the most dominant and nirS the least. The archaeal version of amoA was more abundant than the bacterial. The abundances of nifH, nirS, nosZI and nosZII exhibited similar seasonal dynamics over two years. These genes were more abundant in spring and summer, after which they decreased in abundances during autumn and remained at the relatively lower values until recovering during the next autumn. Bacterial and archaeal amoA gene abundances showed opposite trends throughout the monitoring years, during which bacterial amoA gene abundance decreased continuously after peaking at spring of the first year while archaeal amoA gene abundance had a final increase during the second autumn. Across two years, the field with clay soil harboured the highest abundances of amoA, nifH, nirS and nosZII as compared to both loam fields. In the loam fields, the bacterial amoA genes were more prevalent with conservative tillage while archaeal amoA genes were more abundant in the conventional tillage field. This suggests that nitrifiers of both groups were more responsive to soil tillage compared to other examined functional genes.
In conclusion, the study showed different seasonal dynamics of functional gene abundances involved in soil N cycling over two years. The effect of additional factors linked to the agricultural managements and cultivation of different crops on the functional gene abundance during the two years is currently further analysed.

[P34] NON-KIN INTERACTIONS RESTRAIN THE SPREAD OF EXPLOITING SURFACTANT MUTANTS

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Bacillus subtilis is capable of cooperative group movement over surfaces, referred to as swarming, where secretion of surfactants is essential for bacterial surface spreading. Surfactants are regarded as a public good and surfactant non-producers as cheaters which by avoiding metabolic costs of surfactant production gain a reproductive advantage. The high probability of exploiter proliferation underlies a long-standing dilemma on the evolutionary stability of cooperative behaviours. A potential mechanism predicted to stabilize cooperative behaviours is kin discrimination (KD), whereby individuals help kin and ignore or harm non-kin. However, strong experimental evidence supporting this hypothesis in bacterial groups is lacking. We here use the social bacterium B. subtilis to test whether KD is a cooperation stabilizing mechanism during experimental evolution. We find that surfactant-deficient cheaters (ΔsrfA mutants) are helped by the isogenic co-operator, but after several re-inoculation cycles this strategy leads to “the tragedy of the commons”. In contrast, the swarming deficient cheaters are not helped by the non-kin surfactant producer. Hence, if cheaters and co-operators are swarming together in a mixed swarm (1:1 initial ratio), the cheaters, after encountering the non-kin swarm, lose their reproductive advantage. Moreover, repeated exposure of the wild-type swarmer to encounters with swarms of different relatedness (isogenic, kin, non-kin) revealed that after the 20th re-inoculation cycle, the swarming deficient cheaters evolved more frequently in populations with isogenic or kin encounters compared to populations exposed to non-kin encounters. These results provide strong support to the theory stating that the evolution of cheaters is hindered by kin discrimination.
Cooperative behaviours such as swarming, and biofilm production are important for bacterial survival and stability of microbial communities. Therefore, to improve strategies to manipulate microbial communities for disease control or bioremediation it is important to better understand how cooperation is maintained and evolves.

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[P35] CONTROLLING SPECIES WITHIN A SMALL BACTERIAL COMMUNITY

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Bacteria live in rich communities forming intricate interaction webs. We are often faced with the need to control individual bacterial species, positively or negatively, to solve critical situations like pathogen infections (e.g. by using antibiotics) or destabilized microbiomes (e.g. by using probiotics). Current approaches tend to dismiss the community context in which bacteria live, only considering the species that needs to be targeted, which can lead to treatment failures as the behavior of individual species strongly depends on other species with whom they interact. To determine if ecological knowledge (e.g. interspecies interactions) can guide the design of more efficient control strategies for bacteria imbedded in communities, we used a 4-species synthetic bacterial community composed of: Agrobacterium tumefaciens (At), Comamonas testosteroni (Ct), Microbacterium saperdae (Ms) and Ochrobactrum anthropi (Oa). We compared two approaches to negatively target At: (i) a direct control strategy where the community context was ignored and an antibiotic (ampicillin) was used to eliminate At; (ii) a strategy comprising direct and indirect control, where in addition to the antibiotic, we fed At's competitors (Ct and Oa) with an additional carbon source (citrate). We performed serial transfer experiments in minimal medium on the whole community as well as on individual monocultures and assessed the outcomes of our two strategies over an evolutionary timescale. To our surprise, when we targeted At directly with ampicillin only, it survived systematically within the community, but died in 2 out of 4 replicates when ampicillin was combined with citrate. Interestingly, the lethality of the citrate-ampicillin combination to At was also higher than just ampicillin in monoculture. We hypothesized that citrate could increase the efficacy of ampicillin, due to its low pH. Further experiments showed that an acidic pH increases the potency of ampicillin (lower minimal inhibitory concentration) against At, a phenomenon that has already been described for other bacteria. Our new hypothesis then, is that species that acidify the environment may be used to indirectly control a target species by increasing antibiotic potency against it. Future work will focus on identifying such pH-modifying species that could be administered together with the antibiotic to target our focal species At. If the principle generalizes to other target species, such as human pathogens, our findings will be important for treating gut or urinary tract infections, for example, as tailored antibiotic treatments could be informed by ecological context.

[P36] DISCOVERY OF NEW ANTIBIOTIC POTENTIALS THROUGH CO-CULTURE OF MARINE BACTERIA PSEUDOALTEROMONAS LUTEOVIOLACEA AND VIBRIO CORALLIILYTICUS

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Multidrug-resistant bacterial infections are a major healthcare burden and novel antibiotics are urgently needed. 60% of currently used antibiotics are derived from microorganisms, mostly soil-dwelling Streptomyces, however, marine bacteria have a large untapped potential for producing novel chemistry and can have a key role in combating bacterial pathogens. The purpose of the present study was to explore marine bacteria for novel antibiotic potentials using a co-culture approach, to mimic their natural environment.

Pseudoalteromonas luteoviolacea (strains S2607 or S4060-1) and Vibrio coralliilyticus S2052 have been genome sequenced and are known to produce a broad spectrum of chemicals, and genome-mining by antiSMASH indicate several BGCs associated with unknown chemistry. Both strains produce several antibiotics and other bioactive compounds, for instance, strains S2607 and S4060-1 produced both violacein and pentabromopseudilin and strain S2052 produced andrimid, as mono-cultures. To mimic the natural competition environment and elucidate antibiotic production, we established a stable liquid co-cultivation setup for P. luteoviolacea (strains S2607 or S4060-1) and V. coralliilyticus S2052 allowing these two potent antibiotic-producing bacteria to stably co-exist. The bacteria were co-cultured on an agar surface embedded with Vibrio anguillarum 90-11-287 (fish pathogen) or Staphylococcus aureus 8325 (human pathogen) and the zones of inhibition in the pathogens were larger during co-culture than when the marine bacteria were tested as mono cultures. The same pattern of inhibition was also recorded in the agar well-diffusion assay where supernatants of mono- and co-cultures of the marine bacteria were tested against the pathogens. Furthermore, MIC measurements of co-culture supernatants demonstrated an increased antibacterial activity over mono-culture supernatants.

To reveal the metabolomics profiles, LC-MS and Global Natural Products Social Molecular Networking (GNPS) are used to cluster structurally related compounds with comparable MS/MS fragmentation patterns. Current preliminary metabolomic analysis revealed an augmentation in the production of stimulants in addition to the discovery of potential (novel) metabolites in the co-culture, causing the increased antibacterial efficacy. Most of the discriminating chemical features were related to bioactive potentials, that are reported from P. luteoviolacea (S2607 or S4060-1) here for the first time in co-culture with V. coralliilyticus S2052. Interestingly, this co-culture approach allows us to speed up the discovery of new antibiotics and their associated biosynthetic pathways. Our results suggest an efficient, integrative strategy for exploring chemical richness in co-culture as well as linking chemistry to uncover the full biosynthetic potential.

[P37] SHORT-TERM (CO-)ADAPTATION IN BIOFILMS OF LACTOCOCCUS LACTIS AND LEUCONOSTOC MESENTEROIDES IMPACTS GROWTH PARAMETERS AND INTERSPECIFIC INTERACTIONS

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Aim: In nature, bacteria coexist in polymicrobial complex communities dominated by interspecies interactions. This coexistence contributes to a constant emergence of variants, but their establishment depends on eco-evolutionary dynamics. Spatially structured and heterogenous biofilms enable niche differentiation and local interplay, supporting greater diversity. This study examines the adaptation and evolution of mono- and co-cultured Lactococcus lactis and Leuconostoc mesenteroides in a biofilm-selecting environment.

Methods: Cultures were passaged every 24 h by bead transfer (biofilm) for 16 days. Evolved variants and ancestors were compared in their growth parameters, competitiveness in reestablished co-cultures (quantitative PCR and confocal laser scanning microscopy), and global proteomes.

Results: Evolution experiments displayed that, in biofilm co-cultures, L. mesenteroides predominated, but both species coexisted. Comparative analyses of L. lactis biofilm variants revealed, in general, an increased biofilm formation but, interestingly, increased culture yield and prolonged generation time exclusively in co-evolved variants. Furthermore, the performance of evolved L. lactis variants was evaluated when co-cultured with L. mesenteroides. Biofilm assays showed a higher proportion of evolved L. lactis strains than their ancestor when co-cultivated with ancestral L. mesenteroides. This effect was particularly evident in low L. lactis starting ratios. Interestingly, in co-cultures with evolved L. mesenteroides, L. lactis was strongly reduced. However, evolved L. lactis strains were more persistent than their ancestor in such conditions.
Combined, this shows that coexistence in biofilms selects for variants adapted to the biofilm life cycle and the other member's presence, reflected in their enhanced persistence and prevalence in the mixed biofilm. Preliminary proteomic analyses of mono- and co-cultures suggest that proteomes of mono- and co-evolved L. lactis variants are more distinguishable by the selected ancestor (lineage) than the cultivation method.

Conclusions: This study emphasizes spatially structured biofilms' importance in maintaining species diversity, as interspecies interactions and the biofilm setting were conditional for the emergence of novel phenotypic variants.

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[P38] THE GLOBAL REPRESSORS MVAT AND MVAU REGULATE CRISPR-CAS ACTIVITY IN PSEUDOMONAS AERUGINOSA BY CONTROLLING GROWTH RATE

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CRISPR-Cas is an adaptive immune system of bacteria and archaea that protects against foreign genetic material. CRISPR arrays store the memory of previous infections as short nucleotide sequences called spacers. These are transcribed and processed into mature CRISPR-RNAs that guide the Cas-mediated cleavage of intruding foreign genetic elements, including plasmids. Despite intensive research on CRISPR-Cas function, our knowledge of the factors controlling CRISPR-Cas activity is still limited. The human pathogen Pseudomonas aeruginosa employs the cell-cell signaling system, called quorum sensing, to activate the expression of CRISPR-Cas at high cell density. Additionally, lower temperatures, which slow the growth rate, increases CRISPR-Cas activity in P. aeruginosa. In Escherichia coli, the conserved global regulator H-NS represses CRISPR-cas. While P. aeruginosa encodes two partially functionally redundant h-ns homologs, called mvaT and mvaU, that coordinately act as global repressors, their role in CRISPR-cas regulation in this bacterium has not been explored.

In this study, we show that compared to the parental P. aeruginosa strain, a mutant deficient in both MvaT and MvaU had an order of magnitude higher CRISPR-Cas activity, measured by the efficiency of transformation when encountered with a CRISPR-recognized plasmid normalized to that of a control plasmid. Similarly, cultivation of plasmid-harboring cells revealed that the ΔmvaT ΔmvaU mutant strain had an order of magnitude higher loss of the CRISPR-targeted plasmid compared to the parental strain, suggesting that MvaT and MvaU inhibit CRISPR-Cas activity. However, our preliminary data show no significant difference in cas3 mRNA or Csy4 Cas protein abundance between the ΔmvaT ΔmvaU mutant and the parental strain. Importantly, the ΔmvaT ΔmvaU mutant had a significantly slower growth rate compared to the parental strain. Thus, while our data show increased CRISPR-Cas activity in the absence of both MvaT and MvaU; this could be a direct consequence of the decreased growth rate in the double ΔmvaT ΔmvaU mutant, as a slower growth rate has previously been demonstrated to enhance CRISPR-Cas activity in P. aeruginosa.

[P39] ARBUSCULAR MYCORRHIZAL FUNGI AND AMMONIA OXIDIZER INTERACTIONS IN ORGANIC AND INORGANIC NITROGEN FERTILIZED SOIL

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As nitrogen (N) is essential for plant growth, inorganic and organic N fertilizers are applied to agriculture soil to increase crop yields. However, inefficient use of fertilizer N results in N pollution through nitrate leaching or emissions of the greenhouse gas nitrous oxide. Most plants form symbiotic relationships with arbuscular mycorrhizal fungi (AMF) that could enhance ammonia and nitrate uptake from soil and impact N-cycling. Nitrification is a key step in the N-cycle where ammonia oxidizing bacteria (AOB) and archaea (AOA) convert ammonia from fertilizers into nitrite which is subsequently oxidized by nitrite oxidizing bacteria to nitrate, or by complete ammonia oxidizers (comammox) who perform the full process. Interaction between AMF and ammonia oxidizers (AO) for N could be a mechanism by which nitrification is impacted. Preference of N source by AMF (ammonia over nitrate and from organic N is predicted) and AO groups (AOB likely favouring ammonium-based fertilizer and AOA organic N derived ammonia) could also determine microbial interactions. A model wheat-AMF mesocosm system was used to test the hypothesis that distinct N-cycling microbial communities will be selected due to AO niche differentiation and AMF N uptake. Mesocosms were applied with either inorganic ammonium, slow-release ammonium fertilizer or plant-based organic N. Synthetic nitrification inhibitors (SNIs) were utilized with the aim of partitioning AMF interactions between AO groups, and 15N-ammonium used to trace plant-AMF N uptake. Based on qPCR of N-cycling genes, the relative abundance of AOB increased with all fertilizer additions while, as predicted, significant increase in AOA abundance was greatest after organic N addition, followed by slow-release fertilizer, compared to inorganic or without N. There was a trend of lower relative abundance of AOB and AOA in mesocosms with high AMF colonization relative to uninoculated soil with minimum AMF root colonization in unsterilized soil. As the high AMF system had greater root biomass, AMF may have directly and indirectly contributed to the decrease in AO abundance. Soil applied with SNIs had relatively high ammonium and low nitrate concentrations and the relative abundance of AOB and AOA was significantly lower after SNI application for all fertilizers. However, SNIs did not reveal clear relationships between N concentrations, AO abundances and AMF systems. In conclusion, AMF may impact AO communities across a range of fertilizers. Implications of AMF on AO activity and nitrous oxide emissions are being explored and use of AMF successfully mitigating N pollution from agriculture soil evaluated.

[P40] MICROBIAL INTERACTIONS: UNVEILING SYNBIOTIC EFFECTS ON SALMONELLA IN CHICKEN CECAL MICROBIOTA WITH POLYFERMS FERMENTATION MODEL

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The chicken caecum is inhabited by a diverse population of microorganisms that live in different lifestyles and communities (planktonic cells- lumen and structured biofilms - gut mucosa and food particles). Biofilm formation by Salmonella in the chicken caecum is known to contribute to its persistence in the host, with consequences on transmission into the food chain and environment. Efficient strategies that reduce Salmonella prevalence and transmission are therefore needed.
Here, we aim to investigate the potential of a synbiotic product (prebiotic combined with a multi-species probiotic) to inhibit the growth of the prevalent multidrug resistant (MDR) Salmonella enterica serovar Infantis (S. Infantis) in the chicken caecal microbiota.

We used a novel in vitro experimental approach based on the continuous PolyFermS chicken caecum microbiota model to investigate spatial microbial interactions in biofilm communities in parallel to planktonic communities. Chicken microbiota, S. Infantis and probiotics were immobilized into porous gel beads and continuously cultivated for 12 days. The beads served as an in vitro biofilm microbial growth model and continuously seeded the planktonic community. Pathogen and probiotics were quantified with qPCR, and the community was analyzed with 16S rRNA gene sequencing and the microbial metabolites with HPLC.
Microscopy analysis showed that immobilized bacteria form biofilms and distinct microcolonies in the beads. The effect of the synbiotic on the pathogen fitness during fermentation was microbial lifestyle-dependent. Pathogen inhibition (0.5 Log gene copies/mL) was detected in planktonic but not in the biofilm microbiota. The five probiotic species colonized chicken microbiota during continuous cultivation with the highest quantity in the biofilm microbiota. Compared to the control, the synbiotic promoted the production of fermentation metabolites such as acetate (+23%) and butyrate (+22%). Based on 16S rRNA gene sequencing, the composition differed between planktonic and biofilm microbiota, where synbiotic treatment promoted the relative abundance of Firmicutes (Eisenbergiella taxa) and reduced the relative abundance of Proteobacteria (Escherichia-Shigella and Proteus taxa) compared to controls.

Our results demonstrate that the synbiotic effect on pathogen fitness in a complex chicken caecal microbiota depends on the community lifestyle. Furthermore, the study highlights the potential of the in vitro PolyFermS model to provide insights into complex microbiota interactions in the chicken caeca during pathogen colonization and synbiotic treatment.
These findings represent a crucial starting point for more extensive omics-based analyses aimed at unraveling the underlying mechanisms driving microbial community dynamics and their responses to synbiotic interventions at the microscale.

[P41] BACTERIAL HETEROGENEITY PRODUCED BY REGULATION OF CRISPR-CAS TYPE I-F IMMUNITY

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Bacteria need to protect themselves from infection and killing by bacterial viruses (phages), which outnumber them in larger quantities in diverse environments. For these reasons, bacteria have developed a sophisticated arsenal of defence mechanisms that can protect individual cells or the overall bacterial population. Individual protection is achieved via systems such as CRISPR-Cas, that are adaptive and of great interest for a wealth of biotechnological applications. Despite the large interest, their regulation and control under external signals and extracellular factors is poorly understood. Using Pseudomonas aeruginosa and the endogenous Type I-F systems our model study, we have engineered a series of fluorescence-based reporter plasmids to track and identify different factors that are regulating the CRISPR-Cas loci. Our data shows that this locus is governed by different promoter regions, regulating different aspects of the system, resulting in a heterogenous expression. We observed that growth rate directly modulates expression and that a subpopulation of cells expresses the CRISPR-Cas system at different growth stages maintaining a constant level of immunity. Furthermore, our results show that viral infection can stimulate the CRISPR-Cas promoters under our experiment conditions, suggesting that not only quorum-sensing signals could be regulating the system. Using our reporters, we sorted populations that reflect a higher immunity and susceptibility; and challenged these with high doses of viral particles. We found that viability is slightly affected, while phage production increases in the susceptible population. We identify that when challenged, most susceptible cells had turned into a similar profile to the immune cells where the expression of GFP correlates to an increase of CRISPR-Cas activity. Our experimental findings indicate that herd immunity is important in bacterial communities, allowing for stable coexistence of bacteria and phages. We believe that such transcriptional heterogeneity can afford cells with bet-hedging opportunities that enable them to regulate the system, allowing them to adapt and survive in changing and stressful environments.

[P42] EXPERIMENTAL EVIDENCE FOR THE LARGE-SCALE ADAPTIVE FLEXIBILITY IN PSEUDOMONAS FLUORESCENS SBW25 GENOME

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Bacterial genomes are typically organized according to gene utility; essential genes tend to occur near the origin of replication, while niche-specific genes cluster around the replication terminus. This organization is presumed to enhance evolvability, and hence survival in changing environments [1]. A prominent example is provided by pseudomonads, a diverse bacterial taxon in which the evolutionary flexibility of the terminus region has been inferred by comparing the genome sequences of different strains [2]. We present direct observations of the proposed flexible region undergoing spontaneous, large-scale rearrangements – including duplication and deletion events – across various laboratory evolution experiments with Pseudomonas fluorescens SBW25. Here, we focus on a mutant isolated from an SBW25 culture subjected to six months of nutrient limitation. Whole genome resequencing revealed, among other mutations, a deletion of 213,799 bp encompassing 173 genes around the replication terminus (Δbig). Despite its large size, reconstructing Δbig in the ancestor led to no detectable effect on growth or fitness in a range of fresh, agitated media. However, in static cultures, Δbig reduced the fitness of SBW25. Δbig also exhibited enhanced survival under highly acidic conditions.

Overall, we report a spontaneous deletion of a section of the computationally defined variable region in a Pseudomonas genome with detectable fitness effects in a complex, structured environment.

Long-term starvation is a condition unambiguously encountered by most natural microorganisms [3]. Several important microbial traits, including antimicrobial resistance are direct result of bacterial nutrient stress. There has been an idea that bacterial genomes are suited for “feast-or-famine” like lifestyles where smooth switch between growth and stress response could be achieved [4]. Our results show that the segment of the genome around the terminus can be flexibly adapted to the ambient selection pressures. These results support the preferential arrangement of most niche-specific, accessory genes around the replication terminus, and the resulting flexibility of this region during adaptation to novel niches.

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[P43] FUNGAL HYPHAE MAINTAIN DIVERSITY AND INCREASE HORIZONTAL GENE TRANSFER DURING MICROBIAL RANGE EXPANSION

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As bacterial communities expand across surfaces, they form spatial patterns as a result of natural selection, ecological drift, and dispersal. Individuals located at the expansion boundary have preferential access to nutrients, leading to a sharp decrease in diversity over time. This seems paradoxical given the sheer diversity of microbial species that colonize our planet. We hypothesized that fungal hyphae could help resolve this paradox by providing dispersal pathways for bacterial cells, allowing individuals behind the expansion frontier to colonize unoccupied space.

To test our hypothesis, we conducted an experiment using pairs of fluorescently labeled bacterial strains and a hyphal-forming fungal strain that expanded together across an agar surface. We first allowed the hyphal network to develop and then stopped its growth by transferring it to anoxic conditions, during which the bacteria expanded across the network. We performed this experiment with both competing and cross-feeding pairs of bacterial strains, and in the presence or absence of a conjugative plasmid. We imaged the communities by microscopy and quantified mixing from the resulting spatial patterns.

We found that hyphal networks maintained the spatial mixing of bacterial strains regardless of the type of metabolic interaction imposed between them. The underlying cause was that flagellar motility drives bacterial dispersal along the hyphal network, counteracting the purifying effects of drift at the expansion boundary. Finally, we showed that hyphae-mediated mixing increases the conjugal spread of plasmid-encoded antibiotic resistance. Thus, fungal hyphae are important regulators of bacterial diversity and promote functional novelty during range expansion.

[P44] TECHNICAL VERSUS BIOLOGICAL VARIABILITY IN A SYNTHETIC HUMAN GUT COMMUNITY

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Studying gut microbial communities is becoming a major research focus as growing evidence suggests the critical role microbial communities play in host health and disease. To better understand the mechanisms driving human gut microbial dynamics, low-complexity communities are used which should be grown in well-controlled environments. However, due to technical challenges, replicate time series of defined communities in chemostat-like conditions are rare.

Here, we investigate the reproducibility of human gut community dynamics with a defined community that contains representatives of two enterotypes (Bacteroides thetaiotaomicron and Prevotella copri) using an automated fermentation system that allows for a larger number of biological replicates. We also systematically assessed the technical variability of 16S rRNA gene sequencing. In addition, we counted the total number of cells in each sample using flow cytometry. As an alternative to sequencing, we applied supervised classification to the flow cytometry data to classify events by species, using a bioinformatics tool developed in our lab. Furthermore, we looked at metabolic changes and found that all replicates showed reproducible shifts in metabolite composition.

We show that bacterial dynamics of the communities are highly reproducible, and that the community stabilized within 2-3 days. The initial dynamics are characterized by the fast growth of Bacteroides thetaiotaomicron, followed by an increase of Blautia hydrogenotrophica. These changes occurred together with reproducible metabolic shifts, namely a fast depletion of glucose and trehalose concentration in batch followed by a decrease of formic acid and pyruvic acid concentrations within the first 12 hours after the switch to chemostat mode.

In conclusion, the observed variability in the synthetic bacterial human gut community as assessed with 16S rRNA gene sequencing is largely due to technical variability. The low variability seen in HPLC and flow cytometry data suggests a highly deterministic system. In our experiments, B. thetaiotaomicron reproducibly displays the behavior of a pioneer species that quickly exploits an easily accessible carbon source but is then replaced, to an extent, by slower growing but metabolically more versatile species.

[P45] METABOLIC HETEROGENEITY AND ECOLOGICAL FLEXIBILITY EXPLAIN THE EARLY DISCOVERIES OF COMAMMOX NITROSPIRA

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Nitrification, the aerobic oxidation of ammonia to nitrate was formerly considered a two-step process where ammonia is first oxidized to nitrite by ammonia oxidizers and then nitrite is oxidized to nitrate by nitrite oxidizers. The understanding of nitrification as an obligated division-of-labour process was refuted by the discovery of nitrite oxidizers belonging to Nitrospira genus capable to catalyse both steps of nitrification on their own (Comammox) [1, 2]. Comammox was firstly reported as dominant in limited oxygen environments (where anaerobic ammonia oxidation was also occurring, Anammox) [2], which generated a debate regarding the oxygen requirements for the chemoautotrophic growth of Comammox Nitrospira [3]. Comammox Nitrospira cocultured with Anammox bacteria has the potential to achieve high levels of nitrogen removal with reduced energy consumption for aeration, limited N2O emissions [4] and sludge production.

To explain Comammox selection under limited oxygen environments, we developed an Individual-based Model able to describe Nitrospira and Anammox growth in suspended flocs simulating the cycles of a sequential batch reactor operation until steady state applying the experimental conditions in which Comammox was firstly discovered [1, 2]. All possible activities of Nitrospira were considered: ammonia and nitrite oxidation, Comammox, nitrate-reducing ammonia oxidation and anaerobic nitrite-reducing ammonia oxidation. The last two metabolic activities, although hypothesized in literature [3], have not been observed yet.

In all tested conditions, the co-existence of different metabolic activities of Comammox Nitrospira were observed in steady state, predicting metabolic heterogeneity in limiting conditions of nitrogen and oxygen. Our results show that even extremely low oxygen concentrations (<2 μM) allow for a proportional growth of Nitrospira versus Anammox bacteria similar to the one experimentally observed [2]. Additionally, metabolic heterogeneity explains the observation of transient nitrite accumulation in aerobic environments with higher ammonia availability [1]. The analysis of the ecological interactions between the individuals of the community shows different ecological maps in all tested conditions, suggesting that the adaptability of Comammox Nitrospira comes from the ecological flexibility yielded by its capacity for metabolic heterogeneity.

In sum, the metabolic heterogeneity of Comammox Nitrospira is the mechanistic explanation of the co-existence with Anammox bacteria under hypoxic conditions [2], the dominance of complete nitrification activity in nitrogen limiting environments, and the transient accumulation of nitrite under aerobic conditions [1].

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[P46] NICHE DIFFERENTIATION ENABLES SUCCESS OF THE LUNA CLUSTER ACROSS A RIVER-TO-SEA SYSTEM

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Estuaries are a key interface between terrestrial and marine environments, funnelling freshwater and nutrients from land to sea. Microorganisms inhabiting these environments are strongly stratefied across freshwater and saline niches, necessitating genetic adaptations to tolerate salt stress. Estuaries represent an ideal system to study competition and niche differentiation within microbial lineages related to salt tolerance and resource utilisation. We investigated the microbial ecology of a river-to-sea transect via metagenomics and metatranscriptomics. This included the recovery and reconstruction of 310 bacterial and archaeal genomes, as well as bacterial community transcription. We also genomically characterized a culture collection derived from across the same estuarine system.

Nine filtered water samples were collected along the transect for sequencing and assembly. Metagenome-assembled genomes (MAGs) were identified using a multi-parameter binning approach, with manual curation, and dereplicated at 99% average nucleotide identity. The 310 MAGs recovered were further curated to retain a set of 265 unique MAGs >75% complete with 0-5% contamination, representing 17 phyla. RNA sequences were then mapped to MAGs to determine transcriptional profiles across the estuary.

Analysis of genome coverage distributions revealed a single freshwater-to-marine cosmopolitan bacterium, Aquiluna sp. Ww131 of the Microbactericeae Luna cluster. Aquiluna Ww131 dominated the saline aquatic environment, often constituting >50% of the microbial community. Though Aquiluna Ww131 was present in freshwater, we observed a tradeoff between Aquiluna and several closely related Rhodoluna species whose abundance was greater in this environment. Members of the Luna cluster are known to utilise proteorhodopsin for photoheterotrophy, which may contribute to the success of this lineage in the estuarine system. Transcriptomic evidence further suggests Aquiluna Ww131 encodes a greater proportion of acidic amino acids in secreted proteins to tolerate salt stress in the saline reaches of the estuary, explaining its relative increase in the saline environment. To further study the attributes contributing to the success and niche differentiation of estuarine Microbacteriaceae, 77 isolates were obtained from freshwater, brackish and marine sites, and genomes generated with long-read sequencing technology.

Overall, results suggest competition among freshwater members of the Luna cluster, with members expressing genes for a (photo)heterotrophic mode of life. Extensive genetic adaptations of Aquiluna Ww131, which belongs to a genus originally described from freshwater environments, likely enables its colonisation of the saline reaches of the estuarine system. Moreover, this study highlights the mechanisms through which Luna cluster members contribute to carbon degradation from river to sea.

[P47] UNRAVELING CELL SURFACE COMPONENTS INVOLVED IN PHAGE-HOST INTERACTIONS OF THE DAIRY BACTERIUM STREPTOCOCCUS THERMOPHILUS

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Dairy fermentations are constantly threatened by the presence of bacteriophages, which can lead to acidification failures and a lower quality of final products. Phage infections of Streptococcus thermophilus is a paramount issue, due to the economic importance of this bacterium for cheese and yoghurt production. The aim of this investigation is to understand the role of different cell surface components in phage adsorption and infection.

Firstly, we generate spontaneous phage-hardened variants (PHVs) of industrially relevant S. thermophilus strains. We utilize phages as fluorescent biomarkers to select for PHVs harboring modifications in the cell surface receptors. Following the whole-genome sequencing of the selected bacteria, we examine the mutations and assign to the altered genes a putative role during the phage infection.

Secondly, we apply genome-based approaches to identify bacterial host factors shaping phage sensitivity. Genomic comparison of closely related strains should allow identifying sequence variants determining phage susceptibility and resistance in S. thermophilus strains. Sequence diversity within selected genomic regions can be used as predictor of new host factors for phage sensitivity.

Lastly, we evaluate the role of various cell surface components in the process of a phage infection. To that end, we generate a portfolio of variants with targeted gene deletions. Since genetic approaches commonly applied to domesticated strains are difficult to implement in industrial strains, we continuously develop a genetic toolbox to engineer the industrially relevant S. thermophilus.

Our research provides novel insights towards developing efficient strategies to control phage outbreaks in dairy plants. The identification of phage receptors of S. thermophilus will aid in improving starter rotation schemes as well as the selection of strains for culture development.

[P49] CHANGES OF SIDEROPHORE VARIABILITY AFTER MANIPULATION WITH SOIL NUTRIENTS AND THEIR PRODUCTION BY ISOLATED STREPTOMYCES SPP.

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In spite of comprising a significant part of Earth's mass, iron is not readily accessible to organisms because under oxic conditions poorly soluble ferric oxyhydroxides are formed. To overcome low bioavailability, (micro)organisms evolved a mechanism to scavenge iron from their environment by producing siderophores, low-molecular-weight compounds with high affinity to iron. Siderophores are typically synthetized by non-ribosomal peptide synthetases (NRPSs) or polyketide synthase (PKS) domains that work in concert with NRPS modules. Besides iron uptake, siderophores function in microbial interactions. The study aimed in determining 1) connections between siderophore biosynthetic pathways detected in individual strain genomes and in vitro production 2) relative frequency and variability of siderophores in an in situ experiment. Higher production and more diverse siderophores were expected in strains coming from sites with higher limitation of available iron, i.e. those with alkaline soils. The isolated strains all belonged among Streptomyces sp. We chose this genus because it is a well-known siderophore producer and significant decomposer, and also because Streptomyces is Gram-positive, while most work on siderophores has been done on Gram-negative bacteria. Out of 200 Streptomyces strains coming from various sites, about 15% did not produce siderophores in a simple chromazurol assay. Out of siderophore producers, 10 strains were chosen for genome analyses and further production tests. It was found that the production in vitro was unpredictable although the strains were cultivated seemingly under the same conditions. The genomes revealed desferrioxamines as a predominating pathway and also the produced siderofores were mostly desferrioxamines by LC-MS/MS analysis. The in situ study was manipulating nitrogen and carbon contents in soil by additions of nitrate and cellulose. Metagenome of the upper soil layer showed that the relative frequency of siderophore genes did not change after nutrient additions but a change in the siderophore variability and type was observed particularly after addition of the carbon source. Since siderophores represent "keystone" soil metabolites, the improved understanding of their functioning can expand not only knowledge on competition and cooperation between soil microorganisms, but also on decomposition processes including changes in CO_2 production and soil chemistry of complexation of organic carbon with clay particles by iron. Siderophores also represent a promising tool for bioremediation of contaminated soil or water environments.

[P50] CHANGES IN MICROBIAL BIODIVERSITY IN STREAM BIOFILMS IN AGRICULTURAL RIVERS WITH DIFFERENT LEVELS OF PESTICIDE POLLUTION

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Despite the importance of preserving biodiversity in aquatic microbial communities for ecosystem services, the relationships between microbial diversity and changes in water quality due to chemical pollution, including pesticides, is scarce. This study aims to assess the relative importance of environmental factors (pesticide pollution, nutrients, dissolved organic matter, metals) to the microbiome diversity of stream biofilms as well as their polyunsaturated fatty acids profile (PUFA) as a proxy of food quality. To this aim, stream biofilm in three water courses in Sweden (Höje å, Skivarpån, and M42) were analyzed in autumn, at the end of the crop season. The sites experienced different levels of pollution. We investigated bacterial, fungal, and algal diversity by Illumina sequencing targeting 16S, ITS2, and LSU 23S regions of ribosomal RNA, respectively. Algal biomass and PUFA profile with HPLC and LC-MS, respectively. A mixture of 57 pesticide compounds (dominated by herbicides) was detected in the most polluted sites (Skivarpsån and M42), reaching concentrations of up to 16 µg/L. Correlation analysis showed that pesticide and nutrients pollution were the water quality parameters most strongly associated with changes in microbial structure, diversity, and food quality. Cluster and principal component analyses based on pigments content and microbial biodiversity profiles clearly separated the microbiomes from the three river sites studied. Algae and bacteria, were the most sensitive microbial groups to changes in water quality, resulting in a lower diversity in the most polluted sites (Skivarpsån and M42). The nutritive quality of the biofilm differed among streams, and fatty acids considered high-quality feed such as eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) were also more abundant in pesticide-polluted streams (Skivarpsån and M42). Our results provide field evidence that water pollution (pesticides and nutrients) impacts microbial biodiversity in freshwater ecosystems, which can have negative consequences for the provisioning of services that such microorganisms provide.

[P51] CPX-SIGNALLING DEPENDENT SILENCING OF UREASE FACILITATES SUCCESSFUL ERADICATION OF BIOFILMS IN YERSINIA PSEUDOTUBERCULOSIS

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Introduction: Bacteria form mixed biofilm communities 1. These bacteria are more resistant to antimicrobial agents and are a primary driver of persistent and chronic infections 2,3. We previously demonstrated that the CpxRA two-component regulatory system controls biofilm formation in the enteropathogen Yersinia pseudotuberculosis (Yptb-YPIII) 4.

Aim: To investigate mechanisms underpinning Yptb-YPIII biofilm inhibition by active Cpx-signalling.

Methods: Parental Yptb-YPIII and an isogenic constitutively active "Cpx-locked on" mutant (ΔcpxA) were grown in 96-well microtiter plates and the total protein pool extracted from planktonic (PL) and biofilm (BF) cell populations and then subjected to a comparative proteome analysis. Enriched KEGG pathways identification was then followed up with biochemical and molecular-genetic assays. The qRT-PCR was used to determine gene-specific transcription. Electrophoretic mobility shift assays (EMSA) were employed to measure CpxR~P binding to gene promoters.

Results: Of the 4192 proteins encoded by Yptb-YPIII, our proteome analysis captured 1856 (44.3%) and 1761 (42.0%) proteins from the PL and BF states, respectively with Log2FC ≥ ±1 and P-value ≤ 0.01. Of the 1856 proteins identified in the PL state, 243 (13.1%) were differentially abundant in the ΔcpxA mutant versus parental Yptb-YPIII (109 down-regulated and 134 up-regulated). Of the 1761 proteins identified in the BF state, 610 (34.6%) were differentially abundant in the ΔcpxA mutant versus parental Yptb-YPIII (224 down-regulated and 386 up-regulated). A comparison of the down-regulated proteins from the PL state (109 proteins) with BF state (224 proteins) derived from ΔcpxA mutant revealed 35 proteins that were common to both cell populations. Overrepresented in these 35 were 7 proteins (20%) encoded within the ure-gene cluster responsible for urease production. Biochemical assays on the ΔcpxA mutant validated a deficiency in urease production and activity. The qRT-PCR determined the repression of ure transcription caused by the accumulation of active CpxR~P. EMSA indicated that this was due to direct CpxR~P binding to the ure promoters. Direct Cpx-dependent transcriptional repression of ureR, encoding a AraC/XylS-like transcriptional activator of the ure gene-cluster can also contribute to controlled urease production.

Conclusion: We propose that Yptb-YPIII cohesive biofilm formation is controlled by active Cpx-signalling partly through the silencing of urease production.

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[P52] THE PERENNIAL WHEATGRASS SEED MICROBIOME AND ITS POTENTIAL TO IMPROVE FUTURE PLANT BREEDING STRATEGIES

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Grain production is an important component of agriculture, with edible plant seeds being the foundation of food security, accounting for almost 50% of the global food calories. Yet, modern agricultural techniques are often unsustainable, harmful to the environment and they often contribute to biodiversity loss. Perennial grain cultivation is a feasible alternative for conventional wheat cropping. Perennial plants are more efficient at using water and nutrients because of their deeper, denser root systems, which can develop over time. This is essential for surviving under more frequent and severe weather extremes brought on by climate change. Although specific techniques for cultivating perennial wheat are under development since the 1980s, additional research is needed to increase their productivity. How the currently ongoing, intense breeding strategies affect the seed microbiome is still unknown. Only recently it was shown that seeds are a source of beneficial microorganisms and some of these bacterial endophytes can be associated with plants throughout their entire life cycle, as well as during postharvest storage. These characteristics make them of particular interest for further research. Here we analyzed for the first time the composition of the perennial wheatgrass (Thinopyrum intermedium, L.) seed microbiome, from three different sampling sites, during three consecutive years. The samples were subjected to 16S rRNA gene fragment high-throughput sequencing to determine the taxonomic composition of bacterial communities and their structure. Our data indicates that the seed microbiome of perennial wheat is dominated by the genera Kosakonia and Bacillus, and that variations in the bacterial community composition are mainly driven by the field site, whereas the sampling year only had a minor influence. Furthermore, we analyzed seeds from four breeding cycles, grown in two different locations. We observed a decrease in microbial diversity for each breeding cycle and less interactions among the present bacteria. Overall, this study provides insights into the composition of the perennial wheatgrass seed microbiome and highlights the importance of considering the impact of breeding on microbial diversity. The results could not only have implications for plant health and productivity, but also may influence future breeding strategies.

[P53] TEMPORAL AND SPATIAL CHANGES IN THE EPIPHYTIC AND ENDOPHYTIC APPLE MICROBIOME INDUCED BY VARYING AIR QUALITY

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Air quality is worsening due to anthropogenic activities and has become an increasingly concerning issue not only for humans but also for planetary health. Research has focused mainly on chemical composition, source, distribution and effects on human health of airborne particulate matter (PM), and the impact of varying air quality on plant health is less studied. Since airborne particulate matter can serve as a carrier for microbes, the microbial community composition of apples and dust collected from apple trees at locations with varying air qualities are analyzed to investigate potential responses in the apple microbiome. Therefore, three apples and three dust samples were collected at two sampling times from eleven locations, whereby the air quality of three sites was classified as very good ($PM_{2.5}$<10), five as good ($PM_{2.5}$ 10<x<50), and three as moderate ($PM_{2.5}$>50). The epiphytic and endophytic bacterial and fungal communities of apple pulp, skin, surface, as well the collected dust were analyzed via high-throughput sequencing of amplicons targeting the V4 hypervariable region of the 16S rRNA gene and the internal transcribed spacer (ITS1) region. It became apparent that sample type (p= 0.001, R= 0.73; p= 0.001, R= 0.24) and sampling time (p= 0.001, R= 0.07; p= 0.001, R= 0.04) are significant drivers of the bacterial and fungal community composition (respectively). Fungal communities also significantly differed between individual trees (p= 0.001, R=0.15) and sampling sites (p= 0.001, R=0.11). Different air quality had a significant effect on the beta diversity of the fungal peel community (p= 0.005, R= 0.11) and here also the evenness was increased at sites with good air quality, but no significant effect of air quality could be shown on bacterial communities when analyzing alpha and beta diversity. Nevertheless, analysis of the core microbiome revealed one bacterial ASV of the genus <I>Verticiella</I> that was associated with all apple sample types at very good air quality exclusively. Furthermore, SourceTracker2 showed that endophytic bacterial communities of pulp (5.2%) and peel (56.1%) and epiphytic bacterial communities on the apple (28%), and endophytic fungal communities of pulp (21.6%) and peel (3.3%) and epiphytic fungal communities on the apple (6.1%) originate from dust. By analyzing changes in the epiphytic and endophytic microbial communities of apples caused by the surrounding air, this study provides insight into the effects of anthropogenically driven variations in air quality and its potential consequences for plant microbiomes.

[P54] MICROBIAL ASPECTS OF MACROLIDE ANTIBIOTICS ASSOCIATED WITH LONG-TERM APPLICATION OF WASTEWATER TREATMENT PLANT PRODUCTS TO SOIL

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Macrolide antibiotics are key drugs in the treatment of serious infections, but the emergence of macrolide-resistant pathogens is alarming due to their high consumption (clarithromycin is the second most used antibiotic in the Czech Republic). Macrolides are not fully metabolized in the human body; their residues are excreted in wastewater and are only partially eliminated in wastewater treatment plants (WWTPs). The application of WWTP products to soil for crop irrigation (treated wastewater- TWW) or organic fertilization (sewage sludge) then represents a direct input of macrolides into soil-plant systems. Direct and indirect threats to humans from consumption of contaminated food, the spread of macrolide resistance genes and associated mobile genetic elements, leading to an increase in resistant pathogens need to be identified. Some genes encoding macrolide resistance (ere, ole, mph) encode enzymes that inactivate these substances and thus eliminate them from the environment.
We hypothesize that the input of macrolides into the soil through the application of WWTP products affects the structure of the microbial community in the rhizosphere and, consequently, its functions, causing some bacteria to adapt and accelerate the degradation processes of these compounds. These biodegrading microorganisms and mechanisms may be useful tools for improv bioremediation techniques leading to the removal of macrolide residues in the environment.
Our study focuses on revealing those mechanisms of resistance to clarithromycin (CLR) that may be useful in bioremediation. The experiment with raised beds simulated 4 situations: Phaseolus vulgaris plants were grown in beds with soil (Cambisol, pH(CaCl2) 6.92, DOC 0.02 µq/g dw, DN 0.03 µq/g dw, Cmic 43.31 µq C/g dw) irrigated with either tap water or TWW (min. and max. CLR concentration in TWW were 98 and 670 ng/L), enriched with sewage sludge or composted sewage sludge. The rhizosphere and endosphere (xylem) were studied. Standard culture techniques on solid media revealed an increase in the number of CLR-resistant bacteria only in sewage sludge-enriched soil (3.6 times higher CFU/g of soil compared to control). Surprisingly, a lower ability of bacterial consortia to adapt to the presence of CLR in soil irrigated with TWW compared to control was demonstrated when bacteria were selected on CLR as sole C source. These consortia and isolated Actinobacteria will be screened for CLR-degradation potential. We isolated almost 500 isolates from the rhizosphere, and almost 40 from the endosphere. The project was funded by the Ministry of Agriculture of the Czech Republic (QK21020080).

[P55] ECOLOGICAL PATTERNS OF MICROBIAL SUCCESSION WITHIN THE FIRST SIX YEARS OF LIFE

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The healthy embryo is considered essentially sterile and the first and very important colonization is established by the infant's early contact with the environment. The bacterial composition of the gut is very dynamic during the first year of life. Initially the composition is dominated by early colonizers, while these are later outcompeted by bacterial taxa characterizing a more mature composition. Despite the role of colonization patterns in structuring the human gut microbiota being increasingly appreciated, we know little about the history dependency of initial community assembly and its implications for the maturation trajectory of the infant gut microbiota.

COPSAC2010 is an ongoing Danish cohort study of 738 unselected pregnant women and their 700 children followed from pregnancy week 24 with deep clinical phenotyping and microbiome characterization. Here we used this longitudinal cohort of 700 Danish children to study the gut microbiota dynamics during their first 6 years of life. Using a time-course gene set analysis, we analyzed a complete series of samples by 16S rRNA gene sequencing (n=2699) from 1 week to 6 years and identified four main trajectory clusters for individual genera in the developing gut microbiota and the formation of distinct community states. Using Dirichlet multinomial mixtures modeling three phases can be identified, an initial colonization, transition, and stabilization.

We then associate environmental factors that influence the developmental trajectories and maturation patterns observed in our cohort. Environmental factors include rural vs. urban living, antibiotic intake, duration of breastfeeding, and exposure to siblings or pets. These environmental factors cause temporal changes in the maturation phases and overall changes in community types. The duration of breastfeeding affects the maturation speed by elongating or advancing the maturation phases. Exposure to siblings or pets, rural vs. urban living or the intake of medication such as antibiotics impact the initial colonization pattern and the order in which microorganisms are introduced into the gut. This subsequently affects the developmental trajectories and community assembly over time. In addition, combinations of environmental factors and exposures such as delivery mode in combination with breastfeeding can cause increased changes in the gut maturation. Our results highlight the distinct ecological patterns observed within our cohort and the environmental factors that influence the developmental trajectories and microbial dynamics in early life.

[P56] THE FEATHER MOSS HYLOCOMIUM SPLENDENS AFFECTS THE TRANSCRIPTIONAL PROFILE OF AMINO ACID METABOLISM IN A SYMBIOTIC CYANOBACTERIUM

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Moss–cyanobacteria associations are an important source of new nitrogen into northern environments like boreal forests. Symbioses between mosses and cyanobacteria have been proposed to be based on a mutualist exchange of nutrients, with hosts providing carbon (C) and sulfur (S) while the microbes provide nitrogen (N) to their partners, but few works have explored how the expression of nutrient-related genes is affected by this interaction. This work investigated if and how the symbiosis between the common feather moss Hylocomium splendens and a N2-fixing cyanobiont affects the expression of genes related to nutrient metabolism and uptake in both partners. For this, we collected H. splendens shoots in boreal forests from northern Sweden and isolated a nostocacean cyanobacterium from them. A cell suspension of the isolated cyanobacterium was co-incubated with H. splendens shoots and compared with cyanobacterial and feather moss shoots incubated separately. After one week, we performed acetylene reduction assays to estimate N2 fixation rates and RNAseq to evaluate the transcriptome of the samples. Contact with H. splendens increased N2 fixation rates in the cyanobiont to a level that was almost four times that of the cyanobacterial cells incubated alone. Differential gene expression analyses showed that genes related to N2 fixation, and the biosynthesis of several amino acids were indeed up-regulated in the cyanobiont. However, the biosynthesis of the S-containing amino acids methionine and cysteine was down-regulated while the degradation of selenocysteine was up-regulated. Sulfur uptake was also down-regulated in the cyanobiont. The number of differentially expressed genes in the feather moss was much lower than the cyanobiont, and almost no genes related to nutrient metabolism were affected. These results show that it is possible that, at least in some stages, the cyanobiont receives few if any nutrients from the host in return for fixed N2, suggesting that moss–cyanobacteria symbioses encompass relationships that are more complex and plastic than a constant mutualist flow of nutrients.

[P57] VISCOSIN SYNTHESIS PROMOTES PSEUDOMONAS FLUORESCENS SBW25 ROOT COLONIZATION AND ALTERS MICROBIAL ASSEMBLY IN WHEAT RHIZOPLANE IN A CULTIVAR DEPENDENT MANNER

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The importance of microbial functions for plant health and performance is unquestioned. Beneficial rhizosphere bacteria such as Pseudomonas sp. promote plant growth and provide protection against pathogens. In the rhizosphere, plant-associated pseudomonads are producers of many bioactive compounds, including cyclic lipopeptides, which are particularly well-known for their antimicrobial activities. However, the role of cyclic lipopeptides in the interaction between beneficial bacteria and plants remains underexplored, especially in natural systems. In this study, the model strain Pseudomonas fluorescens SBW25 producing the cyclic lipopeptide viscosin was used to unravel the impact of viscosin on bacterial colonization potential and microbiome assembly at the wheat root. Two varieties of winter wheat were inoculated with either the SBW25 wild-type strain or a viscA mutant deficient in viscosin production. The ability of both strains to colonize the roots of each variety was quantified using qPCR analysis, and plant parameters including plant biomass and height were assessed. Our results indicate that SBW25 wild-type strain has stronger colonization ability than the mutant strain at the rhizoplane. In order to study the impact of viscosin on microbial assembly, rhizobiome16S rRNA gene and 18S rRNA gene amplicon sequencing was performed. Our results indicate that viscosin intervention changed bacterial as well as protist community diversity in a plant cultivar dependent manner. This study provides new insights into the natural importance of viscosin and specifically the role of viscosin in the colonization of plant roots and in shaping the microbial communities associated with different wheat varieties.

[P58] AEROBIC ANOXYGENIC PHOTOTROPHIC BACTERIA ARE UBIQUITOUS IN BOREAL AND ARCTIC PLANT MICROBIOMES

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In addition to plants, algae and cyanobacteria, photosynthetic systems are widely present in evolutionary ancient anoxygenic photosynthetic bacteria, including aerobic anoxygenic phototrophic bacteria (AAPB). AAPB are abundant in aquatic ecosystems in all climates, but they have been also detected in terrestrial ecosystems, and recently, in metagenomes of several plant phyllosphere microbiomes. However, it is currently not known, how common AAPB are in plant microbiomes, what is the role of AAP (if any) in plant-microbe interactions.
We screened phyllospheres and endospheres of over 30 plant species for AAPB by near infrared fluorescence imaging of culturable bacteria. In collaboration with seven high schools in Finland, we sampled in ten distinct locations across latitudal gradient spanning from hemiboreal to oroarctic climate zones in Finland, and additional locations in Svalbard and Greenland.
We show, that AAPB are consistently present in foliar plant microbiomes in these climates, as they were detected in all sampled locations, and in virtually all plant species studied. Most of the characterized isolates represent alphaproteobacterial genera Sphingomonas and Methylobacterium, but AAPB representing betaproteobacteria as well as several novel alphaproteobacterial lineages were also identified. Methylobacterial and novel Rhizobiales isolates were mostly present in the phyllosphere with weak host specificity, while Sphingomonas AAPB showed clear host plant specificity.
Intriguingly, the AAPB detected in this study represent bacterial taxa with plant growth promoting representatives, and several are part of their host plant core microbiota, prompting questions about putative role of AAP in plant-microbe interactions in cold climate ecosystems characterized by strong annual fluctuations of light and temperature. Whole genome sequencing of 60 representative strains revealed presence of complete clusters of photosynthesis genes with PSII-type photosystems, but also suite of diverse photosensors and genes related to plant-microbe interactions.

[P59] PREDATORY BACTERIA INFLUENCE HOST-ASSOCIATED MICROBIAL COMMUNITIES AND C. ELEGANS FITNESS

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According to ecological theory, the presence of higher trophic level organisms such as predators may influence alpha-diversity by reducing dominant members of a community and creating niches for less common species to thrive. Ecosystem stability has been linked to higher alpha diversity; hence the presence of predatory bacteria may have a positive effect on various ecosystems including hosts.

Indeed, a meta-analysis investigating the effect of the presence of the bacterial predators Bdellovibrio and like organisms (BALOs) on distinct host-associated microbial communities corroborated the hypothesized relationship between predator presence and diversity, with BALO presence being associated with an increase in alpha-diversity (1). To validate this correlation, we conducted in vitro experiments with 12- and 50-member microbial communities in the presence of the bacterial predator Bdellovibrio bacteriovorous MYbb2 in liquid medium. Over time, the richness of amplicon sequence variants (ASVs) decreased significantly less in the presence of the predator.

We sought to extend these findings to a host system and chose the hermaphroditic nematode Caenorhabditis elegans as a model host due to the ease of manipulating its microbiota. We performed controlled laboratory experiments and found that Bdellovibrio bacteriovorous MYbb2 presence impacted the 12- or 43-member worm microbiota, with certain community members displaying significant differential abundances when compared to BALO-free controls. Additionally, worms exposed to Bdellovibrio produced a significantly greater number of offspring and lived significantly longer than those from control treatments.

Taken together, these findings underscore the importance of predators in host-associated communities and their impact on host fitness.

(1) Johnke, J., Fraune, S., Bosch, T. C. G., Hentschel, U. & Schulenburg, H. Bdellovibrio and Like Organisms Are Predictors of Microbiome Diversity in Distinct Host Groups. Microb. Ecol. 79, 252–257 (2020).

[P60] SALMONELLA ENTERICA ADAPTS TO AGRICULTURAL ENVIRONMENTS BY ADJUSTED CARBON METABOLISM

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Salmonella enterica serovar Typhimurium (S. Typhimurium), a non-typhi serovar with a broad host range, causes millions of human infections every year, a large proportion of which is resulting from consumption of contaminated agricultural produce. S. Typhimurium has been reported capabilities to persist in numerous agricultural environments for up to several months. As a result, understanding the mechanism employed by S. enterica to persist is of vital importance for establishing precise methods to prevent Salmonella contaminations.
By extracting upregulated genes in S. enterica serovar Typhimurium strain 14028s (S. Typhimurium 14028s) exposed to multiple agricultural environment-mimicking media from published RNA-Seq data, we discovered top KEGG modules all related to central carbon metabolism, including glycolysis and the tricarboxylic acid (TCA) cycle. Consequently, key intermediates of central carbon metabolism were evaluated in both media and cultured S. Typhimurium 14028s cells. Glycerol was the major carbon compound in diluvial sand (DS) soil, while glucose, fructose, and several di-/tri-saccharides were detected in lettuce leaf based medium (LM) or tomato leaf based medium (TM). Multiple intermediates of glycolysis and the TCA cycle were detectable in the Salmonella cells grown in such media, either commonly or specifically. Interestingly, cells harvested from TM and LM performed a more comparable carbon metabolism pattern than cells harvested from DS soil, consistent with the carbon composition of corresponding media. This indicates adjusted carbon metabolism as strategy to persist in agricultural environments. To identify Salmonella genes contributing to its persistence in distinct niches, RT-qPCR was used to verify RNA-Seq results. The pyruvate dehydrogenase subunit E1 encoding gene aceE was speculated important for Salmonella adaptation to leaf-related environment. Persistence assay and competitive index assay with the aceE mutant in tomato leaves confirmed its important role. Another gene, aceB, which encodes malate synthase A, responsible for glyoxylate pathway, behaved opposite, despite of upregulation in Salmonella. Comparing metabolites in aceB mutant with metabolites in S. Typhimurium 14028s cells grown in TM, we observed a fumarate accumulation, possibly via diminished catabolism, as suggested by fumarate reductase RT-qPCR results.
In conclusion, we demonstrated that adjusted carbon metabolism may be a strategy employed by the animal pathogen S. Typhimurium to adapt to agricultural environments, including tomato leaves. During this process, the abundance of fumarate may play an important role. Our research may assist in developing precise means in prevention of Salmonella persistence in agricultural production.

[P61] FIRST COME, FIRST SERVED: DECIPHERING THE COLONIZATION OF THE INFANT GUT ECOSYSTEM

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Directly after birth, the infant's gut provides a vast empty habitat to be colonized by bacteria. With changes in the infants diet and the maturation of its immune system, this constantly changing habitat provides a model system to investigate ecological dynamics. Modeling microbial ecosystems benefits from a diverse set of studies to be successfully applied to unseen data. In [1] a meta-analysis of 28 studies was able to detect a generalizable signal of diseased samples in adult humans independent of the patient's condition.

Although the colonization of the infant's gut has been subject of several studies, comparisons across cohorts, geographic locations and lifestyles are rare.

Here, we aim to define a generalizable model for colonization of the infant's gut. The model will provide insight into the host-microbiome dynamics and microbial ecological succession in a sterile environment.

We use published longitudinal 16S and shotgun sequencing data of children under the age of three years to analyse the colonization of the infant's gut across cohorts, countries, and lifestyles.

We present preliminary results for the 16S-sequencing data of 17 studies including 17823 samples of 2832 individuals.

We implemented a custom method to merge studies on Amplicon Sequence Variant (ASV) level to maintain as much diversity information as possible. We obtained 16322 ASVs with a median length of 233 bp for further analysis. Previously used strategies of merging studies at a specific taxonomic level (genus or family) decreased the reliability of our predictions.

Although cohort specific effects heavily affected microbial profiles, we identified key taxa predictive of the host's age across studies. Our models indicated a significant effect of several metavariables such as birth weight, mode of delivery and feeding practice consistently across all cohorts. However, the importance of gestational age and sex depended on the individual cohort.

This assembled dataset allows a comprehensive analysis of different variables affecting the colonization of a sterile habitat. Our results illustrate the importance of diverse datasets to infer generalizable models for longitudinal microbial dynamics. Next, we will incorporate functional information from shotgun sequencing data into our models. In the future, we aim to use these models to detect deviations from a healthy maturation.

[1] Duvallet et al., Nature Communications 8, no. 1 (December 5, 2017): 178

[P62] ALL IN ONE: THE MICROBIOME OF A ROOTLESS PLANT

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Plant microbiomes diverge among the rhizosphere, endosphere, and phyllosphere, each supporting a unique microbial community. However, two genera of duckweed, Wolffia and Wolfiella, have undergone drastic reduction and simplification, resulting in the world's smallest and fastest growing plants. We have used a newly domesticated edible duckweed, Wolfia globosa, as our model plant. This fast-growing aquatic plant consists solely of leaves floating on the surface of water and was consumed for centuries in the Far East, where it is known as water egg or water lentil. As the name implies, W. globosa is rich in protein, but also contains essential macro and micronutrients (including zinc and iodine), carotenoids, fatty acids (including Omega-3 and-6), phytosterols, and vitamins, especially B12 (cobalamin), which was found to be bioavailable and beneficial for humans. Some of these nutritional benefits are assumed to be produced by the W. globosa microbiome, for instance cobalamin. However, little is known about the diversity, function, and control of this plant microbiome to enable the detection and enhancement of the nutritional benefits in this novel edible crop. We hypothesised that the microbiome of this rootless plant supports a unique endophytic community that acquires properties usually reserved for different parts of the plant. To test our hypothesis, we evaluated the composition and function of the W. globosa microbiome by population-resolved metagenomic analysis and by isolating and identifying the dominating bacterial species. Our results have shown that the endophytes of W. globosa are unique, differing from the epiphytes and growth media communities. The endophytes were dominated by bacteria, but were almost devoid of fungi, protists, or archaea. The diversity of the bacterial endophytes was low and thus 32 full genomes were assembled with members mostly affiliated to a- and g- Proteobacteria. These bacterial species formed a tightly knit community that shared functions and pathways. We identified functions that are usually reserved for the rhizosphere, such as atmospheric nitrogen fixation, mobilization of essential nutrients, and vitamin biosynthesis. We also detected functions that characterize leaf endophytes, such as carotenoids, pigment biosynthesis, or terpenoids production. The isolation and characterization of the dominant bacterial species of W. globosa confirmed the inferred functions. Our in-depth description allows the generation of hypotheses on the microbiome assembly and interactions within the plant holobiont.

[P63] HOST-ASSOCIATED MICROBIOME OF THE GIANT RED SHRIMP: ECOLOGICAL INSIGHTS FOR SEAFOOD TRACEABILITY

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Environmental features can shape the microbial community, paving the way for the use of a "microbial fingerprint" to obtain and predict information about an ecosystem. Thinking outside the box, such microbial signature can be exploited for seafood traceability purposes.
Indeed, seafood traceability is often challenging, especially when the species is the same, but the geographical provenance makes the difference.
Omics tools, such as population genomics and microbiome-based studies, combined with advanced bioinformatic approaches, can provide insightful solutions.
Our case-study is the giant red shrimp Aristaeomorpha foliacea: it has a wide geographical distribution in the world and represents an important economic resource, highly exploited in the Mediterranean Sea. However, new fishing grounds recently joined the global seafood market and began exporting their catch worldwide. This led to a value differentiation of the species in relation to different origin, making it a subject of commercial fraud.
In this study, a molecular characterization of A. foliacea-associated microbiome was carried out, in order to explore its associated bacterial communities and to find any origin-related differences. To do so, we engaged several stakeholders, and we were able to collect georeferenced samples from two relevant fishing areas. These areas are characterized by both economic and environmental diversity: Strait of Sicily, where the Mazara del Vallo red shrimps are caught, and Mozambique Channel.
Here we implemented our phase 1 "SeaTraceOmics project" with hundreds of samples analyzed, comparing environmental microbiome, host-associated microbiome, and population genomic information about the host. Furthermore, we characterized the shrimp diet comparing the two different localities, analyzing the gut content through a DNA metabarcoding approach.
Noteworthy, our results showed that, despite being the same species, we were able to differentiate the two different populations. This was possible through the integration of multiple data sources. Moreover, supervised machine learning approaches allowed the taxonomic characterization of distinct bacterial communities linked to different ecological features, and the extrapolation of predictive patterns.
This study links microbial ecology to traceability and is at the core of "SeaTraceOmics project", constituting a promising ground for the investigation of the ecological footprints influencing the sustainability and safety of seafood in our plate.

[P65] ASSESSING THE BACTERIAL COMMUNITY DYNAMICS IN A TURBOT (SCOPHTHALMUS MAXIMUS) LARVICULTURE FACILITY

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The production of high-quality fish larvae is often hindered by unpredictable mass mortality events. Previous studies have shown that the initial bacterial community of fish is a critical factor in promoting their resilience and survival. However, our understanding of the bacterial colonization and succession process that takes place during the earliest stages of a fish's life is limited. In this study, we aimed to investigate the early-stage microbial recruitment dynamics in newly hatched larvae under intensive larviculture conditions. To achieve this, we implemented a continuous sampling plan spanning five months across seventeen larviculture production tanks (PTs). The PTs were managed in accordance with standard animal husbandry practices commonly used in commercial farming operations. High-throughput sequencing of the 16S rRNA gene was used to investigate the bacterial communities of rearing water, whole larvae, and feed samples collected at 5, 10, 15, and 30 days after hatching. Bacterial community profiles were compared between the production units and sampling time and their structural diversity related to various parameters, such as water quality parameters, sampling date and initial larval density. Our analysis underscores the importance of various environmental parameters, including sampling date, temperature, dissolved oxygen, and feed, in shaping the composition of bacterial communities. We observed a convergence of fish and water microbiomes as the turbot larvae aged, which highlights the complex interplay between the host and its environment. Interestingly, our results also showed that the early stages of larval development (5-10 DAH) are dominated by fast-growing R strategist bacteria belonging to the Vibrionales order (genus Vibrio). In contrast, the bacterioplankton communities are more prominently composed of bacteria affiliated to Flavobacteriales, Rhodobacterales, and Oceanospirillales. Interestingly, this period (5-10 DAH) coincides with higher larval mortality rates. Taken together, our findings suggest the importance of suppressing the development of Vibrionales members during the initial stages of larval development.

[P66] EXPLORING THE EFFECT OF TRIBUTYRIN-SUPPLEMENTED DIETS IN RAINBOW TROUT (ONCORHYNCHUS MYKISS) GUT BACTERIAL COMMUNITIES

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Dietary supplementation with butyrate, a short chain fatty acid, has been associated with beneficial effects on fish health, as well as improvements in the ability of carnivorous fish to tolerate diets with an increasing plant-based content. The direct use of butyrate salts in feeds is nonetheless challenging: its strong odor and bitter taste make it less appealing to fish. Tributyrin is a non-volatile and stable triglyceride and as a metabolic precursor of butyrate, it can potentially substitute butyrate salts in diet formulations for aquaculture fish. In this study, we studied the effects of TBT-supplemented plant-based diets on rainbow trout (Oncorhynchus mykiss) digestion, including their gut bacterial composition, putative functioning, digestive enzymes, and chyme metabolome. An experimental feeding trial was conducted in an accredited facility for laboratory animal science for 50 days with triplicate tanks per diet. Fish were hand-fed twice-daily a basal control diet (Cont) or diets supplemented with tributyrin at 0.1% (m/m) (TBT1), 0.2% (TBT2) or 0.4% (TBT4) until apparent satiety. Sampling was conducted in 24-h postprandial fish. Gastro-intestinal tract activities of the digestive enzymes α-amylase, lipase, trypsin and chymotrypsin were measured in individual fish. Bacterial community composition data for each tank was obtained with high-throughput sequencing of the 16S rRNA gene using DNA extracted from pooled digesta samples. This data was used to predict functional profiles with the Tax4Fun2 package in R. Metabolome profiles of the digesta of individual fish were obtained by proton (1H) NMR spectroscopy. Our results showed that TBT supplementation had no significant effect on fish gut bacterial composition, digestive enzyme activities, or metabolic profile when compared to the non-supplemented control diet. The overall bacterial communities were dominated by bacteria affiliated to the Mycoplasmatales, Lactobacillales and Enterobacterales orders. An analysis of the most abundant taxa showed that diets with the highest TBT concentrations (TBT2 and TBT4) selectively reduced the abundance of an enterobacterial bacterial population related to Klebsiella pneumoniae, a potential fish pathogen. Furthermore, the predicted functional profiles of the bacterial communities indicated that increased levels of TBT were associated with enriched KEGG pathways related to antibiotic and secondary metabolite production, as well as depleted pathways related to pathogenesis. These specific effects of TBT on gut bacterial communities are intriguing and encourage further studies to investigate the potential of this triglyceride to promote pathogen suppression in the fish gut environment.

[P67] GENOME RESOLVED METAGENOMICS REVEALED THE IMPACT OF AMR STRAIN COLONIZATION AND ANTIBIOTIC TREATMENT IN COMPLEX MICROBIOTA IN VITRO.

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Microbiota of human and animal gastrointestinal tracts represent complex ecosystems and important reservoirs of AMR genes – i.e., resistome- contributing to the emergence and spread of multi-resistant pathogenic bacteria.
While the composition of the microbiota and resistome in human and animal gastrointestinal tract (GIT) has been documented, the drivers influencing the resistome have been poorly investigated. The potential of exogenous strains carrying AMR genes and different antimicrobial (AM) pressure in shaping GIT resistome structure and bacterial population dynamics remains unclear.
To address those questions, we designed in vitro PolyFermS continuous fermentations enabling controlled and replicated experiments. Chicken and human microbiota were inoculated with AMR strains isolated from chicken meat (i.e., ESBL E. coli and VRE E. faecium) and submitted to antibiotic treatment (i.e., cefotaxime and vancomycin).
We characterized the dynamic of taxonomic and metabolic activity of bacterial communities and in silico-mobilome classification, genome resolved metagenomics approaches were used to describe resistome structure, bacterial populations dynamics, AMR genes evolution and potential transfer among bacterial taxa.
Our results revealed that inoculated strains were not able to colonize microbiota models without AM selective pressure. While the abundance of Enterobacteriaceae appeared to drive the resistome in the chicken model, a dose-dependent increase of AMR gene abundance was observed in human microbiota indicating a potential co-selection of multidrug-resistant strains. Altogether, this study provides insights in strain-level dynamics, changes in the resistome structure, evolution of resistance genes and factors associated with variations of dissemination potential.

[P68] SALT□INDUCED STRESS TOLERANCE DURING WHEAT SEED GERMINATION IS IMPROVED WHEN VARIOVORAX SP. STRAIN P1R9 IS APPLIED IN BACTERIAL CONSORTIA

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EEndophytes associated to plants in extreme environments are attracting increasing interest as microbes to improve growth and stress tolerance of agricultural plants impacted by climate change. Here, we isolated and characterized endophytic bacteria with plant growth promoting (PGP) traits from plants in two extreme Chilean biomes (Atacama Desert and Chilean Patagonia). Out of 376 isolates, 42 were characterized as halotolerant auxin-producers (2–51 mg L-1) and 1-aminocyclopropane-1-carboxylate (ACC) degrading bacteria (15–28 µmol αKB mg protein-1 h-1). Most efficient isolates were tested as single strains, dual and triple consortia or in combination with known PGP rhizobacterial strains (Klebsiella sp. 27IJA and 8LJA) for their impact on the germination of salt exposed wheat seeds. Isolates with the highest germination index (GI) belonged to the genera Variovorax, Bacillus, Staphylococcus and Curtobacterium, with strain Variovorax sp. P1R9 showing highest effects. Most effective consortia were further challenged in greenhouse experiments for their effects on physiological and biochemical responses of wheat plants exposed to salt-induced stress (0.15 M and 0.25 M NaCl). Both consortia contained the new endophytic isolate Variovorax sp. P1R9. They showed significant PGB traits as expressed e.g. by higher biomass (41%) and reduced lipid peroxidation (33-56%) than in non-inoculated salt stress exposed plants. Although underlying mechanisms remain elusive, our data suggest that the application of endophytic Variovorax sp. P1R9 as a member of PGP consortia may improve plant salt stress tolerance and, hence, contribute to environmentally responsible solutions to counteract negative effects of desertification or salinity.

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[P69] HOT SPOTS IN ARCTIC SOILS: ARE ANCIENT ARCTIC SETTLEMENTS POSSIBLE RESERVOIRS FOR KNOWN OR POTENTIAL PATHOGENIC AGENTS?

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The history of Greenland is marked by repeated waves of Paleo-Inuit immigration from North America. From the 10th to 15th century, Norse settlers immigrated from Europe and flourished in southwest Greenland with the introduction of domestic livestock. The different Inuit and Norse cultures created middens by dumping and accumulating domestic waste. Today, animal bones, excrements, mollusc shells and other artefacts associated with past human occupation are a valuable resource for archaeologists to study these past societies' diets, habits, life, and death. However, these archaeological features might also represent unique microbial reservoirs of organisms from mixed origins (soil, human or animal associated) within an organic rich matrix- A latent source of microbes, including potential pathogens, that is currently exposed to climate change. Drastic increase in temperature observed in Greenland leads to thawing of the protective permafrost layer and midden material may be carried away by enlarged melting water from snowdrifts. Rising sea levels and loss of sea ice also accelerates the erosion of middens that are most often situated along the coast and might be washed out into the sea. This raises the question whether these sites represent a disease emergence threat. Especially that the increase in plant production in West Greenland is opening for the development of sheep farming in the Nuuk area, exactly where sheep farms were situated during the Norse era and abandoned for the past 500 years. In the VEO project, we aim to evaluate if ancient Arctic settlements are possible hot spots for pathogenic agents, and if potential pathogens may spread to the surrounding environment. Using metagenomics, we compared the microbial communities of middens from different age and location in West and South Greenland (Paleo-Inuit, Norse, and Modern Inuit middens) to pristine surrounding soils. We found that even after hundreds of years, the middens harbor a distinctive microbial signature enriched in host-associated and/or pathogenic bacteria belonging to Clostridium and other firmicutes; commensal bacteria from human faecal microbiome C. massilliamazoniense, Ramboutsia hominins and Eubacterium tenue, food poisoning agent C. perfringens, opportunistic pathogens Paeniclostridium sordellii causing toxic shock in humans, or agents associated to rare infant or adult botulism such as C. baratii and C. thermobutyricum. The next step is to identify and characterize pathogenicity markers, relatedness to currently circulating pathogens as well as model potential dissemination.

[P70] DIVERSITY AND CONNECTIVITY OF FUNGAL COMMUNITIES IN DATE PALM ROOTS AND SOIL ARE DISTINCT AND IMPACTED BY IRRIGATION WATER SOURCES

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Date palm (Phoenix dactylifera) is a widely cultivated crop in arid agroecosystems, where the impact of irrigation water sources on below-ground fungal communities remains poorly understood. In this study, we characterized the soil and root-associated fungal (RAF) communities of date palms under different irrigation regimes (freshwater vs. saline groundwater) using ITS2 metabarcoding. Our results reveal that RAF diversity was lower compared to soil, and RAF communities were distinct and highly connected. We found that the fungal communities in both soil and root compartments were significantly impacted by irrigation water sources. Water pH and electrical conductivity (EC) were identified as the primary structure factors in both compartments, while soil pH and EC chemistry contributed to the additional factors in the soil. We observed that drift (stochasticity) was the dominant process affecting both root and soil compartments under saline groundwater irrigation, with a higher relative importance in roots than soil. Furthermore, specific saprotrophic genera, including Acrocalymma, Coprinopsis, Myrothecium, Chaetomium, and Preussia, were significantly enriched in roots and soil under saline groundwater irrigation. Our findings suggest that the RAF communities are complex and connected, and that saline groundwater irrigation selects specific fungal communities that are suitable for promoting host growth under extreme conditions of saline agroecosystems. Our study provides important insights into the role of irrigation water sources in shaping fungal communities in date palm roots and soil, which may have implications for improving crop productivity in arid agroecosystems.

[P71] PSEUDOMONAS KOREENSIS STRAIN 69RS COLONIZES RICE PLANTS AND INCREMENTS PHOSPHATE BIOAVAILABILITY: RESULTS FROM IN VIVO EXPERIMENTS

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Rice (Oryza sativa, L.) is one of the most important cereal and staple food crop and its production needs to further enhance due to the increase of world's population. The demand for production is impaired by nowadays lower water availability under climate change scenarios. Concomitantly, cultivation under aerobic management affects mineral fertilizers bioavailability, phosphate especially. For these reasons, it is important to acquire innovative and eco-friendly agrotechnologies to preserve productivity and sustainability of rice production. Plant growth-promoting rhizobacteria (PGPR) can be the key position improving soil quality and various nutrients bioavailability, in particular of different phosphate forms present in soil. The present work aims at determining the efficacy of PGPR inoculation of rice in the solubilization of inorganic phosphate, as determined in pot experiments.
More than 200 bacterial strains were isolated from rice rhizosphere and characterized for different PGP activities including solubilization of three inorganic phosphate forms, mineralization of phytate, nitrogen fixation, indole 3-acetic acid production, aminocyclopropane-1-carboxylate deaminase activity, siderophore production and extra-cellular polymers production. In in-vitro experiments, the best performing bacterial strain belonged to Pseudomonas koreensis. P. koreensis strain 69RS was able to solubilize different mineral phosphates (tricalcium, Fe and Al) and to mineralize the organic one (phytate), these abilities being confirmed by the presence of functional genes gcd, phoD and phnX. The phosphate solubilizing activity as quantified by the molybdenum blue method was major for AlPO4 followed by Ca (CO3)2PO4 and FePO4. Green Fluorescent Protein transformants of strain 69RS were used to follow rice seedling colonization in growth pouches experiments and successively in pot experiments. The strain was able to colonize rice plants and growth substrates, as evidenced by confocal microscopic analyses. Particularly, in the growth pouches experiment the bacterized seeds showed a significant increase in the rice root growth. Pot experiments with Ca (CO3)2PO4 as only P source confirmed that the presence of strain 69RS increased rice growth and phosphate bioavailability.
This study highlighted that P. koreensis strain 69RS possess important PGP activities, it successfully colonizes plant tissues and is persistent into the rice rhizosphere system. The strain can increment phosphate bioavailability and improves root growth thus deserving further attention as bioinoculant in water-saving cultivation schemes.

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[P72] GENOME-RESOLVED METAGENOMICS SUGGESTS AN UNANTICIPATED ROLE OF RHODOTHERMALES SYMBIONTS OF MARINE SPONGES IN BENTHIC BIOGEOCHEMISTRY AND CHITIN DEGRADATION

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Chitin ranks as the most abundant polysaccharide in the oceans yet knowledge of shifts in structure and diversity of chitin-degrading communities across marine niches is scarce. Differential degradation pathways, utilization, and turnover have been recently hypothesized to dictate the processing of chitin across marine biotopes, highlighting that the microbiomes of sessile marine invertebrates may play a decisive role in the degradation of this major biopolymer [1].

Marine sponges are foundational, widely distributed benthic organisms populated by diverse and dense bacterial communities. Although a general role in polysaccharide degradation has been proposed for several sponge symbiont lineages, knowledge of their capacity to degrade and utilize chitin is limited. This study employs genome-resolved metagenomics to delineate the community of potential chitin degraders and utilizers within the marine sponge microbiome.

Twelve microbial metagenomes from Spongia agaricina, Spongia officinalis, and seawater were each assembled and binned into 137 metagenome-assembled genomes (MAGs) which were subsequently classified into 18 prokaryotic classes across 12 phyla. The capacity to degrade the large chitin polymer via hydrolysis is likely a bottleneck function within the sponge symbiotic consortium, as endo-chitinase domains were encoded by 12 (8.76%) MAGs only. Instead, the potential for chitin hydrolysis into dimers and monomers, degradation via deacetylation into chitosan, and utilization of the chitin monomer N-acetylglucosamine were frequent among symbionts, being annotated on 47 (33.58%), 107 (78.10%) and 73 MAGs (53.28%), respectively.

Remarkably, MAGs in the order Rhodothermales (class Rhodothermia, phylum Rhodothermota) possessed all the chitin degradation and utilization domains examined here, along with enrichment in chondroitinase and heparinase-encoding genes indicative of glycosaminoglycan degradation capacity. Collectively, these features suggest that these symbionts occupy a unique micro-niche involving the utilization of complex carbohydrate sources and peptidoglycans that are structural components of marine sponges. Comprehensive phylogenetic assessments further revealed a robust clade composed exclusively of endo-chitinase domains from sponge-specific, uncultured symbionts of the order Rhodothermales, strengthening the notion of chitin hydrolysis as an adaptive feature of these symbionts to living in the in-spongia milieu. Genome-wide, phylogenomic inference helped circumscribe the Rhodothermales order further by uncovering three sponge-specific candidate families formed exclusively by uncultured symbionts of marine sponges, sharply expanding our view of Rhodothermales

diversification, host range, and biogeographical occurrence within these animals. Our findings suggest Rhodothermales symbionts as keystone members of the marine sponge holobiont and global players in benthic biogeochemistry owing to their versatile carbohydrate metabolism.

[1] Raimundo et al. Microbiome 2021, https://doi.org/10.1186/s40168-020-00970-2

[P73] THE FLAGELLAR TRANSCRIPTIONAL REGULATOR FLHC OF ACIDOVORAX AS A MODERATOR OF PLANT-MICROBE INTERACTIONS

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The beta-proteobacteria of the genus Acidovorax are mostly known as plant pathogens, although some species (eg. A. radicis) can promote plant health. However, most strains adapted to a broader variety of environments and fall in a spectrum from opportunism to mutualism. In a previous study, we found that type III (T3SS) and VI secretion systems (T6SS) delineate the border between plant associated and free-living Acidovorax strains, being present in most of the former and almost absent in the latter (1). We also observed that two A. delafieldii isolates, differing only by the presence of the flagellar transcriptional regulator flhC (flhC+, flhC-) gene in their genome, caused opposite effects on plant growth. Upon re-inoculation of their host of origin, Lotus japonicus, flhC- promoted plant growth, whilst flhC+ reduced it. Here, we studied the role of flhC in moderating the transcriptional response of the two A. delafieldii isolates to a cocktail of plant metabolites. We cultivated the flhC+ and flhC- strains in media supplemented with extracts from L. japonicus roots. Comparative transcriptome analyses revealed a relative expression increase of several genes coding for T6SS in flhC-. Interestingly, along with flagellar assembly and chemotaxis, flhC+ showed increased expression of T3SS genes. Given the constitutive costs and the reduced need for motility, the antigenic potential of the flagella, and the ecological relevance of T3/T6SS, purifying selective forces acting on the flagella in the host microbiome are expected to relax. Under this assumption, we posit that mutations or loss of flhC are more likely to happen in host-associated bacteria. To test this, we mined flhC and flhD sequences from 4163 reference genomes, belonging to 38 phyla, of which 1640 and 1495 were isolated from hosts and the environment respectively. We studied both sequence alignments in an evolutionary phylogenomic framework, and we found evidence of positive diversifying selection acting on flhC, but not flhD, in the host-associated genomes. Our study provides evidence for a transcriptional balance between T3 and T6SS regulated by FlhCD and hints at a selective disadvantage of a fully functional FlhCD in host-associated bacteria.

1. Siani, Roberto. Microbial Genomics 7.12 (2021).

[P74] CHANGES OF ENDOPHYTIC BACTERIAL COMMUNITIES BETWEEN SEEDS AND GERMINATED SEEDS OF FOUR COMMERCIAL VEGETABLES FAMILIES

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Aim: It is widely recognized that plant microbiomes are crucial for the development and growth of plants. In this context, seeds harbor adapted endophytic bacteria which can be vertically transferred to the progenies, including plant growth-promoting (PGP) endophytic bacteria. In this context, Chile is among the Top–10 seed exporters at global level, where 46% of exported seeds are vegetables. However, the composition and assembly of seed microbiomes in Chilean vegetable seeds and early stages of seedlings have been scarcely investigated. The aim of this study was exploring the changes on the composition, PGP traits and biocontrol (BC) activity of endophytic bacterial communities (EBC) contained in seeds (S) and germinated seeds (GS) of four families of Chilean demanded vegetables.

Methods: The EBC associated with disinfected S and aseptically GS (2 weeks) of four vegetables families (Apiaceae, Asteraceae, Brassicaceae, and Solanaceae) were evaluated by 16S rRNA metabarcoding analysis. Alpha- and beta-diversity was evaluated by QIIME1 and PCoA, respectively. In addition, endophytic culturable bacteria were isolated from S (50 isolates) and GS (97 isolates) and characterized based on their PGP traits and BC activity against three certified plant pathogens (Xanthomonas sp., Pseudomonas syringae pv. syringae and Pseudomonas viridiflava).

Results: Statistical differences ($p < 0.05$, t–test) in alpha diversity (Chao1, Shannon and Simpsons indexes) of EBC between S and GS were not observed, except in Solanaceae family. Members of Pseudomonadota were dominant (32 to 89%) in S, followed by members of Actinomycetota (24 to 3%) and Bacillota; (3 to 18%) phyla. Pseudomonadota was also found as the dominant phylum (23 to 93%) in GS, followed by Bacillota; (37 to 89%) and Actinomycetota (5 to 34%). Significant differences (adonis, $p < 0.05$) in EBC between S and GS were revealed by PCoA in Asteraceae, Brassicaceae and Solanaceae families, except in Apiaceae family. Bacterial isolates showing PGP traits were observed in both S and GS from all studied vegetables families. BC activity against at least two assayed pathogens was observed in 74% and 82% of isolates from S and GS, respectively.

Conclusions: Differences in EBC between S and GS were revealed by 16S rRNA metabarcoding analysis. Isolates from S and GS showed PGP traits and biocontrol of plant pathogens.

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[P75] INSIGHTS INTO THE CULTURABLE BACTERIAL COMMUNITY OF LONG-TERM AQUARIUM TROPICAL OCTOCORALS

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Coral reefs are highly complex marine ecosystems. Although there is still much to unveil, it is known that reef communities host diverse microbial consortia, both taxonomically and functionally, that play key roles in coral fitness. Due to climate change, dysbiotic processes linked to elevated temperatures are a major force of change in coral-dominated ecosystems and have led to severe coral mass mortality events, resulting in biodiversity loss. A shift from stony coral-dominated reefs towards octocorals (Subclass Octocorallia) has been registered in several geographical areas; nevertheless, octocorals are still affected by climate change and diseases. To better understand the symbiotic relationships that octocorals establish with microorganisms, in this study, we determine the taxonomic composition of culturable, octocoral-associated bacteria in a long-term aquarium mesocosm. Three octocoral species, Litophyton sp., Lobophytum sp. and Sinularia sp., were sampled from a 19 m3 live coral aquarium from Oceanário de Lisboa, operating since 1998, and their associated bacterial community cultured on diluted marine R2A and Marine Agar media. A total of 90 bacterial strains were isolated. 16S rRNA gene-based phylogenetic analyses grouped the isolates into six bacterial classes, 14 orders and 26 classified genera. Additionally, six unclassified isolates were obtained, two of them likely presenting new bacterial families in the Alteromonadales and Cellvibrionales orders, and the other four likely presenting a new gammaproteobacterial order. The collection comprised multiple, harder-to-cultivate cultured genera such as Endozoicomonas (6 isolates), Fictibacillus (2 isolates), Ureibacillus (2 isolates) and Flammeovirga (1 isolate). Most genera were only obtained from one octocoral species, indicating host-specificity. Moreover, possible new bacterial species in the genera Amylibacter, Aquimarina, Dietzia, Endozoicomonas, Ferrimonas, Fictibacillus, Flammeovirga, Gracilibacillus, Halomonas, Nocardioides, Pelagibius and Ureibacillus were identified. The Endozoicomonas genus, in particular, has been frequently reported as associated with multiple coral species, and suggested as a core symbiont of the healthy holobiont. The preservation of core symbionts of healthy corals in captivity highlights the possibility of using sustainable, man-made ecosystems as repositories of stable and healthy coral microbiomes, which may be applied as coral probiotics to improve wild corals' health. Our study showcases the phylogenetic uniqueness of culturable, bacterial symbionts of tropical octocorals maintained for long-term periods in controlled mesocosms. This emphasizes the key role that aquarium facilities can have in coral reef restoration and as source of new bacterial species worth further research.

[P76] COMPOSITION AND FUNCTIONALITY OF ZOOPLANKTON-ASSOCIATED BACTERIA ACROSS THE BALTIC SEA SALINITY GRADIENT

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Zooplankton and bacterioplankton are two key components of the pelagic community that perform key ecological functions, such as nutrient cycling and trophic transfer. Compared to free-living bacteria in the ambient water, higher concentration of bacteria associates within zooplankton create hotspots of interactions (Grossart et al., 2013). Associated bacteria are assumed to benefit their hosts in various environments, assisting in acclimating to dynamic ambient conditions and combating environmental stress (Li et al., 2021; MacKe et al., 2017). To understand the ecological consequences of the zooplankton microbiota, we investigated the composition and functions of zooplankton-associated bacteria and how they change along the salinity gradient in the Baltic Sea. Zooplankton were sampled from six stations within the Baltic Sea and two stations in the West Coast of Sweden. 16S Metabarcoding sequencing and Phylogenetic Investigation of Communities by Reconstruction of Unobserved States (PICRUSt2) were implemented for bacteria community and function analysis (Douglas et al., 2020). We found common bacterial phyla: Proteobacteria, Bacteroidetes, Firmicutes and Actinobacteria in different zooplankton hosts across all sampling stations. However, family composition of each phylum showed high variance across host and station. Function analysis suggested that core metabolic pathways existed in zooplankton from different locations, but the bacteria taxa contributing to those pathways were different across location. Further, location-specific bacterial metabolic pathways were detected. These results suggested that zooplankton and location both significantly influence the microbiome composition and functionality. This is consistent with the assumption that environmental inoculation and host-filtration both determined the assembly of associated bacterial communities (Tang et al., 2010). By implemented bioinformatic prediction of function of metabarcoding data with different reference databases, this research highlights the importance of specific reference databases for symbiotic bacteria research.

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[P77] LATE EVENTS OF THE INTRACELLULAR LIFE CYCLE OF PISCIRICKETTSIA SALMONIS ARE REQUIRED FOR THE PATHOGEN INFECTIVITY

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Piscirickettsia salmonis is a facultative intracellular pathogen and the causative agent of piscirickettsiosis, a systemic infection of salmonid fish. Fish acquire the infection via horizontal transmission from fish-to-fish, without the need for physical contact. After invasion, P. salmonis actively replicates in macrophages and monocytes, and its ability to enter and multiply within host cells is essential for pathogenesis. We report that at late stages of the intracellular life cycle P. salmonis triggers a transcriptional response like that of bacteria in the stationary growth phase. Thus, P. salmonis decreases its transcription and energy metabolism and, at the same time, induces the expression of components associated with nutrient starvation response, stress resistance and virulence factors, suggesting its differentiation into a form more virulent and potentially more resistant to environmental stresses. To study the differences in infectivity between different intracellular stages of P. salmonis an in vitro infection model that uses a macrophage-like cell line was employed. Viable bacteria were recovered from the intracellular medium at different times post-infection to assess the replication rate and cytopathogenicity of P. salmonis in new target cells. Our results indicate that bacteria extracted from infected cells at late stages of their intracellular life (10 days post-infection) significantly increase their replication in the new cells (up to 100 times) and their cytopathogenicity (up to 55% more) when compared with bacteria extracted at early stage of infection or with extracellular P. salmonis grown in broth. By confocal microscopy, recruitment of Lamp-1 and Rab-7 proteins into the P. salmonis containing vacuole was detected, suggesting at this late stage of infection, P. salmonis locates within a phagosome-like compartment, facing starvation and probably acidic conditions. These results provide insights into the regulatory mechanisms induced during the intracellular life cycle of P. salmonis that confer the bacterium the ability to leave the host cell, survive in hostile environmental conditions, and re-infect a new target cell. We propose that the capability of differentiate into a more resilient form constitute an important virulence trait evolved by P. salmonis as part of its natural history.

[P78] CULTIVAR-DEPENDENT ENRICHMENT OF PSEUDOMONAS MICRODIVERSITY AND CYCLIC LIPOPEPTIDE BIOSYNTHETIC GENE CLUSTERS

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Plants are well known to recruit and maintain a unique rhizosphere microbiome in a symbiosis that provides important functions for the plant. However, modulation by the plant of the taxonomic and functional microdiversity of its microbiome is unresolved, despite the unexplored potential within highly diverse genera, e.g., Pseudomonas. Here, we employed an integrative approach combining culturomics, comparative genomics, microscopy, and plant assays to determine the degree of cultivar-dependent Pseudomonas taxonomic, biosynthetic, and functional micro diversity in the wheat (Triticum aestivum) rhizosphere. A Pseudomonas strain library of 552 isolates was built from two cultivars of wheat with contrasting resistance to the plant pathogen Fusarium culmorum. Through full-length 16S rRNA gene sequencing of the strain library, we found that the resistant cultivar, which was also shown to have thinner roots, harbored a more diverse Pseudomonas community compared to the sensitive cultivar, shown to have thicker roots. Genome mining of all 48 unique Pseudomonas genomes from the strain collection did not reveal a difference in the abundance of secondary metabolite (SM) biosynthetic gene clusters (BGCs) between the cultivars. However, a sequence-based similarity network analysis of BGCs showed that the Pseudomonas community from the resistant cultivar harbored a greater diversity of BGC families. Additionally, SM prediction demonstrated that the BGC for an antifungal cyclic lipopeptide (CLP), lokisin, was enriched in the resistant cultivar while the BGC for the motility CLP viscosin was enriched in the sensitive cultivar. Confocal microscopy of representative strains revealed that Pseudomonas harboring the lokisin BGC preferentially colonized the crevices between root cells while Pseudomonas harboring the viscosin BGC preferentially colonized roots along the outer surface of root cells. When these strains were re-inoculated onto Fusarium-infected wheat seedlings, Pseudomonas producing lokisin protected both cultivars against infection while Pseudomonas producing viscosin protected only the susceptible cultivar against infection. Taken together, we conclude that two different wheat cultivars have the capacity to enrich their microbiomes with distinct Pseudomonas micro diversity, populated by strains with alternative colonization strategies and harboring CLP BGCs that differentially modulate plant health. Overall, our results demonstrate the limitations of generalizing microbial genera in microbiomes and highlight the importance of intragenus micro diversity to microbiome functionality.

[P79] ESTABLISHING A WHEAT-PROMOTING MICROBIAL COMMUNITY

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Important crops cultivated across the world are subject to yield losses due to pests and diseases, as well as climate change, resulting in high rates of undernourishment and food insecurity. Plant growth-promoting rhizobacteria represent a sustainable solution to boost crop resiliency. Understanding the interactions between plant roots and key bacterial groups, and especially bacterial communities, is crucial to harness their potential. Here, we focus on developing a wheat growth-promoting rhizosphere microbiome. This is pursued through successive wheat cultivation and repeated re-inoculation of the root microbiome of wheat plants displaying different growth using non-stressed and stressed conditions. Under the appropriate stress factor, the plants may be prompted to shape their rhizosphere microbiome, which in turn can inform a more targeted investigation of specific bacterial groups. 16S amplicon sequencing reveals how the selection lineages influence the microbiome compositions, especially in the rhizosphere. Specific bacterial groups which differ between the different lineages may contribute to determine the selected plant phenotype and are therefore excellent starting points to validate synthetic communities used for testing microbe-plant interaction. This study delineates a top-down approach for investigating larger microbial communities for biocontrol, starting from a successive cultivation with selection of a plant parameter. Our method is a valuable complementary approach in addition to single strain studies for the assembly of microbial communities for plant biocontrol and bio stimulation.
This project is part of INTERACT within the Collaborative Crop Resiliency Program (NNF19SA0059360) funded by the Novo Nordisk Foundation.

[P80] THE PHYTOBIOME OF THE MEDICINAL PLANT ORIGANUM VULGARE: LINKING THE BACTERIAL ENDOPHYTIC COMMUNITIES TO THE ESSENTIAL OIL

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Aim: Antimicrobial resistance is a global concern associated with high morbidity and mortality. Multidrug-resistance bacteria (MDR) may be untreatable with conventional antibiotics; hence, it is important to prioritize the development of alternative therapies. Medicinal and aromatic plants represent a natural source of bioactive molecules. In particular, antibacterial and antifungal activities have been reported for plants belonging to the Origanum genus and their essential oils (EOs). One of the advances in addressing these issues is the discovery that microorganisms residing inside the plant may contribute to the production of metabolites of pharmaceutical interest. The aims of this work are i) to characterize the bacterial endophytic community associated with Origanum species and subspecies to select a collection of bacteria able to synthesize antimicrobial molecules, and ii) to understand if the EO aroma profile might be influenced by the presence of the endophytes and/or if some EO compounds might be synthesized by the endophytes themselves.
Methods: Endophytic bacterial communities were isolated from different plant compartments. EOs were hydro distilled from the same plants and their chemical composition was determined by gas chromatography coupled with mass spectrometry (GC-MS). The structure and the composition of the bacterial communities were investigated through a Random Amplified Polymorphic DNA (RAPD) analysis and 16S rDNA gene sequencing. Endophytes' ability to inhibit the growth of a panel of MDR human pathogens was also evaluated through the cross-streaking method.
Results: The analysis of RAPD profiles and the bacterial taxonomic affiliation revealed a high degree of biodiversity and a low degree of strains sharing among Origanum subspecies and the different compartments of the same plant. Cross-streaking experiments revealed endophytes' potential to synthesize antibiotics, including volatile organic compounds (VOCs). The analysis performed with GC-MS revealed that some of the VOCs synthesized by endophytes are also present in the EO chemical profile of the host plant.
Conclusions: Data obtained suggest the existence of selective forces able to determine the structure and composition of the microbial community associated with different medicinal plants but also with different compartments of the same plant. The ability of endophytes to produce volatile molecules able to inhibit the growth of MDR bacteria highlights the enormous biotechnological and pharmacological potential

of endophytes. It might also suggest their involvement in the determination of the EO aromatic profile of the plant with which they are associated.

[P81] ANTARCTIC PSYCHROTOLERANT BACTERIA ENHANCE PLANT TOLERANCE TO COLD STRESS AND CAUSE METABOLIC CHANGES IN TOMATO

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Plant-associated microorganisms can protect plants against cold stress, promoting physiological responses of acclimation to low temperatures in crops. Bacteria associated with Antarctic plants are an understudied source of beneficial microorganisms and the use of these bacteria may represent a sustainable strategy for the protection of crops (e.g. tomato) against cold stress. However, scarce information is available on the molecular mechanisms underlying this process. This work aims at understanding the physiological mechanisms activated by psychrotolerant bacteria on tomato plants and to identify plant metabolites involved in the mitigation of cold stress. Two psychrotolerant bacteria isolated from the Antarctic plant Colobanthus quitensis (Hafnia sp., Pseudomonas sp.) and a well-studied endophyte (Paraburkholderia phytofirmans PsJN) were selected for their ability to promote tomato shoot growth at low temperatures. In particular, Hafnia sp. produced indolacetic acid in presence of tryptophan at 25°C and at 4°C. To characterize the ability of these bacterial isolates to affect plant metabolism under cold stress, surface-disinfected tomato seeds were inoculated, while mock-inoculated seeds were used as control. Four-week-old plants were exposed to 4°C for 7 days in the dark and incubated at 25°C for zero (control), two and four days to allow recovery from the stress treatment. Metabolites were analyzed with high performance liquid chromatography coupled with high resolution mass spectrometry (HPLC-HRMS) analysis. Bacterium-inoculated plants showed lower concentrations of phenylalanine-derived polyphenolic compounds compared to mock-inoculated plants. Interestingly, bacterium-inoculated plants accumulated higher content of putative phenylalanine-containing dipeptides compared to mock-inoculated plants, suggesting that these compounds may play a role in cold tolerance. The study suggests that Antarctic bacterial isolates were able to modulate phenylalanine metabolism in cold-stressed tomato plants. A deeper knowledge of the mechanisms activated by beneficial bacteria in tomato plants will open the possibility to develop new sustainable strategies to protect the plants against cold stress.

[P82] THE EFFECTS OF MICROPLASTIC POLLUTION ON THE MICROBIOME OF THE FRESHWATER KEYSTONE SPECIES DAPHNIA MAGNA

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Aim: The aim of this study was to investigate the impact of anthropogenic stress in the form of microplastics on the Daphnia microbiome.

Methods: Daphnia magna and bacterioplankton were sampled from 15 ponds across Flanders (Belgium), which were subjected to varying levels of pollution (from nature reserve to city ponds). Additionally, parameters such as NPOC, pH, temperature, chlorophyll concentration and dissolved oxygen were measured. Daphnia and bacterioplankton samples were sequenced with 16S metabarcoding and Illumina shotgun. For the latter, the reads were quality filtered, host-depleted and co-assembled per location with megahit. To identify potential plastic-degrading enzymes, HMM motifs were generated from experimentally confirmed enzymes compiled from literature and publicly available databases. These custom-made motifs were then used to screen the contigs for potential presence of plastic-degrading enzyme homologues with focus on PLA, PET and Nylon. Proteins with a bit-score higher than 100 and e-value less than 0.0001 were considered significant and inspected further.

Results: In total, 65 Daphnia specimens and 95 samples of bacterioplankton were collected. We also found that some specimens of Daphnia collected from polluted city ponds contained unidentified plastic fibers in their guts. The Daphnia microbiome differed significantly from corresponding bacterioplankton communities (p-value < 0.01). Alpha diversity indices were not significantly different across locations and pond categories except for Citadelpark, a city pond which was significantly less rich than other sampled ponds. Water reservoir ponds, city ponds and samples from the Lange Rode Vijver pond formed distinct groups in an ordination (permanova, p-value < 0.01). Environmental parameters were regressed on the community matrix amounting to 50% of the observed variance (RDA, Adjusted-R2: 50%, p-value < 0.01). A PET-enzyme homologue was found in Daphnia's microbiome sample from a polluted city pond and was identified as an alpha-beta hydrolase with a 71.26% similarity to that of Roseateles depolymerans, a known PET-degrader.

Conclusions: We found that Daphnia can accumulate plastic fibers in its gut and that its microbiome is distinct from surrounding bacterioplankton. The microbiome composition differs across location and pond categories, but its main driving factor is still unknown. Future work will involve measuring microplastic concentrations to further resolve these differences. The presence of potential plastic-degrading enzymes in the shotgun data is encouraging but given noise from the host genome, the resolution was not adequate to fully elucidate the degradative potential of Daphnia's microbiome in response to plastics.

[P83] UNCOVERING CANNABIS SEED ENDOPHYTIC DIVERSITY AND COMPOSITION ACROSS VARIETIES

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Seed endophytes are specialized microorganisms that thrive in plant tissues and can persist for multiple generations. These endophytes establish a symbiotic relationship with their host and are crucial for the host's germination, adaptability, and overall health. Our study examined the seed core microbiome of 46 Cannabis varieties and found that it is mainly comprised of six bacterial endophyte genera: Pantoea, Ralstonia, Pseudomonas, Bacillus, Kosakonia, and Rhodococcus. This core microbiota accounts for 58.6% of the variations in microbial composition between cultivars. Host-related characteristics, such as inflorescence type, and chemotype, influenced the microbial structure and composition of Cannabis varieties. We also identified bacterial biomarkers that can differentiate between these host-related features. Using a Bayesian method to analyze microbial communities, we estimated the fraction of potentially inherited seed microbiota for some varieties. Understanding how plant breeding can influence microbial communities within Cannabis seeds can provide a basis for developing new strategies that improve plant growth and performance by aiding in the selection and propagation of beneficial microbes, leading to healthier crops that are able to withstand environmental stressors and promote sustainable agriculture.

[P84] GENOME STREAMLINING OF SPONGE-ASSOCIATED AMMONIA-OXIDIZING ARCHAEA

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Ammonia oxidizing archaea (AOA; order Nitrososphærales) are common symbionts of marine sponges. As part of the sponge holobiont, AOA are vital for the removal of nitrogenous waste products from the sponge tissue via the oxidation of ammonia to nitrite, the first step of nitrification. In comparison to free-living AOA, genomes of sponge-associated AOA show specific adaptations to facilitate symbiosis (i.e., eukaryotic-like proteins for host-symbiont recognition, mixotrophy via the uptake of branched-chain amino acids). Despite their exclusively symbiotic lifestyle, genome sizes of previously studied sponge-associated AOA (1.80 ± 0.30 Mbp) are typically larger than free-living AOA (1.53 ± 0.36 Mbp). Here, we characterize a novel sponge-associated AOA group with genome sizes of 0.86 ± 0.04 Mbp, representing the smallest AOA genomes described so far. The functional consequences of reduced genome size were evaluated using comparative genomics and genome-centered metatranscriptomics. The genomic information was complemented with nitrification rate measurements and symbionts were quantified and visualized in adult sponges and larvae. The reduced AOA genomes revealed representative genome characteristics of endosymbionts such as low GC content, high coding density, and low pseudogenization. Although they lack otherwise typical functional adaptations of symbiotic AOA, key genes for ammonia oxidation, energy flow, carbon fixation, vitamin, and amino acid biosynthesis were preserved and actively transcribed. The extremely reduced genomes can serve as minimal genome proxies for ammonia-oxidizing archaea due to their fully functional core metabolism. Hence, these newly discovered genomes can help to elucidate unresolved steps of the archaeal ammonia oxidation pathway.

[P85] DISTRIBUTION OF POLYUNSATURATED FATTY ACID GENES AMONG EARTHWORM GUT MICROBIOTA TO UNDERSTAND RARE-LIPID PROVISIONING IN THE TERRESTRIAL FOOD CHAIN

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In-land production of long-chain polyunsaturated fatty acids (PUFAs) is considered to stem primarily from eukaryotic microorganisms such as the thraustochytrid algal protists that inhabit freshwater ecosystems. In aquatic environments, a linear relationship is seen between PUFA content and organism taxonomic hierarchies – from algae to invertebrates to vertebrates. The scenario by which strictly terrestrial organisms obtain PUFAs such as eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) is incompletely understood, given that there are claims about conversion bottlenecks and insufficient denovo synthesis. These observations have translated into a booming supplements industry to market EPA- and DHA-containing oils derived from marine organisms. Curiously, soil bacteria such as members of the γ-Proteobacteria and certain myxobacteria, such as Sorangium cellulosum and Aetherobacter fasciculatus contain genetic and enzymatic competences to denovo synthesize PUFA using an iterative type I fatty acid synthase (FAS)/polyketide synthase (PKS)-like synthase system. Additional soil bacterial candidate producers are coming to light as metagenomic data unearths uncultured genomic information on environmental microorganisms. The link between microbial and faunal food webs in soils is often linked by earthworms, and curiously, their gut-soil and muscle tissue have increasing concentrations of EPA and DHA relative to surrounding bulk soil, implicating microbial synthesis within the earthworm intestinal tract. Therefore, we have interrogated earthworm metagenomic datasets, archived full genomes of sequenced organisms, and generated a novel dataset on earthworm microbial composition and gene expression from local organisms to explore the connection between microbial PUFA synthesis activity in terrestrial ecosystems that overlaps with animal host microbiomes. These data provide insights about how the terrestrial trophic chain may be supplied by bacterial activity at its base and explores the hypothesis that PUFA production is activated by stochastic stressful conditions, and constrains trait dispersal. The understanding of this process and extent of producer networks is important for answering open questions in mammalian evolution, as well as for biotechnological efforts to more sustainably source nutritional PUFAs.

[P86] THE MARINE BRYOZOAN CONOPEUM SEURATI REPRESENTS A NICHE FOR TROPODITHIETIC ACID (TDA) PRODUCING PHAEOBACTER

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Marine invertebrates have long been a source of bioactive compounds; however, several studies have indicated that such secondary metabolites are often produced by associated bacteria. Thus, there is a great interest in understanding the role of bacterial secondary metabolites and their producers, in their association with marine invertebrates. This requires that researchers decipher the microbial communities of such invertebrates, and the purpose of the present study was to describe the microbiome associated with a marine bryozoan specimen collected at a Danish coastal location. In previous studies, potent secondary metabolite producers have been isolated from these bryozoans. Based on morphology and 18S rRNA amplicon sequencing bryozoan specimen was identified as the calcified Conopeum seurati (order: Cheilostomatida). Amplicon sequencing of the 16S rRNA gene revealed that members of the Myxococcota and Spirochaetes phyla were uniquely associated with C. seurati, with an unclassified spirochaete ASV (Amplicon Sequence Variant) being the most predominant member. Secondly, we found that Phaeobacter sp. was among the most abundant bacterial members of C. seurati, and present in higher relative abundance than in the surrounding environments. Using a qPCR assays, we detected tropodithietic acid (TDA) genes originating from Phaeobacter sp., in the C. seurati microbiome corresponding to $6.3x10^4$ cells per gram of C. seurati, which was >5x higher that in the surrounding environments relative to total bacteria. However, we could not chemically detect TDA using LC-MS. Lastly, we initiated the profiling of the metabolomic landscape of C. seurati, where fungal metabolites and various pigments were among the annotated metabolic features. These results highlight that C. seurati represent a natural habitat of bacteria having the potential to produce TDA, and therefore holds great potential to be used in the future to help understand the ecological importance of TDA and the bacteria that produce them.

[P87] MICRO-SCALE INTERSPECIFIC INTERACTIONS SHAPE SPATIAL ORGANIZATION OF MULTISPECIES BIOFILMS FORMED ON ARABIDOPSIS ROOTS

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Microorganisms colonizing plant roots co-exist in complex, spatially structured multispecies biofilm communities. However, little is known about the spatial interactions of species in multispecies biofilms formed on plant roots. Here, we used an established four-strain multispecies biofilm model (Stenotrophomonas rhizophila, Paenibacillus amylolyticus, Microbacterium oxydans and Xanthomonas retroflexus, termed as SPMX) on Arabidopsis roots to study the impact of multispecies biofilm on plant growth and its spatial organization on the roots. SPMX co-culture notably promoted root development and plant biomass. Co-cultured SPMX increased root colonization and formed multispecies biofilms, structurally different from those formed by monocultures, revealing emergent properties of the SPMX consortium. Combining 16S rRNA amplicon sequencing and fluorescence in situ hybridization with confocal laser scanning microscopy (FISH-CLSM) analysis, we found that the composition and spatial organization of the four-species biofilm significantly changed over time. Interestingly, P. amylolyticus as monoculture failed to form biofilm on plant roots, but highly enhanced its population and enlarged aggregates when residing in the four-species biofilm. Co-localization analysis revealed a potential spatial pattern where the other three biofilm members were enriched around P. amylolyticus at microscales facilitating interspecific interactions. Our findings highlight that species individually showing low colonization and biofilm formation may benefit from interspecific interaction with other community members and hereby become important drivers of spatial organization and dynamics in complex communities on plant roots. Understanding the interplay between interspecific interactions and spatial organization in the rhizosphere will increase future chances to manage microbial communities for plant growth promotion and control of plant pathogens.

[P88] BACTERIAL LAG PHASE SHORTENING BY METHYLATED COMPOUNDS - A PRIMER FOR PHOTOTROPH-HETEROTROPH INTERACTIONS?

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Marine heterotrophic bacteria depend on organic carbon that is mainly produced by microalgae. However, microalgal carbon production relies on light and nutrients and is thus subjected to temporal dynamics. Consequently, heterotrophic bacteria must endure prolonged phases of starvation under conditions of low microalgal productivity but require to rapidly activate their metabolism with the onset of microalgal growth. A rapid metabolic activation is key for bacteria to outgrow co-occurring heterotrophs that compete for the same resources.

We found that minute amounts of methylated compounds, which are abundantly produced by microalgae, activate the metabolism of starving bacteria. We investigated the underlying mechanism in the bacterium Phaeobacter inhibens; a member of the Roseobacter group that is commonly associated with microalgae. Briefly, we conducted growth experiments using bacterial pure cultures that were grown with glucose and supplemented with algal metabolites. The growth experiments revealed that nano- to micromolar concentrations of S- and N-methylated compounds, such as dimethylsulfoniopropionate (DMSP) and glycine betaine, induce a marked shortening of the bacterial lag phase. We further applied RNA-sequencing of algal-bacterial co-cultures and of lag phase bacteria, as well as 13C-labeled metabolomics, gene knockouts, biochemical analyses and stoichiometric calculations. The experiments revealed that the bacterium assimilates methyl groups during the lag phase. Specifically, methyl groups are assimilated via the methionine cycle, which stimulates the downstream synthesis of spermidine; a metabolite known for its role in growth regulation. Of note, we also detected lag phase shortening by methylated compounds in other bacteria, such as Bacillus subtilis.

We conclude that methyl groups are a limiting building block during the lag phase of bacteria. To overcome this bottleneck, bacteria have evolved strategies to harvest methyl groups from donor molecules that are available in the environment. Interestingly, photosynthetic organisms produce methylated compounds in high abundances, and are therefore major sources for methyl groups. Thus, our findings reveal a novel function for methylated compounds in phototroph-heterotroph interactions.

[P89] WATER PH DISTINCTLY ALTERS SOIL AND ROOT-ASSOCIATED BACTERIAL COMMUNITIES AND STOCHASTIC PROCESSES DOMINATE THEIR ASSEMBLY IN DATE PALM (PHOENIX DACTYLIFERA)

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Aim: Date palms are extensively cultivated in arid agroecosystems, wherein the knowledge of the influence of irrigation water on the diversity, community structure, assembly process, and interactions of soil and root-associated bacteria (RAB) is scant.
Methods: Soil and root samples were collected from date palm farms in the United Arab Emirates. To investigate the bacterial diversity and communities, 16S rDNA (V3-V4) metabarcoding was employed, followed by assembly process analysis and co-occurrence analysis.
Results: We found lower bacterial diversity in root and distinct community patterns between compartments, wherein irrigation water pH (soil and root) and soil electrical conductivity (EC) (soil) were significant factors. Bacterial communities were found to assemble mainly through stochastic processes (dispersal limitation: 39.39 and 20.47 %; drift: 38.17 and 47.37 %, in soil and root respectively). The co-occurrence patterns of RAB communities were less complex and highly connective compared to soil and the abundance of OTUs, in specific modules of root (Bacillus, Streptomyces and Gaiella) and soil (Pedomicrobium) correlated positively with water pH.
Discussion: Roots exhibited lower bacterial diversity likely due to the “root effect”. Water pH amplifies host selection pressure and transiently selects (Xiong et al. 2021) distinct bacterial communities depending on pH variations, which assemble mainly through dispersal limitation (Chen et al. 2020) and drift (random birth and death events) (Ramoneda et al. 2020), while EC structure bacterial communities through salinity filtering (O’Brien et al., 2019) in soil. Higher resource availability in roots alters co-occurrence patterns from a highly complex to a less complex form; while niche partitioning determines the occurrence of highly connective co-occurrence patterns in the roots (Qian et al. 2019). The higher abundance and positive correlation of taxa/modules to water pH which are putatively involved in nitrogen cycling (aerobic denitrification and assimilatory nitrate reduction) point to their importance in agroecosystems.
Conclusions: Our results imply that bacterial communities in soil and root compartments are distinctly affected by the irrigation water pH rather than soil pH. Perturbations in water pH allows growth of specific bacteria, which leads to a reduction in the complexity of communities in the soil-root continuum that assemble mainly through stochastic processes. Our findings improved understanding of belowground microbiomes and has significant implications for nutrient cycling, specifically the nitrogen cycle, in arid agroecosystems.

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[P90] COMPOSITIONAL CHANGES OF A SPECIES-RICH NATURAL COMMUNITY WITH CONTRASTING NUTRIENT AVAILABILITY UPON PSEUDOMONAS PROTEGENS INVASION IN THE WHEAT RHIZOSPHERE

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Beneficial bacterial rhizosphere inoculants are of great interest in agriculture for their potential to promote plant growth and health. Nonetheless, the invasion of the rhizosphere microbiome often results in a suboptimal or transient colonization governed by a variety of factors that influence the fate of the inoculant. Due to the high complexity of rhizosphere microbiomes, studies involving species-rich communities rather than individual isolates or synthetic communities are needed. The aim of this work was to analyse early compositional changes in a soil species-rich natural bacterial community, both in exponential nutrient-rich or stationary nutrient-limited growth conditions (i.e., growing and stable communities, respectively), upon invasion of the plant-beneficial inoculant Pseudomonas protegens in bulk soil and in a wheat rhizosphere environment. We used 16S rRNA amplicon sequencing to analyse the microbiome composition, structure and diversity. Succession trajectories were followed for nine days, comparing the effect of nutrient availability, environment and inoculation pattern based on Bray-Curtis dissimilarities and non-metric multidimensional scaling analyses. Microbiome assembly processes were assessed based on the β-nearest taxon index of whole community null models. Ecological association inference networks of taxa were determined using sparse inverse covariance estimation. Finally, competition analyses between relevant Pseudomonas isolates were performed. P. protegens successfully established in all conditions tested, being more abundant in the rhizosphere of the stable community. Nutrient availability was a major factor driving microbiome composition and structure as well as the underlying assembly processes. While access to nutrients resulted in communities being mainly assembled by homogeneous selection, stochastic processes dominated in the nutrient-deprived conditions. An increased rhizosphere selection effect was observed in nutrient-limited conditions, resulting in higher numbers of enriched ASVs. The inoculation with P. protegens produced discrete changes, some of which involved other Pseudomonas. Direct competition between Pseudomonas strains only partially succeeded to replicate differences observed in the microbiome.
The results obtained in this study show that nutrient availability is a major driving force of microbiome composition, structure, and diversity both in bulk soil and the wheat rhizosphere and determines the assembly processes governing early microbiome development. The successful invasion of the inoculant was facilitated by the wheat rhizosphere and produced discrete changes among other members of the microbiome. Direct competition between Pseudomonas strains only partially explained microbiome changes and revealed that indirect interactions or spatial distribution in the rhizosphere or soil interface could be crucial for the survival of certain bacteria.

[P91] THE EFFECT OF MICROBIOME IN NON-SYMBIOTIC CORAL ON THE LARVAE SETTLEMENT

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Corals play a crucial role for sustainable marine ecosystems and global carbon cycle. Thus coral restoration is important in ocean engineering. The function of coral microbiome is more critical in non-symbiotic, azooxanthellate corals and the complex of community leads to healthy coral life and also the pattern of abundance and diversity shapes coral resilience and adaptation to environmental change. The high latitude corals are facing a decline or a distribution change by increased seawater temperature like as coral reefs around tropical area encountering a tremendous decline and bleaching event. In this study, we investigated the microbiome composition in azooxanthellate coral and compared it between corals under reproduction period and non-reproduction period and also habitat seawater and non-habitat seawater with seasonal variation. In addition, we examined the effect of microbiome on the coral larvae settlement. The majority of OTUs significantly shifted in corals under reproduction period and in coral habitat seawater indicated distinction in the relative abundance of bacteria compartment/ site-wise. Richness and diversity were higher, and more taxa were enriched in the corals under reproduction period and coral habitat seawater in summer. Flavobacteria and alphaproteobacteria dominated corals under reproduction period and coral habitat seawater in summer. Flavobacteriaceae and Oceanospirillaceae showed the most dramatic difference between corals in reproduction period and non-reproduction period. Flavobacteriaceae and rhodobacteriaceae showed the biggest composition difference between coral habitat seawater and non-habitat seawater. In the larvae settlement experiment, coral larvae settled on the microbiome coated surface 70% higher than non-coated surface and the normal polyp development were enhanced in group on the microbiome coated surface. We suggest that coral restoration through their microbiome could be a self-sustaining tool in worldwide coral decline. This work was supported by Marine Biotics project (20210469) funded by Ministry of Ocean and Fisheries, Korea.

[P92] VISCOSIN FROM PSEUDOMONAS FLUORESCENS SBW25 IS REGULATED BY ORPHAN LUXR REGULATORS THAT BIND TO PLANT PHENOLICS AND QUORUM-SENSING MOLECULES

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The plant-beneficial bacterium Pseudomonas fluorescens SBW25 produces viscosin, a cyclic lipopeptide (CLP) important for root colonization. While several studies have shown the plant beneficial effects of viscosin, knowledge of the regulation of this CLP in plant-microbe interactions is limited. In the plant-pathogenic Pseudomonas fluorescence 5064, the production of CLPs is regulated through quorum sensing (QS), a mechanism where a LuxR transcriptional regulator binds to an acyl homoserine lactone (AHL) signaling molecule, forming a complex that induces the transcription of the target genes. Recently, it has been demonstrated that the LuxR regulators LasR and RhlR from Pseudomonas aeruginosa, can bind to plant-derived phenolics and flavonoids. Inquisitively, in the plant pathogenic bacterium Pseudomonas syringae, the production of the CLP syringomycin is enhanced in the presence of the plant phenolics arbutin, salicin, and phenyl. However, it was not tested interaction through LuxR regulators. In the non-AHL producer strain, P. fluorescens SBW25, the LuxR-type transcriptional regulators ViscAR and ViscBCR are involved in viscosin regulation. Still, their mechanisms are unknown, as they lack the conserved amino acids that bind to AHLs. We hypothesize that plant phenolics and/or AHLs in complex with ViscAR/BCR regulators or other uncovered LuxR regulators may trigger the up regulation of viscosin biosynthetic genes. To investigate this, we tested a range of plant phenolics and quorum-sensing molecules as potential triggers for viscosin biosynthesis using LuxR bioreporter assays in combination with molecular docking computations.
We found that ViscAR up-regulates the expression of viscA in the presence of salicin (P=0.0076) and regulates its own expression in the presence of 3OC6 (P=0.0076). Additionally, ViscBCR also up-regulates the expression of viscA in the presence of 3OC6 (P=0.0131) and helicin (P=0.0243). Moreover, we discovered an orphan LasR homolog that showed a hierarchical role by inducing the expression of viscAR (P=0.0444) in the presence of 3OC12. Furthermore, LasR can directly regulate viscA in the presence of 2-benzoxazolinone (BOA) (P=0.0164) and viscB in the presence of salicin (P=0.0017). Structural models of ViscAR and ViscBCR showed that these regulators harbor non-archetypical ligand-binding domains able to bind to AHLs and plant phenolics.
The findings suggest that ViscAR, ViscBCR, and the LasR homolog can recognize both bacterial and plant signals and induce the expression of viscosin biosynthetic genes. These results demonstrate that bacteria can sense their environment through LuxR solos, which are not limited to binding to quorum-sensing molecules.

[P93] INSIGHTS INTO METABOLIC SPECIALIZATION, NICHE PARTITIONING AND CHITIN TURNOVER IN THE OCTOCORAL MICROBIOME

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Octocorals (Octocorallia, Cnidaria) are an integral part of benthic marine ecosystems. They increase habitat complexity and biodiversity and play key roles in coastal food chains, helping to regulate primary and secondary production. They are found in association with various microorganisms. Our previous work showed that the octocoral microbiome is distinct from the environmental surroundings, host genus-specific, and undergoes complex structural changes in the transition to the dysbiotic state [1]. However, the role of bacterial symbionts that populate octocorals is still poorly understood. To shed light on their metabolic capacities, we examined 66 high-quality metagenome-assembled genomes (MAGs) spanning 30 prokaryotic species, retrieved from microbial metagenomes of three octocoral species and seawater [2].

Symbionts of healthy octocorals were affiliated with the taxa Endozoicomonadaceae, Candidatus Thioglobaceae, Metamycoplasmataceae, unclassified Pseudomonadales, Rhodobacteraceae, unclassified Alphaproteobacteria and Candidatus Rhabdochlamydiaceae. Phylogenomics inference revealed that the Endozoicomonadaceae symbionts uncovered here represent two species of a novel genus unique to temperate octocorals, here denoted Candidatus Gorgonimonas eunicellae and Candidatus Gorgonimonas leptogorgiae. Their genomes revealed metabolic capacities to thrive under suboxic conditions and high gene copy numbers of serine-threonine protein kinases, type III-secretion system, type IV-pili, and ankyrin-repeat proteins, suggesting excellent capabilities to colonize, aggregate, and persist inside their host. Contrarily, MAGs obtained from seawater frequently lacked symbiosis-related genes.

All Endozoicomonadaceae symbionts harbored endo-chitinase and chitin-binging protein-encoding genes, indicating that they can hydrolyze the most abundant polysaccharide in the oceans. Other symbionts, including Metamycoplasmataceae and Candidatus Thioglobaceae, may assimilate the smaller chitin-oligosaccharides resulting from chitin breakdown and engage in chitin deacetylation, respectively, suggesting possibilities for substrate cross-feeding and a role for the coral microbiome in overall chitin turnover. We also observed sharp differences in secondary metabolite production potential between symbiotic lineages. Specific Proteobacteria taxa may specialize in chemical defense and guard other symbionts, including Endozoicomonadaceae, which lack such capacity.

We identify a thus-far unanticipated, global role for Endozoicomonadaceae symbionts of corals in the processing of chitin, a major component of the natural zoo- and phytoplankton feed of octocorals. We conclude that niche partitioning, metabolic

specialization, and adaptation to low oxygen conditions among prokaryotic symbionts likely contribute to the plasticity and adaptability of the octocoral holobiont in changing marine environments. These findings bear implications for our understanding of symbiotic relationships in marine environments and benthic ecosystem functioning.

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[P94] EXPLORING THE CORRELATION BETWEEN POST-LARVAL PHENOTYPIC TRAITS OF TURBOT (SCOPHTHALMUS MAXIMUS) AND THEIR BACTERIAL COMMUNITIES.

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Current research supports the notion that the composition and functional ecology of the gut microbiome plays a crucial role in shaping the development, functioning, and resilience of host organisms. Some studies have shown that changes to the associated bacterial community are correlated with certain phenotypic host traits (i.e., size). However, in the aquaculture sector, it is unclear whether the overall composition of the host-associated bacterial community can affect host phenotype (e.g., size, skeletal deformities, and pigmentation) as nutrition does. In intensive larviculture systems for turbot (Scophthalmus maximus), a significant proportion of fish can develop undesirable features over time, such as poor growth, deformities, and reduced pigmentation. Advances in larval nutrition have decreased the amount of sub-optimal fish reared, but no studies have yet examined whether the composition of the bacterial community is linked to the turbot's phenotype. This study aimed to investigate, for the first time, the potential correlation between post-larval phenotypic traits of turbot (Scophthalmus maximus) and bacterial community composition. To achieve this goal, post-larvae fish (30 days old) were collected from twenty larviculture production tanks (PT) in an industrial larviculture facility. Fish were categorized into groups based on three phenotypic traits: size, pigmentation, and deformity. Altogether, five groups were established: "large", "small", "deformed", "unpigmented" and "normal". A total of twenty fish were sampled for each category, one representative from each group in each PT. High-throughput sequencing of the 16S rRNA gene was performed on DNA extracted from the whole fish. Our results showed that there was no significant association between host phenotypic traits, host bacterial composition (PERMANOVA: $P>0.05$), or diversity (Kruskal-Wallis: $P>0.05$). Nevertheless, the abundance of certain taxa was found to be significantly associated with some groups (GLM-ANOVA: $P<0.05$). Pairwise comparisons between groups (EMMEANS: $P<0.05$) showed that bacteria assigned to the genus Pantoea (family Erwiniaceae), a probiotic candidate for marine aquaculture, had a significantly higher abundance in "normal" fish than in all other categories. In addition to this, bacterial populations related to Sulfitobacter and potential fish pathogens related to the genus Aliivibrio were more abundant in "small" fishes compared to "large", "normal" and "unpigmented" fishes. In general, our results indicate, neither deformity nor pigmentation traits were related to bacterial composition, indicating that other factors (e.g., host's genetics, or nutrition among other things) may be more important for the development of these traits. Yet, members of the Sulfitobacter and Aliivibrio genera may have adverse impacts on fish growth.

[P95] ANTIBIOTIC RESISTANCE IN MARINE BACTERIAL COMMUNITIES FROM SALMON FARMING AND PRISTINE MARINE SITES

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Global aquaculture production has experienced continued growth. In Chile, the world's second largest producer of salmon, aquaculture has undergone a rapid development by taking advantage of Chilean geography with large areas of fjords protected from open sea. Intensive aquaculture increases the predisposition to bacterial infections and the excessive use of antibiotics. Natural environments such as the ocean are reservoirs of antibiotic resistance genes (ARG), and the extensive use of antimicrobial compounds selects resistant bacteria. In the present work, the relative abundance of taxa, ARG composition and the antibiotic resistance phenotypic profiles of the bacterial communities was determined in an intensive aquaculture zone (AFS, aquaculture farm site) and a pristine zone (PSh, Pacific shore) far from aquaculture centers, in the Chiloé island, X region, Chile. In each zone, the total DNA of the surface bacterial fraction was purified and sequenced and cultured in a battery of media with different compositions. Through a metagenomics analysis of the communities, GRA prediction was performed using CARD. A total of 750 and 574 ARGs were identified in PSh and AFS metagenomes, respectively, and only 398 genes were shared between the sites, and unexpectedly, the total abundance of ARGs was higher in PSh. In both sites, the most abundant antibiotic families were beta-lactamics, aminoglycosides, multidrug, tetracyclines, vancomycin, and peptides. We inspected the ARGs related to antibiotics used in the salmon industry (florfenicol FFC, flumequine FLQ and oxitetracylcine OTC), and found that genes conferring resistance to flumequines were more abundant in AFS, but those associated with resistance to phenicols and tetracyclines were more abundant in PSh. In addition, the culturable fraction of the bacterial communities was subjected to susceptibility tests to antibiotics used by the salmon industry, and we observed a higher frequency of antibiotic resistant colonies for FFC, FLQ and OTC in the AFS site, ranging from 102 to 106 increase depending on the culture media used. Taxonomic analysis revealed an increase in bacterial richness in the antibiotic resistant culturable community from the AFS, and an overall enrichment in Gama-proteobacteria order Rhodobacterales and Cyanobacteria orden Synechococcales in AFS compared to PSh. This indicates that different bacterial taxons are carrying ARGs in the AFS and the PSh sites, and despite the higher abundance of ARGs in PSh metagenome, the higher frequency of resistant colonies in AFS shows that the presence of ARGs is not the only factor to evaluate antimicrobial resistant potential in a bacterial community.

[P96] CATCH ME IF YOU CONJUGATE! PLASMID RECOVERY FROM COMPLEX ECOSYSTEMS USING SECRETED PILUS MACHINERY AS A HOOK

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Aim
Plasmid conjugation plays an essential role in facilitating bacterial genomic plasticity and adaptation, including the acquisition and transfer of antimicrobial resistance (AMR) determinants amongst clinical pathogens from pre-existent environmental reservoirs. Hence, knowledge on the diversity and dynamics of conjugative plasmids in complex ecosystems is instrumental to develop new strategies to tackle the global AMR crisis. The current study aims to develop a workflow to enrich and capture bacterial cells containing a conjugative plasmid from environmental samples, followed by genomic sequencing and bioinformatic analyses to investigate the diversity of conjugative plasmids and identify the native hosts of these recovered plasmids.
Methods
Green Fluorescent Protein (GFP)-displaying MS2 bacteriophage and SybrGold-stained Pf3 bacteriophage were employed to fluorescently tag the bacterial cells expressing conjugative pili and sort the tagged cells from a complex microbial mixture using flow cytometry. The genomic DNAs of the sorted cells would be processed with genomic sequencing of single cells, PacBio long-read sequencing of pooled samples and DNA-methylation-profile-based binning.
Results
The GFP-displaying MS2 phage particles tagged a significantly higher proportion of the F'-plasmid-containing Escherichia coli population than the plasmid-free E. coli population and was able to differentiate the former from the latter in an artificial mixed population containing the two strains in flow cytometry assays. Similar results were achieved with the SybrGold-stained Pf3 phage particles in distinguishing between Pseudomonas aeruginosa PAO1/RP1 and the plasmid-free counterpart in a mixed culture.
Conclusions
It was feasible to use the plasmid-specific bacteriophage (MS2, Pf3), either genetically displaying GFP or being stained with the fluorescent dye SybrGold to enrich the cells containing a conjugative plasmid from a simple bacterial mixture. Proof-of-concept experiments using these two phages are underway to isolate and sequence plasmid-containing bacterial cells from more complex natural environments.

[P97] PLASMID-SPECIFIC PHAGES TO REDUCE THE SPREAD OF CONJUGATIVE PLASMIDS CARRYING ANTIMICROBIAL RESISTANCE GENES IN THE ENVIRONMENT

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Bacteriophages are the most abundant biological entities on our planet and play an important role in the environment by infecting and killing bacteria. Plasmid-specific bacteriophages (PSP) infect bacteria carrying self-transmissible plasmids by recognizing the plasmid-encoded conjugal pilus. Conjugative plasmids often carry antimicrobial resistance genes (ARG); therefore, PSP may offer opportunities to reduce plasmid carriage, dissemination of ARG, and the concomitant appearance of multi-resistant superbugs. Here we aimed to isolate, characterize, and evaluate novel PSP with the potential to be used to reduce the load of conjugative plasmids in environmental communities. We used an avirulent strain of Salmonella enterica carrying IncP, IncH or IncN plasmids to enumerate and isolate PSPs from wastewater, verifying that these isolates were non-somatic (i.e., failed to infect the same strain without plasmids) and then checking their host range against other bacteria/plasmid combinations. In addition, PSPs were inoculated into serial cultures of wastewater bacterial communities to assess their dynamics and their impact on the communities. Our results demonstrated that PSP are abundant in hospital and domestic wastewater and are significantly more abundant than somatic phages against S. enterica. The most abundant PSPs are IncH and IncN-specific. Sequence analysis revealed that depending on the plasmid used for isolation, the PSP isolates were either DNA phages from Kalamavirales or Caudovirales orders (IncP or IncN-specific) or RNA phages (IncH-specific). The phages isolated on Salmonella carrying an IncN plasmid belong to a novel genus and infect diverse bacterial strains carrying conjugative plasmids from several incompatibility groups including IncH, IncI, IncN, IncP, IncW and IncX, a plasmid host range larger than described for all other PSPs. Lab inoculation of PSP to wastewater communities indicate that they can multiply on wastewater bacteria with an impact on microbial composition. Therefore, we propose that our PSP may control the population of conjugative plasmid in environmental communities, opening opportunities to reduce the spread of ARG from conjugative plasmids in such environments rich in bacteria.

[P98] ANTIBIOTIC RESISTANCE PATTERNS AND PERMISSIVENESS TOWARDS RESISTANCE PLASMIDS OF AEROMONAS IN RESIDUAL WATERS

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Aeromonas is a genus of Enterobacteriaceae, prevalent in wastewater and often detected in aquatic environments. Some are opportunistic pathogens of humans and animals. Mobile antimicrobial resistance (AMR) determinants, such as plasmids, are also frequently detected in Aeromonas. These properties allow Aeromonads to be considered as an indicator of the dissemination of antimicrobial resistance between humans and the environment. Therefore, the aim of this study was to investigate the diversity and AMR profile of Aeromonas in Denmark, as well as their ability for uptake of resistance plasmids. Based on this information, we aimed to assess the contribution of wastewater treatment plant (WWTP) discharges to the spread of antibiotic resistance in the environment.

Aeromonas spp. were enumerated from both raw sewage and effluents of several WWTPs in Denmark, as well as from upstream and downstream locations of the receiving water bodies. The taxonomic diversity of the retrieved Aeromonas covered 13 species with A. media predominant in all samples. A. media, A. veronii and A. caviae were more abundant in wastewaters and river downstream the WWTP than upstream, while A. veronii were only detected in the effluent and downstream. Through susceptibility testing, we showed that resistance to piperacilin-tazobactam, cefepime, and tetracycline was detected downstream of WTTP, but not upstream. In addition, solid mating were performed between diverse Aeromonas strains and three model donors carrying the broad host range plasmid pKJK5. A. media, A. veronii, A. salmonicida, A. caviae and A. allosaccharophila can obtain plasmids from several donors with relatively high transfer rates. The fact that A. media, the species most detected in our sampling, is highly permissive to conjugal plasmids suggests that they could contribute significantly to environmental dissemination of mobile resistance genes.

Overall, we conclude that WWTP discharges may enhance the dissemination of antibiotic-resistant Aeromonads into surface water, thereby increasing the risk of spreading antibiotic resistance in the environment.

[P99] BIRMINGHAM INCP-1A PLASMIDS REVISITED: RP4, RP1 AND RK2 ARE IDENTICAL AT THE NUCLEOTIDE RESOLUTION

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Aim
The plasmids RP1 and RK2 were originally identified in burn wound isolates of Pseudomonas aeruginosa and Klebsiella aerogenes, respectively, that were isolated in 1969 at the Birmingham Accident Hospital (UK). The RP4 plasmid, that was described in Escherichia coli J53 and distributed from Datta et al., 1971[1], was considered as a mislabeling of RP1 at some stage. Two early studies found that RP4, RP1 and RK2 were undistinguishable using DNA duplex electron microscopy and restriction enzyme pattern analysis. Nonetheless, it is uncertain whether these plasmids, also called 'Birmingham IncP-1α plasmids', are identical at the nucleotide level. Given that they are widely used as a model to study host-plasmid interaction and plasmid conjugation, it is important to sequence the RP4, RP1 and RK2 plasmids to provide a high-quality reference sequence for future studies.
Methods
RP1 and RP4 plasmids were extracted from P. aeruginosa PAO1/RP1 and E. coli J53/RP4, respectively, and sequenced using Illumina paired-end short-read sequencing. Furthermore, the sequencing reads for one RK2 and two RP4 plasmids were retrieved from the public database from three previous host-plasmid evolution studies. The plasmid sequences were assembled using UniCycler and compared to each other or to the current RK2 reference sequence (BN000925.1) using progressiveMauve. The newly assembled RP4/RP1/RK2 sequence was annotated using Prokka and manually examined.
Results
Assembling the sequencing reads showed that RP1, RP4 and RK2, each had a 60095-bp circular contig and were found to be identical at the nucleotide resolution. In one exception, the RP4 plasmid from one previous study had an additional 10-kb insertion of Tn6048 between parC and parD. The newly assembled RP1/RP4/RK2 sequence had 32 mismatches when comparing with the current reference sequence, some of which might lead to misinterpretation in previous studies, such as 9 mismatches in the kanamycin resistance-encoding gene aphA, a frameshift mutation in korF and the in-frame fusion of the two genes, pecM and orf2, caused by a homologous recombination event.
Conclusions
RP4, RP1 and RK2 are identical, providing confidence to unite the knowledge obtained from studies of each plasmid over decades. Strikingly, the plasmid sequence is highly conserved despite being distributed to different labs over 50 years and propagated in different bacterial hosts, supporting the notion that the bacterial host tends to adapt to the RP4/RP1/RK2 plasmid rather than the reverse. Lastly, an updated high-quality annotated plasmid sequence was provided for the scientific community to use as a reference sequence.
[1]PMID: 4945193

[P100] FUNCTIONAL INVESTIGATION OF THE PLASMID-ENCODED PUTATIVE ANTIMICROBIAL RESISTANCE GENE LSA(B) FROM PRIESTIA MEGATERIUM

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Background: When commercializing bacterial strains like Bacillus sp. for animal feed applications or plant health applications, it is required that the strains are free of acquired antimicrobial resistance genes that could potentially spread to pathogenic bacteria and thereby add to the pool of resistance genes that may cause treatment failures in humans or animals. On the other hand, if antimicrobial resistance is intrinsic in a bacterial species, the risk of spread horizontally to other bacteria is considered very low (1). An in-silico assessment of a Priestia (formerly Bacillus) megaterium strain revealed a putative lsa(B) lincosamide resistance gene (with 81% identity) on a 179 kb plasmid, but since the species P. megaterium is intrinsically resistant towards lincosamides, the functionality of the gene needed to be evaluated in another genetic background.

Aim: Assess the ability of the putative lsa(B) to confer resistance against lincosamide antibiotics. For this purpose, the putative lsa(B) was cloned into an expression vector for B. subtilis 168 and further characterized.

Methods: The putative lsa(B) was further assessed for functionality and potential to transfer horizontally by use of genome assessment and NCBI searches. Furthermore, the putative lsa(B) was amplified by PCR and cloned into a high copy Bacillus expression vector under the control of an IPTG-inducible promoter. Minimal inhibitory concentrations (MIC) were determined for the B. subtilis 168 containing the empty vector, the strain containing the lsa(B) expression plasmid and for the wildtype P. megaterium strain as previously described (2).

Results: Searches against NCBI database found genes annotated as lsa(B) in both P. megaterium and P. aryabhattai (>99% identity and 100% coverage) and in 13% (5/38) of P. megaterium strains. The putative lsa(B) had the known recognition sites for the lsa gene class (5) and susceptibility testing with the lsa(B) expression strain showed a slightly increased susceptibility against clindamycin (4-fold increase), suggesting that the encoded Lsa(B) protein confers reduced susceptibility against lincosamide antibiotic.

Conclusion: The putative lsa(B) gene present on a plasmid in P. megaterium is most likely functional, but due to conferring low level of reduced susceptibility towards lincosamides and only found in a few species, it seems of less clinical relevance.

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[P101] SYSTEMATIC ANALYSIS OF BACTERIAL DEFENSE SYSTEMS

ACROSS PLASMIDS

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Many plasmids can transfer horizontally among cells, thus providing bacteria with access to a vast environmental gene pool. While much attention has been given to the role of plasmids in disseminating antibiotic resistance genes, less is known about their involvement in the transfer of other critical phenotypic traits. A growing body of work shows that a considerable fraction of the accessory genes in bacteria is dedicated to defense functions against their genetic parasites, e.g., bacteriophages. The variability of the immune system arsenal among related taxa suggests that defense systems are prone to horizontal exchange, yet little is known about their mobility. Here, we carried out a census of all known bacterial defense system components across publicly available plasmid sequences, focusing on characterizing their prevalence, diversity, and distribution. Overall, we find that many plasmids carry defense system components, highlighting the evolutionary and ecological importance of plasmids in disseminating defense systems across bacterial communities.

[P102] ENGINEERED GFP-BASED REPORTER STRAINS FOR SELECTION INDEPENDENT EXOGENOUS ISOLATION AND TRANSFER STUDIES OF CONJUGATIVE PLASMIDS FROM ENVIRONMENTAL COMMUNITIES

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Conjugation of broad host range plasmids, such as those belonging to incompatibility group P-1 (IncP-1), play a critical role in the horizontal transfer of genes in bacterial communities. Currently, however, exogenous isolation of wildtype plasmids is selection dependent. This means that wildtype plasmid that do not carry antibiotic resistance genes are not well characterized despite representing the vast majority. Also, while environmental communities have been shown to be highly permissive towards introduced IncP-1 plasmids, it remains unknown how efficient environmental communities can transfer their wildtype plasmids. In this study, we developed fluorescence-based reporter strains to isolate environmental IncP-1 plasmids in a selection independent manner. The reporter strains were tested against conjugative plasmids from a range of different incompatibility groups. We found that our reporter system is specific to all tested IncP-1 subtypes. The reporter was validated by detecting and capturing plasmids from samples taken from influent wastewater. When the reporter strains acquired IncP-1 plasmids, GFP expression was induced and the transconjugants could be isolated by fluorescent activated cell sorting. In addition, this enabled us to quantify how efficiently environmental communities could transfer IncP-1 plasmids. Our study provides a novel and convenient method to capture and study conjugative IncP-1 plasmids from environmental communities- an approach that likely can be applied to any type of plasmid.

[P104] SUB-INHIBITORY GENTAMICIN POLLUTION INDUCES GENTAMICIN RESISTANCE GENE INTEGRATION IN CLASS 1 INTEGRONS IN THE ENVIRONMENT

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Antibiotics at sub-inhibitory concentrations are often found in the environment. Here they could impose selective pressure on bacteria, leading to the selection and dissemination of antibiotic resistance, despite being under the inhibitory threshold. The goal of this study was to evaluate the effects of sub-inhibitory concentrations of gentamicin on environmental class 1 integron cassettes in natural river microbial communities. Gentamicin at sub-inhibitory concentrations promoted the integration and selection of gentamicin resistance genes (GmRG) in class 1 integrons after only a one-day exposure. Therefore, sub-inhibitory concentrations of gentamicin induced integron rearrangements, increasing the mobilization potential of gentamicin resistance genes and potentially increasing their dissemination in the environment. This study demonstrates the effects of antibiotics at sub-inhibitory concentrations in the environment and supports concerns about antibiotics as emerging pollutants.

[P105] CORRELATION BETWEEN ANTIBIOTIC RESISTANCE GENES PREVALENCE IN HOSPITAL WASTEWATER AND THE QUANTITY OF ANTIBIOTICS USED IN THE CLINICAL SETTINGS

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Background: Antibiotic resistance is one of the biggest threats to global health nowadays. The development of bacterial resistance to antibiotics is closely related to the success rate of infectious disease therapy. It has significantly increased morbidity and mortality rates [1]. One of the most common places for the development and spread of antibiotic resistant bacteria and antibiotic resistance genes due to the high use of antibiotics is the hospital. Hospital wastewater is an important reservoir of antibiotic resistance that has the potential to accumulate resistance genes [2,3].

Aim: This study aimed to measure the copy number of antibiotic resistance genes in hospital wastewater and correlate it to the antibiotics used in the clinical settings.

Methods: This study was carried out at a referral hospital in Indonesia. Inlet hospital wastewater samples were taken randomly twice each week for 6 weeks of monitoring. The wastewater samples were filtered, followed by DNA extraction. The copy number of 36 antibiotic resistance genes (8 ESBL resistance genes, 7 carbapenem resistance genes, 14 aminoglycoside resistance genes, and 7 quinolone resistance genes) was quantified using a high-throughput real-time polymerization chain reaction (HT-qPCR) system. The HT-qPCR quantification results were analyzed and visualized using the ResistApp platform. Data on antibiotic use in the clinical setting was obtained from the Department of Pharmacy during the inlet wastewater sampling period.

Results: Antibiotic resistance genes were detected over time in inlet hospital wastewater samples. Aminoglycoside resistance genes were the most abundant genes detected in hospital wastewater ($p< 0.001$). Beta-lactam antibiotics, including cephalosporins and carbapenem, were the most prescribed antibiotics in the clinical setting, followed by quinolones and aminoglycosides ($p=0.024$). We found a significant correlation between the prevalence of antibiotic resistance genes in hospital wastewater and the quantity of antibiotics used in hospital ($p=0.004$). However, the correlation between each group of antibiotics was not statistically significant (cephalosporins, $p=0.326$; carbapenem, $p=0.102$; aminoglycosides, $p=0.145$; quinolones, $p=0.275$).

Conclusions: The prevalence of antibiotic resistance genes in hospital wastewater was correlated with the quantity of antibiotics used in clinical settings. Monitoring of the quantity of antibiotics use in the clinical setting might be performed through hospital wastewater samples, however further investigation with more samples is required to evaluate the correlation of each antibiotic group.

Keywords: Antimicrobial resistance, Hospital wastewater, Indonesia

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[P106] HOW DO PLASMIDS OVERCOME THE DEFENSE SYSTEMS OF RECIPIENT BACTERIA?

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Bacteria have developed diverse defense systems to prevent the invasion of mobile genetic elements, including CRISPR-Cas and restriction-modification systems. However, horizontal gene transfer of mobile elements persists across species. Hence, it is not surprising that plasmids and other conjugative elements have developed various anti-defense genes that can counteract microbial immunity. We explored an extensive set of conjugative elements from diverse environments and discovered that anti-defense systems, encoding anti-CRISPR, anti-restriction, and anti-SOS, are highly overrepresented in the leading region of plasmids. This largely uncharacterized region is the first to enter recipient cells during conjugation. Further, we found that anti-defense systems in the leading region tend to cluster into “islands” that contain various combinations of anti-defense and anti-defense-related proteins. Early expression of the leading regions’ genes, even before the transfer is complete, is enabled by special single-stranded promoters, which are prevalent in the islands we identified. Our results suggest that anti-defense islands on conjugative elements are expressed upon entry, promoting rapid protection against host defense systems. Uncovering these islands and characterizing them may considerably improve our understanding of the repertoire of anti-defense genes, plasmid dissemination, and the intricate co-evolution of plasmids and their hosts.

[P107] INFLUENCE OF ZOOPLANKTON ON DNA DEGRADATION AND NATURAL TRANSFORMATION IN FRESHWATER MICROCOSMS

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The spread of antibiotic resistance genes (ARGs) by natural transformation has been recently pointed out to occur in aquatic environments, one of the largest ARGs reservoirs. Nevertheless, knowledge on this topic is still scarce, mainly because of the difficulty in tracking this phenomenon. Considering that in aquatic ecosystems the zooplankton plays a crucial role, and it closely interacts with bacteria, the aim of this study was to evaluate its influence on extracellular DNA (eDNA) fate and acquisition by bacteria through natural transformation. Experiments were carried out in microcosms of artificial lake water, using two model organisms, i.e., Daphnia obtusa as the zooplankton model and Acinetobacter baylyi BD413 as model natural competent bacterium. We observed that the presence of alive individuals of D. obtusa resulted in the degradation of plasmidic DNA, as supported by gel electrophoresis and quantitative PCR analysis. Moreover, we found that the addition of water in which Daphnia and its microbiome were allowed to release compounds among which proteins (i.e., secretome) led to a gradual modification of plasmidic conformation following an animal concentration-dependent trend. Through a LC-MS/MS based proteomics approach, we identified proteins released by Daphnia and its microbiome. The presence of proteins with functions related to DNA binding and degradation suggested the involvement of Daphnia and its associated microorganisms on eDNA degradation. Then, we monitored the ability of the model bacterium A. baylyi BD413 to acquire a plasmidic DNA incubated in presence and absence of D. obtusa and in presence of molecules released by the zooplankton. While the presence of Daphnia individuals lowered the transformation frequency due to plasmid degradation, as observed before, in presence of Daphnia-released molecules an increase of transformation frequency was found, suggesting that a more accessible plasmid conformation might be available for the bacterial uptake. In conclusion, this work revealed that zooplankton and/or its microbiota, in a concentration-dependent way, degraded free DNA and changed DNA topology, thereby affecting its uptake by bacteria. This study suggested that the zooplankton could have an important role on the diffusion of antimicrobial resistance in freshwater.

[P108] SINGLE-CELL ANALYSIS REVEALED THAT PROMA PLASMIDS WERE ORIGINALLY POSSESSED BY DIVERSE BACTERIA IN NATURAL ENVIRONMENTS

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PromA is a group of plasmids proposed in 2009[1]. It includes conjugative and broad-host-range plasmids that are widely distributed in nature[2]. PromA plasmids usually lack previously known accessory genes (e.g., antimicrobial resistance genes, pathogenic genes, and/or metabolic genes), therefore, they have been rarely found with their host phenotype(s). The objective of this study is to identify the original hosts of the PromA plasmids to understand how these plasmids behave in nature and what their importance is. Here, we developed a culture-independent, single-cell analysis method to identify the original hosts using water-in-oil (w/o) droplets, digital PCR, and targeted droplet sorting. Multiplex droplet digital PCR was performed in w/o droplets. Each droplet contained a single cell of environmental microorganism extracted from the lake sediments (Lake Sanaru, Shizuoka, Japan), the specific primers and fluorescent TaqMan probe to amplify and detect the replication initiation protein (repA) gene of the PromA plasmid, and the primers for amplifying a part of 16S rRNA (V3-V4) genes. When the repA was present and amplified in a droplet, green fluorescence of FAM could be detected. We analyzed a total of 1,076,983 droplets (323,094 droplets were calculated to contain environmental microbes). As a result, 413 droplets exhibited strong fluorescence indicating that they contained PCR products. These fluorescent droplets were dispensed one by one into each well of a 96-well plate using On-chip Droplet Selector® (On-chip Biotechnologies). Then, the nucleotide sequences of the 16S rRNA genes in the fluorescent droplets were determined. We analyzed 116 droplets, resulting in 31 strains belonging to at least 16 different genera as original host candidates of the PromA plasmids. This fact reaffirmed that the PromA plasmids were distributed in a broad range of bacteria in nature. In addition, six genera were repeatedly detected from separate droplets, suggesting the robustness of the method. Notably, Gram positive bacteria (11 genera), not previously identified as hosts of the PromA plasmids by cultivation-dependent methods, were obtained as candidates. Two candidates showed less than 90 % identity with the 16S rRNA (V3-V4) genes in the public database (NCBI and SILVA), suggesting that they might be unidentified bacteria that have not been isolated before. These results indicate that horizontal gene transfer via PromA plasmids might also occur among uncultured or nonculturable bacteria.

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[P109] BIOMARKERS FOR MONITORING ANTIBIOTIC RESISTANCE IN AQUATIC ENVIRONMENTS

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The occurrence of antimicrobial resistance raises concerns as a human health threat that can be propagated through the environment. Wastewater discharge into the environment is an important source for antibiotic resistant bacteria (ARB) and antibiotic resistance genes (ARGs). Sewage collection and urban wastewater treatment plants (UWTPs) are major barriers that reduce environmental contamination by sewage-derived pathogens and nutrients. However, the continuous discharge of ARB and ARGs through wastewater, including when well-functioning UWTPs are available, is unavoidable. Regular and integrated antibiotic resistance monitoring in both wastewater and receiving water bodies would contribute to improve control measures. However, monitoring processes are not harmonized being the choice of suitable biomarkers a first limitation.

In this study, we tested 10 selected potential antibiotic resistance biomarkers, which have been described has being associated to humans, and rare in clean environments- intI1, sul1, ermB, ermF, aph(3'')-Ib, qacEΔ1, uidA, mefC, tetX and crAssphage. The public database MGnify (https://www.ebi.ac.uk/metagenomics/; hosted by EMBL-EBI), was screened using the filters corresponding to origin- human gut, wastewater, sewage, and fresh water. These biomarkers and the 16S rRNA gene were monitored by quantitative PCR (qPCR) tested in raw wastewater, activated sludge, treated wastewater and surface water (upstream and downstream the UWTP) samples, collected from different countries (Portugal, Czech Republic, Denmark, The Netherlands, and Israel).
The abundance of the 10 potential biomarkers decreased on average by up to 2.5 log-units gene copies/mL of sample from raw wastewater to surface water, due to treatment and/or dilution in surface water. A clustering analysis of samples based on biomarkers abundance, grouped the samples according to the (waste)water type. This classification was confirmed when 12 anonymous (waste)water samples were analysed in a blind test.
The tested biomarkers were observed to differentiate different types of samples, permitting the assessment of wastewater treatment efficiency or of impacts of UWTPs discharge or others in aquatic environments. The selection of suitable biomarkers

that can typify different water sources and levels of ARG contamination, along with harmonized qPCR procedures, can facilitate regular and integrated legal requirements to antibiotic resistance monitoring in wastewater and related aquatic environments.

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[P110] COMPARATIVE ASSESSMENT OF POST-SECONDARY TREATMENT PROCESSES TO REDUCE THE BACTERIAL LOAD IN TREATED DOMESTIC EFFLUENTS

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Aim: This study aimed to compare distinct post-secondary treatment processes- sand filters with coagulant, UV, ultra-filtration, reverse osmosis, ozonation, and plasma technology- to reduce the bacterial load in treated effluents.
Methods: The secondary effluent of a full-scale urban wastewater treatment plant with an activated-sludge process was subjected to different treatment processes. An initial characterization included raw wastewater (RWW), secondary effluent (sTWW), secondary effluent after sand filter with addition of coagulant (ssTWW), after UV disinfection (tTWW), and the inlet and outlet of a final effluent reservoir (RES_in; RES_out). Later, secondary effluent samples were examined after distinct processes: ultra-filtration, reverse osmosis, ozonation, and plasma technology. Adequate wastewater volumes were filtered through polycarbonate membranes (0.22 µM, porosity) for total DNA extraction. Total coliforms, total bacteria and the intI1 gene (based on qPCR 16S rRNA gene), and standard wastewater quality parameters were examined. The bacterial community and the pattern of antibiotic resistance genes were analyzed for the main treatment stages, while specific antibiotic resistance biomarkers are being examined for other samples.
Results: Bacterial diversity or antibiotic resistance profiling clustered the samples in three groups: RWW, sTWW+ssTWW+tTWW, and RES_in+RES_out. The most abundant phyla (>8%) along the system were Pseudomonadota, Bacillota, Campylobacterota, Actinomycetota, and Bacteroidota. In general, antibiotic resistance and related genes significantly decreased ($p<0.01$) between RWW and TWW, and after sand filtration with coagulant. Log removal values ranged between 0.8 to 1.5, with the 16S rRNA genedecreasing from 7.7 log-units copies/mL inRWW to 6.8 in sTWW and 5.7 in ssTWW The intI1 gene presented quantities about 1 log-unit below in the different types of water. UV radiation or storage did not cause significant variations. Among the tested processes, ultra-filtration provided the highest E. coli log removal (4.8 log-units/volume), followed by ozonation (2.9 log-units/volume), and plasma technology (1.6 log-units/volume). The removal values were lower when estimated based on 16S rRNA or intI1 genes. Ultrafiltration (9 m3/h) presented higher log removal values than ozonation (28.8 g O3/m3) and plasma technology.
Conclusions: Available secondary post-treatment technologies can produce treated effluents with different microbial quality levels. Results show that apart from E. coli, other contaminants can be removed with different efficiencies, and processes such as ultrafiltration or reverse osmosis may be required to achieve trace levels of bacterial contaminants, in particular antibiotic resistance genes. The post-treatment selection should consider the final intended reclaimed water uses, and respective risk analysis.

[P111] PHD CANDIDATE

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While most studies in antibiotic resistance have been done with single or co-cultures, we know that bacteria live in multi-species communities where many interact with each other.
Interspecies relationships like horizontal gene transfer are key factors in the evolution and spread of antibiotic resistance genes.
In this study, we built a system of bioreactors simulating the human-gut environment. These bioreactors were inoculated with a poly-microbial community from a stool sample and cultured until reaching a steady state. Later on, a donor strain carrying a plasmid conferring resistance against trimethoprim (TMP) was added to the steady state cultures. During this part of the experiment, TMP was also pumped into the bioreactors with the liquid medium.
16S rRNA amplicon sequencing and long read metagenomics were used to monitor the whole community along the experiment.
HGT was detected through a dual fluorescence system within the plasmid and donor cells allowing us to identify both, donors and transconjugants. Transconjugants were then extracted from the whole population using fluorescence activated cell sorting (FACS) and identified via 16 rRNA gene sequencing.
Preliminary analyses highlight that the HGT is pervasive among members of this community with a significant proportion of taxa containing the plasmid by the end of the experiment. This single-cell resolution understanding of HGT will give us insight into the ecologial impact of antibiotic on the whole community

[P113] ECO-EVOLUTIONARY FEEDBACKS SHAPE THE EVOLUTION OF CONSTITUTIVE AND INDUCIBLE DEFENCES

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Organisms have evolved many different defense mechanisms that are either constitutive (always active) or inducible (elicited by parasites). Fitness trade-offs associated with these defenses tend to manifest accordingly (i.e., constitutive, or infection-induced), and consequently, organisms are predicted to invest more in constitutive defenses as the infection risk increases, and less in induced defenses. However, the short-term transient evolutionary dynamics of these different resistances has remained unstudied, and it is unclear if and how competition between these orthogonal defense strategies when resistance first emerges impacts their long-term evolution. Bacteria and their phages offer a tractable system to study this: bacteria can acquire constitutive resistance by mutation of the phage receptor (surface mutation, sm) or induced resistance through their CRISPR-Cas adaptive immune system. Using a combination of theory and experiments we demonstrate that the mechanism that establishes first has a strong advantage because it weakens selection for the alternative resistance mechanism. As a consequence, ecological factors that alter the relative frequencies at which the different resistances are acquired have a strong and lasting impact: high growth conditions promote the evolution of sm resistance by increasing the probability of rate of spread of the receptor mutation events during the early stages of the epidemic, whereas a high infection risk during this stage of the epidemic promotes the evolution of CRISPR immunity, since it fuels the (infection-dependent) acquisition of CRISPR immunity. This work highlights the strong and lasting impact of the transient evolutionary dynamics during the early stages of an epidemic on the long-term evolution of constitutive and induced defenses, which may be leveraged to manipulate phage resistance evolution in clinical and applied settings.

[P114] NANOPORE SEQUENCING AS A TOOL TO ANALYSE THE DIVERSITY OF BACTERIOME AND RESISTOME OF WASTEWATER AND SEWAGE SLUDGE

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Aim: The spread of antibiotic resistance is ranked by the World Health Organization among the world's major threats today. Wastewater treatment plants (WWTPs) were recognized as key sites for the spread of antibiotic resistance due to the presence of antibiotic resistance bacteria (ARBs) and antibiotic resistance genes (ARGs). Thus, knowledge of the diversity of ARG in various complex WWTP matrices and its correlation with the diversity of bacterial species is extremely important in defining the core ARB species most likely to contribute to the spread of ARG. Nanopore sequencing (NS) is a portable massive parallel sequencing device developed by Oxford Nanopore Technologies (ONT), which generates long sequencing reads. The aim of this study was to test the suitability of NS in determining the resistome diversity of various WWTP matrices and to correlate it with the present bacteriome.
Methods: We analyzed four different samples of three different matrices from WWTPs (activated sludge, anaerobic sludge, filtered treated wastewater). DNA was isolated by three different kits (DNeasy PowerSoil, QIAGEN; FastDNA™ Spin Kit, MP Biomedicals; DNeasy PowerWater, QIAGEN) and sequenced using the ligation sequencing kit with barcoding. Fastq sequences were analysed by ONT software tools such as MinKNOW and EPI2ME, by open-source software platforms such as Galaxy, ResFinder and different R packages.
Results: The most abundant bacterial genera in tested samples were Accumulibacter, Coprothermobacter, Defluviitoga, Dechloromonas, Escherichia, Luteimonas, Methanosarcina, Nostoc, Pelosinus, Pseudomonas and Thauera (in alphabetical order). The most frequently detected ARGs were mutations in genes gyrA, gyrB, parC, parE or rpoB; followed by genes encoding different efflux complexes (e.g., complexes Mex-Opr, SmeABC or CeoAB-OpcM), ribosomal tetracycline protection proteins (e.g. TetG, TetM, or Tet32), enzymes inactivating aminoglycosides or beta-lactamases of types as IMP, OXA or LCR-type or genes encoding sulfonamide resistant dihydropteroate synthase (sul1). We also compared the results obtained by different software tools and approaches including MAG assembly for the detection of the ARG's hosts.
Conclusions: This study proves that nanopore sequencing is a suitable tool for analyzing bacteriome and resistome diversity. This is a very versatile tool allowing us to adapt the depth and complexity of analyses to the user's expertise and designed purposes.
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[P115] IMPACT OF BIOFILM STRUCTURE AND PHENOTYPES ON PLASMID TRANSFER AND STABILITY

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Background: Bacteria can use horizontal gene transfer (HGT) to rapidly adapt to changes in their environment by directly transferring mobile elements like e.g., plasmids. This can ensure their survival even under otherwise detrimental conditions such as exposure to antimicrobials. It is also widely accepted that biofilms are the primary lifestyle of bacteria and they have been proposed to be a hotspot for HGT because of the high density of individuals and structural stability. Here we investigate the transfer of plasmids in biofilms to identify factors limiting or promoting conjugation as well as the ability of well-established biofilms to act as spatiotemporal reserves for plasmids.

Methods: Two different dual-labelled plasmid systems were used for detection of transfer or stability, respectively, enabling detection of plasmid transfer or plasmid loss by using flow cytometry and confocal laser scanning microscopy. Proteomics and knock-out mutants were used to confirm observations linked to increase in plasmid uptake as well as the use of individual-based modelling to eliminate possible mechanisms.

Results: The results showed how biofilms can act as spatiotemporal reserves for plasmids, which thereby persist even under non-selective conditions. We further observed that the dynamic structure of biofilms directly impacts the population of plasmids that are retained. We also identified an additional determinant of plasmid transfer – flagella- which are required for motility by many bacteria. We demonstrate that their absence or altered activity can lead to enhanced plasmid spread. Furthermore, we demonstrate the utility of mathematical modelling to eliminate hypothetical explanations.

Conclusions: Our findings highlight biofilms as conducive towards maintaining genetic diversity as well as shedding light on the complex effects of flagella on bacterial conjugation in biofilms. Understanding the process impacting and maintaining genetic diversity of accessory functions is an important step to restrict spread of unwanted plasmid associated genes such as those encoding resistance.

[P116] HORIZONTAL GENE TRANSFER IN THE PRESENCE OF METALS IN A CONSTRUCTED MICROBIAL COMMUNITY

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Aim: The spread of antibiotic resistant bacteria and antibiotic resistance genes in the environment is a global concern for human health. The human use of antibiotics and metal containing fertilizers causes the release of antibiotic and metal resistance genes into agricultural soils and wastewater, which triggers the development of antibiotic and metal resistance in microorganisms. It is believed that the increase of metal concentrations in soils and wastewater might affect the antibiotic resistance of the bacteria populating these environments. To test that assumption, this research will concern the effect of metals on the transfer of antibiotic resistance genes in the microbial community.
Methods: An artificial microbial community from different strains will be constructed. The communities for the horizontal gene transfer experiments will include the plasmid donor carrying a broad-host-range conjugative plasmid which encodes resistance to kanamycin, ampicillin, and tetracycline. A series of laboratory evolutionary experiments will be conducted, during which the microbial community will be exposed to sub-inhibitory concentrations of metals ions (copper or nickel) and cultivated with the plasmid donor. The hosts of antibiotic resistance will be detected using epicPCR (Emulsion, Paired Isolation and Concatenation PCR).
Results: At the present time, the artificial microbial community is being constructed and preliminary experiments on the effect of metals on adaptation to antibiotics are being planned. The composition of the community at various stages of the experiment will be determined, which will provide information on the effect of various concentrations of metals on the composition of microbial communities. EpicPCR will allow us to determine the bacteria carrying the antibiotic resistance genes.
Conclusions: There are studies suggesting that the presence of metals favors adaptive antibiotic resistance and transfer of antibiotic resistant genes; however, those studies usually examine the effect of metals on the resistance of single microorganisms rather than of a bacterial community. This study will provide more information on the relationship between metal and antibiotic resistance, as well as understanding of the mechanisms and dynamics of antibiotic gene transfer. The identification of hosts and recipients of the antibiotic resistance genes could be beneficial for finding solutions against antibiotic-resistant infections.
References: Hultman et al. (2018) FEMS microbiology ecology 94.4: fiy038; Spencer et al. (2016) The ISME journal 10.2: 427-436.

[P117] ANTIBIOTIC-RESISTANCE AND HERBICIDE-UTILIZING BACTERIA IN SURFACE SEDIMENTS OF THE LAKE VILLARRICA (SOUTHERN CHILE)

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Aim: Lake Villarrica has a high relevance for economy and tourism of southern Chile; however, an increase of nutrients in water has been observed the last few years. As result the lake basin was declared as 'Satured Zone' and a 'Decontamination Plan (DP)' is being developed by Chilean Government. In this context, emerging contaminants, such as antibiotics and pesticides, derived from anthropogenic activities (aquaculture, agriculture, urban centers, etc.) are continuously released in the lake basin, where sediments can act as a reservoir of both antibiotic-resistant and herbicide-utilizing bacteria, which are not considered in the DP. The main of this study was exploring the occurrence of bacterial populations harboring antibiotic-resistance and herbicide-utilizing traits in sediments from the Lake Villarrica.

Methods: Total DNA was extracted from surface sediments collected during 2020 and 2021 samplings. Antibiotic resistant genes (ARG) to beta-lactam (bla-TEM), tetracycline (tetM) and amphenicol (catA1), and herbicide-utilizing genes (HUG) to atrazine (atzA) and glyphosate (phnJ) were evaluated by qPCR. In addition, 41 strains showing antibiotic multi-resistance (30 µg amoxicillin ml-1, 25 µg chloramphenicol ml-1 and 25 µg oxytetracycline ml-1) on agar plates were isolated from 2021 sampling. Then, the presence of ARG and HUG as well as the antibiotic-resistance spectra was screened by qPCR and diffusion discs (DF) technique in all isolates, respectively.

Results: qPCR revealed higher abundances of 16S rRNA gene copies g-1 sediment in 2021 (1010 to 1011) than 2021 (106 to 107). The occurrence of ARG and HMG ranged from 101 to 104 gene copy g-1 sediment. The culturable bacteria ranged 1 to 2×106 CFU g-1 sediment with higher counts of antibiotic-resistant bacteria on agar plates with amoxicillin (2 to 9×105 CFU g-1 sediment) and amoxicillin plus chloramphenicol (0.6 to 1×106 CFU g-1 sediment). By using qPCR, most of isolates were positive for atzA (90%), phnJ (76%). Despite a minor proportion of isolates were positive for antibiotics catA (24%) and tetM (29%), most of them showed resistance to oxacillin (1 µg), cefotaxime (30 µg), erythromycin (15 µg), and vancomycin (30 µg) by DF technique. In contrast, most of isolates also showed sensibility to kanamycin (30 µg), gentamicin (10 µg), ciprofloxacin (5 µg) and nalidixic acid (30 µg).

Conclusions: Bacterial populations in surface sediments of the Lake Villarrica harbor ARG and HMG, where culturable bacteria showed multi-resistance to β–lactams, cephalosporin, macrolides, chloramphenicol; but sensibility to aminoglycoside and quilonones.

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[P118] CULTURABLE BACTERIAL COMMUNITY AND THEIR PATHOGENIC POTENTIAL ALONG THE EXPANSION OF FROZEN TO ICE-FREE SOIL ZONES OF ECOLOGY GLACIER, ANTARCTICA

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Antarctic Peninsula is recognized as the most rapidly warming region on the planet and contain many ice-free areas. However, the consequences of climate change, such as ice melt, on these edaphic (soil-associated) microbial communities are poorly understood. Expanding these ice-free zones could lead to the discovery of previously unknown microorganisms carrying novel virulence factors, multidrug-resistant (MDR) strains, and resistance genes (RGs). In this context, Ecology Glacier is rapidly retraining, driving an expansion of terrestrial Antarctic ice-free zones for colonization by pioneer microbes and plants. Here, we used the culturable-dependent approach to compare the abundance of culturable bacterial community with pathogenic potential along the expansion of frozen to ice-free soil zones of the Ecology Glacier from King George Island, Antarctica. There, iced (P; permafrost), icetransient (M; moraine), and ice-free (R; rhizosphere) soils of Ecology Glacier were obtained in 2022 during the Antarctic Scientific Expedition (ECA 59, INACH). Our results demonstrated a significant ($P > 0.05$) higher bacterial counts from rhizosphere (from 2.9×105 to 8.2×105 CFU g-1 dw) than moraine and permafrost (from 1.4×103 to 6.4×103 CFU g-1 dw),
especially on R2A media across the 30 d of incubation. A total of 49 different bacterial strains were isolated (10, 20 and 19, moraine, permafrost and rhizosphere-associated isolates, respectively) identified as being genetically unique by enterobacterial repetitive intergenic consensus (ERIC)-PCR. Subsequently, virulence and antibiotic resistance assays were performed, the results of which revealed that the virulence factors such as hemolytic activity (70%), pyocyanin production (53%) and lecithinase activity (42%) was more frequent in rhizosphere strains than in those from moraine and permafrost. Additionally, the Dnase activity (11%) was only observed in strains isolated from moraine. In contrast, the isolates collected from moraine samples were resistant to several antibiotic groups in comparison. with those observed from rhizosphere and permafrost strains. High rates of antibiotic resistance were observed to bacitracin (84.7%), lincosamides (82.9%), cephalosporins (82.2%) and oxazolidinones (74.4%). Finally, we identified the strains Pseudomonas sp. P11 and Stenotrophomonas sp. M84, which carrying more than two virulence factors and had high resistance antibiotics (more than 10 different groups). This study demonstrated high rates of antimicrobial resistance and the presence of virulence factors in Antarctic ice-free soils.
Acknowledgment: Anillo project (mBioClim) cod. ACT210044

[P119] DYNAMIC OF ANTIMICROBIAL RESISTANCE GENES' PREVALENCE IN HOSPITAL WASTEWATER IN A REFERRAL HOSPITAL IN INDONESIA: A PILOT STUDY

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ABSTRACT

Background: Antimicrobial resistance is a significant concern worldwide [1]. Hospital wastewater is regarded as a hotspot for antimicrobial resistance, allowing antibiotic resistance genes (ARGs) to be transferred horizontally between pathogens and commensal bacteria [2,3]. Study on ARGs in hospital wastewater in Indonesia is scarce. Aim: This study aimed to monitor the prevalence of ARGs in hospital wastewater in a referral hospital in Indonesia.

Methods: Twelve wastewater samples were collected from an influent wastewater treatment plant. The samples were taken randomly twice per week during October 2021 and November 2021. DNA was extracted from wastewater samples. A high throughput qRT-PCR technique was used to measure the copy number of beta-lactam (n=15), aminoglycoside (n=14), and quinolone (n=7) resistance genes in hospital wastewater, respectively.

Results: The prevalence of total antibiotic resistance genes in hospital wastewater was highest in week one and lowest in week six of the six weeks of monitoring ($p<0.001$). Quinolone, and aminoglycoside resistance genes were the most prevalent in weeks one and two ($p=0.029$ and $p=0.004$), and week four ($p<0.001$).

Conclusion: The prevalence of ARGS was dynamic during the six weeks of monitoring. Wastewater treatment might play a role in the existence of ARGs in hospital wastewater [4,5]. Quinolone and aminoglycoside resistance genes were more abundant in hospital wastewater than beta-lactam resistance genes. The abundance of ARGs in hospital wastewater may reflect the prevalence of multidrug resistant organisms circulating in clinical settings. Further investigation is required to develop an early warning system for multidrug resistant organism outbreaks through hospital wastewater analysis.

Keywords: antimicrobial resistance; Indonesia; wastewater

[P120] GENETIC CHARACTERIZATION OF CARBAPENEM NON-SUSCEPTIBLE ACINETOBACTER BAUMANNII BLOOD ISOLATES IN INDONESIA USING OXA-51 SEQUENCE-BASED TYPING

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Aim: Hospital-acquired infections, including bacteremia due to carbapenem non-susceptible Acinetobacter baumannii (CNSAB), have been reported to increase worldwide. The A. baumannii strains involved are usually limited to a few clonal lineages. Therefore, grouping epidemic A. baumannii strains into a specific clonal lineage is important. Multilocus sequence typing (MLST) is the gold standard method for detecting clonal dissemination of A. baumannii, but this examination is very complex. Therefore, examination using single locus-based typing (SBT) of the OXA-51-like gene, unique to A. baumannii, is effective for application. This study aimed to identify genetic characteristics using OXA-51-like SBT CNSAB isolates isolated from blood in several hospitals in Indonesia.

Methods: The sample for this study included 110 non-repetitive CNSAB isolates grown from routine blood cultures. Samples come from 12 hospitals in Indonesia. CNSAB is an intermediate or resistant isolate to one of the carbapenem antibiotics (meropenem, imipenem, doripenem). Sequencing was performed using BigDye® Terminator v3.1 Cycle Sequencing Kit (Applied Biosystems). The sequence results were matched to a reference gene using the BLAST program by PubMed National Center for Biotechnology Information. The neighbor-joining method is used to construct a phylogenetic tree using the MEGA 11 application.

Results: SBT OXA-51 from 110 isolates in the study were found to have various types of OXA-51-like genes consisting of OXA-66 (74/110; 67.3%); OXA-69 (27/110;24.5%); OXA-870 (4/110; 3.6%), OXA-94 (2/110;1.8%); and OXA-508,-873,-64 (1/110;0.9%). OXA-66 and OXA-69 genes belong to the worldwide lineage 2 (WW2, complex clonal CC2) and WW1 (CC1). We identified OXA-66 as the major OXA-51-like gene, consistent with several other reports from the United States, South America, Europe, Turkey, and China. In

addition, OXA-66 and OXA-69 were also associated with a particular epidemic lineage of A. baumannii.

Conclusions: Our results showed a successful predominance of international clone 2 in Indonesian hospitals. These findings emphasize the urgent need for effective measures to control the spread of A. baumannii in this country. In addition, more studies are essential to explore the local genetic diversity and the molecular epidemiology of carbapenem-resistant A. baumannii.
Keywords: Acinetobacter baumannii, Indonesia, Carbapenem resistance, blaOXA-51 sequence-based typing

[P121] QUANTIFICATION OF SEDIMENTARY BACTERIAL ANTIBIOTIC RESISTANCE AND DEGRADATION GENES ALONG A MAJOR SCANDINAVIAN RIVER RECEIVING SEVERAL MUNICIPAL WWTP DISCHARGES

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The high usage of antibiotics in human and veterinary medicine calls for higher-than-ever-before concentrations of antibiotics in hospital and municipal wastewaters making wastewater treatment plants (WWTPs) a sink for antibiotic pollution and a hotspot for bacteria carrying antibiotic resistance genes (ARGs) and antibiotic degradation genes (ADGs). However, the extent to which municipal WWTPs discharge these genes in receiving rivers and their capacity to prevail in the environment is still not resolved. The aim of this project is to characterize the occurrence of ARGs and ADGs in sediment bacteria along the River Göta Älv, one of the largest in Sweden with ~ 100 Km of length, from the source in Lake Vänern to the estuary in the Skagerrak sea, and the impact of WWTP discharges on ARG and ADGs emissions into the river. The response of ARGs and ADGs in river bacterial sediment upstream and downstream the WWTPs of the cities Vänersborg (~ 24 000 inhabitants), Trollhättan (~ 46 000 inhabitants), Lilla Edet (~ 4 800 inhabitants) and Gothenburg (~ 533 000 inhabitants) were compared. Gene screening was also done in sludge and effluent samples from the WWTPs of these cities. Quantitative PCR was used to determine the occurrence of ten genes. These include, four ARGs (SulI, tetA, ermB, and qnrS) and four ADGs (SadA, tetX, mphA and BlaCTX-M), conferring resistance/degradation to sulfonamides, tetracyclines, macrolides, quinolones, and beta-lactam antibiotic classes respectively, as well as two bacterial reference genes (16S rDNA and rpoB). Based on our preliminary results, all genes were present in the sludge from the WWTP in Gothenburg. Further results will allow us to assess if those genes prevail in river sediment and the contributions of WWTP discharges on the spread of ARGs and ADGs into a large Scandinavian River.

Key words: antibiotic resistance genes, real-time PCR, wastewater treatment plants, aquatic contamination, antibiotrophy

[P122] MOLECULAR INSIGHTS INTO TYPE IV-A CRISPR-CAS SYSTEMS AND THEIR ROLE IN PLASMID-PLASMID COMPETITION

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Bacteria and their mobile genetic elements are locked in a constant evolutionary arms race for survival and co-existence. Many bacteria employ CRISPR-Cas systems as an adaptive immune strategy to combat invasive mobile genetic elements, such as phages or plasmids. In contrast to all other described types of CRISPR-Cas, type IV loci are enriched on conjugative elements. Bioinformatic data suggests that these systems may play a primary role in plasmid competition dynamics, rather than participating in host adaptive immunity. Because type IV CRISPR-Cas systems lack an adaptation module and a nuclease effector, the underlying molecular mechanisms for spacer acquisition and interference remain poorly understood. In this study, we show that a type IV-A CRISPR-Cas system, which is encoded by an IncHI1B/FIB plasmid, utilizes the adaptation machinery from its host, Klebsiella pneumoniae, to acquire new spacers. Moreover, we explore the interference of this system with conjugative plasmids and its potential for biotechnological applications.

[P123] THE FEATURES OF INCP/P-1PLASMID WOULD BE PREDICTED BY K-MER COMPOSITIONS

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Conjugative transfer of plasmids can spread various genes among different bacteria. It is important to determine which bacteria can acquire plasmids by conjugation for understanding bacterial evolution and adaptation mechanisms. In silico analyses have reported that nucleotide composition of plasmids and their known host chromosomes is usually similar[1]. However, it's not well understood whether the similarity in nucleotide compositions between plasmids and bacterial chromosomes can be used to predict which plasmids are hosted by which strains. Thus, the objective of this study is to elucidate whether the features of plasmid replication, maintenance and conjugative transfer in different bacterial strains could be predicted by using the nucleotide compositions. Ten IncP/P-1 plasmids[2] (subgroup β, η, θ, κ, λ, μ, ο, ρ, τ) were isolated from different environments and showed different nucleotide compositions (e.g., 18% difference in GC contents). First, conjugation assay and stability assay were performed with Escherichia coli and Pseudomonas putida. In parallel, the dissimilarity of k-mer composition between IncP/P-1 plasmid and these chromosomes were calculated as Mahalanobis distance[1]. As a result, k-mer compositions of three plasmids (subgroup η, ρ, and μ), which could not be replicated in P. putida, were not similar with that of the Pseudomonas chromosome. Furthermore, the stabilities of these plasmids were different in host by host. The θ-subgroup plasmid could be stably maintained in E. coli, whereas the β and o-plasmids could not. Interestingly, the k-mer composition of the chromosome of the host in which the plasmid was stably maintained was like that of the plasmid. Next, conjugation assays were performed with the three IncP/P-1 plasmids (subgroup β, o, and θ) and the artificial bacterial community (composed of 25 different strains). The transconjugants were isolated by a cell sorter with GFP as a reporter protein. As a result, P. putida was the most abundant transconjugants for β and o-subgroup plasmids, whereas Aeromonas cavie and E. coli were the most for θ-subgroup plasmid. The type of transconjugant was consistent with the host in which the plasmid was maintained stably and showed similar k-mer compositions. These results indicate that k-mer compositions of plasmids and chromosomes of their suitable hosts, which plasmids can be transferred, replicated, and stably maintained, were more similar than non-suitable host ones and that the suitable hosts could be predicted by using k-mer compositions.

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[P124] ARGS AS EARLY INDICATORS OF BREACHES IN NANOFILTRATION (NF) SYSTEMS

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AIM:
This study explored the possibility of using antibiotic resistance genes (ARGs) as early indicators of breaches in nanofiltration NF membrane systems. Specifically, the study sought to evaluate ARGs' removal efficiency under normal and breached operational conditions. The ultimate goal was to determine the feasibility of using ARGs as a reliable indicator of NF system integrity.

METHODS:
Laboratory- and pilot-scale nanofiltration (NF) systems were used to investigate the impact of membrane breach (which helps in the free passage of pollutants) on the spread of total bacteria (as indicated by 16S rRNA) and two ARGs, sul1 and tetO, in biologically treated greywater. NF modules were evaluated both under normal and breached conditions. Samples were collected from both the feed and permeate. DNA was extracted from the samples, and genes were quantified absolutely with qPCR, and serial dilutions of gBlock Gene Fragment were used as standard.

RESULTS:
Under normal operational conditions, substantial removal of all targeted genes upon nanofiltration was achieved as expected. In the laboratory-scale reactor, log removals for 16S rRNA, sul1, and tetO were 3.9±0.4, 4.5±0.4, and >2.8±0.3 (tetO levels in the permeate <LOD), respectively, with initial feed concentrations of 7.5±0.1, 4.9±0.1, and >2.8±0.03 log CN.L-1 for 16S rRNA, sul1, and tetO, respectively. In the pilot-scale experiment, log removals of 5±0.3, 5±0.3, and >4.3±0.1 were achieved for 16S rRNA, sul1, and tetO, respectively, with similar feed concentrations to the laboratory-scale experiment.

However, when at least one fibre of the NF membrane module was broken, the monitored genes were not removed. Instead, a drastic reduction in log removal was observed for all genes. In the laboratory-scale experiment, log removals were 0.2±0.4, 0.2±0.3, and 0.2±0.3 for 16S rRNA, sul1, and tetO, respectively, with feed concentrations of 7.1±0.08, 4.3±0.01, and 3.0±0.08 log CN.L-1 for 16S rRNA, sul1, and tetO, respectively. In the pilot-scale experiment, log removals were 0.2±0.4, 0.2±0.3, and 0.3±0.5 for 16S rRNA, sul1, and tetO, respectively, with similar feed concentrations to the intact NF systems.

CONCLUSIONS:
The study concludes that ARGs can potentially serve as indicators of NF integrity. However, the full potential of ARG qPCR to detect membrane breaches depends on the levels of resistance genes in the sample and their detection limits.

[P125] TRANSMISSION OF ANTIMICROBIAL RESISTANCE IN THE GUT MICROBIOME OF SUBSOCIAL INSECTS

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Background: The rapid increase of antimicrobial resistance (AMR) poses a major threat to global health. The emergence and dissemination of AMR through the commensal microbiome aggravate the problem due to the potential horizontal gene transfer of AMR genes to pathogens. Several mathematical models explore the effect of AMR transmission by commensal bacteria, yet, experimental studies on between-host transmission are rare and challenging to set up.

Aim: This study examines a subsocial gregarious cockroach species (Pycnoscelus surinamensis) as a possible animal model for AMR transmission experiments. The first objective is to demonstrate that antimicrobial treatment has a detectable effect in the gut microbiome with metagenomic sequencing. The second objective is to determine whether AMR genes can spread and establish in untreated individuals after contact with treated ones.

Methods: For the first objective, one cockroach population was split into two, with one group being treated with tetracycline daily for eight days. For the second objective, the two populations were subsequently colour-marked and mixed for eight more days. Gut samples (triplicates) were taken from all groups throughout the experiment. DNA was extracted with the QIAamp Microbiome Kit to deplete host DNA, and then shotgun sequenced with Illumina, NovaSeq platform. The raw reads were trimmed and then mapped with KMA against the ResFinder database for AMR genes and a genomic database for taxonomic identification. The metagenomic counts were used to estimate α-diversity and β-diversity at different levels, the latter with compositional methods for ordination and differential abundance.

Results: Our results show that antimicrobial treatment disrupted the treated hosts' gut microbiome, reducing the bacterial community's evenness and increasing the relative abundance of tetracycline resistance genes. Additionally, the relative abundance of tetracycline resistance genes was higher in the untreated hosts that interacted with treated individuals compared to the untreated control. Finally, the treated hosts that were mixed with the untreated hosts exhibited recovery of their bacterial diversity compared to the treated controls.

Conclusions: Metagenomic analysis of the cockroach gut microbiome captured shifts in the AMR gene profile and bacterial community composition due to antimicrobial treatment, as well as changes due to bacterial transmission between individuals. Gregarious cockroaches are thus a promising candidate for experiments on AMR transmission and can be used to validate previous theoretical transmission models.

[P126] THE EFFECT OF COPPER AND SILVER EXPOSURE ON CONJUGATION AND THE INFLUENCE OF ENUMERATION METHOD ON THE TRANSFER FREQUENCY

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There is evidence that antibacterial biocides at subinhibitory concentrations in aquatic environments may stimulate the dissemination of antimicrobial resistance via conjugation. However, we are lacking an understanding of the types of chemicals that exert that effect, the type of conjugal plasmids, donor/recipient combinations that display it, and the molecular basis of the underlying mechanism.

We initiated the first step towards assessing the effect of frequently used- and environmental relevant biocides on conjugation. To achieve this goal, a conjugation assay was developed using the model IncPα RP4 plasmid harbored by E. coli MG1655 as the donor strain and plasmid-free E. coli MG1655 as the recipient. Cell densities were at approximately 108 cells/ml, and matings were carried out in PBS to avoid growth bias and exposure to the biocides was done at two different sub-inhibitory concentrations. Non-exposure controls (timepoint 0 hour and end-of-experiment timepoint at 4 hours) were used as the baseline for the detection of conjugal stimulation. An antibiotic-cocktail pretreatment was implemented to eliminate the large background of transfer events stemming from high cell densities that allow conjugation occurrence on transconjugant selective medium. Enumeration of transconjugants, recipient and donors were carried out using two different methods; (i) traditional plate count method and (ii) MPN method in order to identify the method that is less subject to bias and/or higher throughput.
We tested exposure to Cu(II) at 0.25 µg/ml and 0.025 µg/ml as well as Ag(I) at 0.005 µg/mL and 0.0005 µg/mL corresponding to approximately 1/10 and 1/100 of the minimal inhibitory concentration of E. coli. Exposure to these antimicrobials did not result in statistically significant stimulation of horizontal gene transmission compared to the non-exposure control. This is in contrast with previous studies.

We note that estimates of transfer frequencies spanned three-order magnitude depending on the method of enumeration (Plating: 1E-04 to 1E-06 T/R and MPN: 1E-07 to 1E-09 T/R). This is because donor and recipient pairings are not killed immediately upon contact with selective medium allowing donors and recipients to continue the mating process on both solid-surface and in liquid. In addition, we emphasize the importance of including enough replicates to minimize variability.

This optimized conjugation assay will be instrumental in systematic testing of the risk of AMR plasmid dissemination associated with environmental concentrations of biocides.

[P127] A WHOLE FAMILY OF REVERSE TRANSCRIPTASES WITH ANTI-PHAGE DEFENSE FUNCTIONS

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Aim:
Reverse transcriptases (RTs) are enzymes present in all three domains of life that polymerize DNA strands using RNA as a template. In this study, we provide a comprehensive and systematic analysis, discovery and classification of prokaryotic RTs belonging to the Unknown groups (UG) and Abortive Infection (Abi) RTs and propose their clustering into a highly diverse and widespread lineage of RTs associated with antiphage defense functions. In addition, we demonstrate the requirement of ncRNA for such function, and the presence of RTs of this family in a large group of previously unknown virus infecting species present in the human microbiome.

Methods:
In this work, we combined computational and experimental approaches to uncover, classify and characterize the Reverse Transcriptases belonging to the UG/Abi family, including evolutionary and comparative genomics analyses, protein structure predictions, phage plaque assays and non-coding RNA sequencing.

Conclusions:
Our study provides evidence for the existence of a highly diverse and widespread lineage of prokaryotic RTs associated with defense functions, which plays an important role in virus-host conflicts. Our findings expand our understanding of RT diversity and provide a basis for further investigation into the role of UG/Abi RTs in prokaryotic defense against bacteriophages.

[P128] HEAVY METALS MAY INCREASE ARG PERSISTENCE IN THE ENVIRONMENT

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The OneHealth approach requires determining the risk that antibiotic resistance genes (ARGs) in the environment pose to human health. ARGs in the environment provide opportunities for human microbiome bacteria and human pathogens to acquire resistance to antibiotics through horizontal gene transfer. Heavy metals can impose a selective pressure on environmental bacteria and select for antibiotic resistance, since ARGs can co-occur with metal resistance genes. In addition, cross-resistance mechanisms between metals and antibiotics such as efflux pumps have been observed. Since metals persist longer than antibiotics in the environment, they may exert a selective pressure on environmental bacteria for longer periods than antibiotics and could cause an increase in the persistence of ARGs in the environment. In our study, river water microcosms were exposed to heavy metals (copper and zinc), fluoroquinolone antibiotics (ciprofloxacin and ofloxacin) and both for 30 days to evaluate the persistence of ARGs using shotgun metagenomic analysis of the extracted DNA. Higher abundances of efflux pumps conferring resistance to ciprofloxacin and ofloxacin were observed in samples exposed to metals, both in the presence and absence of antibiotics than in controls. Therefore, copper and zinc could select for and maintain fluoroquinolone resistance in the environment by cross-resistance. The ARGs that showed an increase in abundance in heavy metal-contaminated river water depended on whether antibiotics were present or not. Therefore, both metals and antibiotics conferred resistance, although the resistance profiles were not the same. Thus, our results are consistent with heavy metal persistence leading to the maintenance of ARGs in the environment.

[P129] THE RELATIONSHIP BETWEEN RESTRICTION MODIFICATION AND ANTIMICROBIAL RESISTANCE GENES

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Aims: This project aims to investigate the association between bacteria's most ubiquitous antiviral defences, restriction modification (RM) systems, and acquired antimicrobial resistance (AMR) genes. RM systems are agnostic to the fitness costs and benefits associated with mobile genetic elements (MGEs) and past experimental studies have shown they can present barriers to conjugation[1], and transformation[2]. However, a between-taxon bioinformatic study has counterintuitively found positive associations between RM systems and MGEs[3]. Here we expand on this work to test the relationships between RM systems and the highly clinically important mobile genes, acquired AMR genes. We make notable improvements, such as utilising a much larger dataset, allowing us to conduct within-taxon phylogenetically controlled analyses, and by testing the influence of genetic linkage between AMR genes and RM systems on observed relationships.

Methods: We utilised all publicly available genomes from the NCBI's refseq database for key human pathogens, including Pseudomonas aeruginosa. Using the tools PADLOC and abricate, we searched them for RM systems, and AMR genes, respectively. Phylogenies were constructed using the genetic distance estimator MASH, and the r package ape. Genetically linked RM systems and AMR genes were identified using Coinfinder. Finally, we conducted within-taxon phylogenetically controlled analyses using the Bayesian modelling tool mcmcGLMM.

Results: For Pseudomonas aeruginosa, the relationship between both type I and type II RM systems and AMR genes is positive, yet when we remove occurrences where AMR genes and RM systems are genetically linked, for type I systems the association's strength is greatly reduced, and for type II systems it is removed. Analyses with other species are ongoing.

Conclusions: The perhaps counter-intuitive positive association between RM systems and acquired AMR genes in Pseudomonas aeruginosa is largely explained by genetic linkage, suggestive of certain RM systems and AMR genes co-occurring on MGEs. As RM systems can function as toxin-antitoxin systems with the ability to stabilize MGEs, this co-occurrence with AMR genes is of potential concern.
From functioning as a barrier to HGT, to potentially occurring on clinically relevant MGEs, understanding the interplay between bacterial defense systems and AMR genes is key in understanding how pathogens might acquire novel drug resistance in the future, and may even help to identify active interventions for this global health crisis.

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[P130] QUANTITATIVE RESISTOME DYNAMICS IN THE HUMAN GUT DURING EARLY LIFE

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The spread of antimicrobial resistance is a major public concern due to its implications in health care. Meanwhile, microorganisms that harbor Antimicrobial Resistance Genes (ARGs) are ubiquitous in nature and they have been found to reside even in the gut of antibiotic-naïve infants. Even though many of those resistant microorganisms are not inherently dangerous for the host, the presence of many ARGs in the human gut can act as an AMR reservoir due to the ability of ARGs to be transferred to other microorganisms through horizontal gene transfer. Studying the abundance and composition of ARGs in the infant gut can be of major importance as it allows us to understand the transmission route of ARGs in the beginning of life.

To understand the dynamics of the resistome, the collection of ARGs, during the first 5 years of life, we have sampled 56 mother-infant pairs. The infants were sampled longitudinally at 8 time points from 1st week of life up to 5 years and shotgun metagenomics was used for the detection of ARGs in the samples. In addition, the total microbial load was measured by flow cytometry to allow for quantitative measurement of the ARG load.

We observed that the relative abundance of ARGs was higher during the first year of life but decreased at 12 months and start resembling more the ARG abundance detected in maternal samples. When the microbial load was considered, the highest abundance of ARGs was detected at six months of age. The ARG richness was higher at the first months of life and start decreasing at 12 months. The opposite trend was observed for the ARG containing bacterial taxa, which were fewer during the first months of life, but their numbers start increasing at 12 months of age. Mode of delivery was associated with the ARG composition during the first months of life, whereas having older siblings was associated with the composition in infants older than 12 months. Furthermore, the nucleotide similarity of the infant and maternal ARGs was compared to define the number and load of ARGs that were transferred from mother to child.

The longitudinal sampling of the infant gut in combination with the quantitative information revealed a peak in ARGs at 6 months of age and indicated that distinctive environmental factors can affect the ARG composition during different periods in early life.

[P131] PLASMID METAGENOMIC: INSIGHT INTO THEIR CRYTIC ECOLOGY

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Plasmids are extrachromosomal self-replicating genetic elements that play a key role in bacterial ecology and evolution by shuttling diverse host-beneficial traits between bacteria. However, our understanding of plasmids is still limited, particularly in the human gut microbiota, and little is known about how they are acquired and become established in infants. In this study, we explored a longitudinal fecal metagenomic dataset obtained from 98 Swedish children who were followed during their first year of life. For this, we developed a bioinformatics pipeline for the complete sequence assembly and annotation of plasmids, together with the identification of plasmid contigs. We found that gut plasmids in these children were extremely diverse, particularly in the first four months of life, and this diversity decreased with maturation of the gut microbiota. Bacteroides and Bifidobacterium were identified as major hosts of transmissible plasmids in the early human gut microbiota. Additionally, we discovered that plasmids played a substantial role in expanding the gene repertoires of their bacterial hosts: approximately a quarter of unannotated plasmid genes were found only on plasmids and not on chromosomes. Together, our results provide the first characterization of the early acquisition and development of plasmids in the infant gut microbiome. Their diversity and abundance in the first months of life could benefit a variable and rapidly proliferating microbiota by providing increased adaptability in a highly competitive environment.

[P132] DIFFERENTIAL RESPONSES OF THE GUT MICROBIOME AND RESISTOME TO ANTIBIOTIC EXPOSURES IN INFANTS AND ADULTS

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Abstract
Despite their crucial importance for human health, little is known about how the gut resistome changes with age or in response to antibiotic treatment across ages. Here, we used fecal metagenomic data from Danish infants and young adults to fill this gap. The gut resistomes were characterized by a bimodal distribution driven by E. coli composition. The typical profile of the gut resistome differed significantly between adults and infants, with the latter distinguished by higher gene and plasmid abundances. However, the predominant antibiotic resistance genes (ARGs) were the same. Antibiotic treatment reduced bacterial diversity and increased ARG and plasmid abundances in both cohorts, especially core ARGs. The effects of antibiotic treatments on the gut microbiome lasted longer in adults than in infants, and different antibiotics were associated with distinct impacts. Overall, this study broadens our current understanding of gut resistome dynamics and the impact of antibiotic treatment across age groups.

[P133] EXOGENOUS ISOLATION OF EXTENDED SPECTRUM B-LACTAMASE-CARRYING PLASMIDS IN CROATIAN WASTEWATER

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Extended Spectrum β-lactamases (ESBL) are enzymes conferring resistance to antibiotics that are significant for human medicine such as penicillin, 1st to 3rd generation cephalosporins and monobactams. ESBL genes are often situated on plasmids that can easily be transmitted within the bacterial community, e.g., from environmental bacteria to human pathogens thus posing a threat to human health. To understand the potential of wastewater as a medium suitable for the occurrence of horizontal gene transfer events of clinically significant genes to recipient bacteria we performed exogenous isolation of ESBL- carrying plasmids. The experiments were performed in biparental mating with bacteria from effluent wastewater of 7 wastewater treatment plants (WWTPs) in Croatia as donors and E. coli CV601 strain as recipient. E. coli CV601 was resistant to kanamycin (KAN) and rifampicin- (RIF) with gfp gene coding for green fluorescence protein. Effluent wastewater was filtered to concentrate the cells and mixed with the recipient overnight culture. Mating experiments were set up on membrane filters and incubated overnight on plates supplemented with cycloheximide (CYC 100 mg/L) at 28°C. After incubation, suspended mating mixtures were spread plated on LB agar supplemented with KAN (50 mg/L), RIF (50 mg/L), CYC (100 mg/L) and 2 mg/L of cefotaxime to ensure the selection of resistant transconjugants with acquired ESBL genes. Transconjugants were identified by green fluorescence emission and confirmed by BOX-PCR fingerprints. Antibiotic susceptibility profile was determined by disk diffusion. The presence of 9 different ESBL genes was tested by PCR. Plasmids were assigned to known incompatibility groups by PCR-based replicon typing (PBRT). In total, 18 transconjugants from experiments with effluents of 5 different WWTPs were identified. Their antibiotic susceptibility profile confirmed resistance to penicillin and 3rd generation cephalosporins. PCR screening revealed the presence of ESBL genes: blaTEM, blaCTX-M-1 group and blaGES. All the transconjugants carried both blaCTX-M-1 and blaTEM genes, while one transconjugant had all 3 mentioned genes. PBRT revealed the presence of broad-host-range plasmids that belonged to IncL/M complex, N and CoIE groups which were previously recorded to carry ESBL as well as other antibiotic resistance genes. The results indicate that wastewater is a suitable environment for the dissemination and transfer of antibiotic resistance genes.

[P134] DAIRY SLURRIES APPLICATION AS SOIL AMENDMENT DRIVES RESISTOMES ENRICHMENT IN SOUTHERN CHILE FARMS

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Aim: Dairy slurries are commonly applied as soil fertilizer amendments due to their content of P, N, and organic matter (%OM). However, selective antibiotic pressure in dairy cattle rumen enrich slurries with antibiotic-resistant bacteria, and antibiotic resistance genes (ARGs). In this study, we explored ARG communities in the slurry, rhizosphere, and bulk soil-continuum in four Chilean dairy farms to assess resistomes enrichment driven by slurries application.

Methods: Thirteen ARGs (tetA, tetG, tetM, tetQ, tetW, tetX, sul1, sul2, blaTEM, blaCTX-M, blaOXA-1, ermB, and dfrA1) and two-related integrase genes (intl1, intl2) abundance was explored in slurries, rhizosphere, and bulk soils via qPCR. In parallel, the composition and structure of total bacteria communities was determined via 16S rRNA Illumina MiSeq. Finally, the resistome enrichment was confirmed by tetX and blaTEM variants sequencing with Oxford Nanopore.

Results: Twelve of thirteen assayed ARGs in this study were detected on all sample types. Only blaOXA-1 was absent in bulk soils. An abundance declining trend towards soil depth was observed for twelve of the thirteen ARGs (from ~10^6 in slurries, to ~10^1 copies per g sample [CPGS]). In contrast, tetX abundances were higher in rhizosphere and bulk soils on three farms (~10^4 in slurries, ~10^6 CPGS in soil samples). The farm without increasing abundances revealed no difference between slurry, rhizosphere, or bulk soil ($P \leq 0.05$). The ARG/16S rRNA ratio showed strong soil association to tetX, whereas slurries were clustered by ermB, tetQ, and tetM. This separation was confirmed as sample type dependent by PERMANOVA ($P \leq 0.05$), PCoA and RDA (~70% variance explanation). On 16S rRNA communities, slurries were less diverse than soils. Slurries were dominated by Firmicutes and Bacteroidota, whilst soils had larger proportions of Actinobacteria, Proteobacteria, Acidobacteria, and Chloroflexi with different ratios associated to sample type (rhizosphere or bulk soil). Amplicon sequencing revealed the relative variants abundance of blaTEM and tetX genes as equivalent for all samples. The blaTEM, communities were dominated by TEM-229 and TEM-116 variants (~15-20% for TEM-229, and ~11-20% for TEM-116, respectively). Likewise, tetX was distributed as tetX3 (50%), tetX4 (30%) and tetX5 (20%).

Conclusions: Despite having different bacterial communities (in terms of composition, structure, and total ARG abundances), the relative proportions of tetX and blaTEM genes confirmed that slurries have enriched the resistomes of these soil bacterial communities.

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[P135] ANTIBIOTIC RESISTANCE GENES AND MOBILE GENETIC ELEMENTS IN THREE DIFFERENT SOILS IRRIGATED WITH UNTREATED OR TREATED WASTEWATER

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Wastewater reuse for irrigation mitigates water scarcity and promotes agricultural productivity. However, pollutants in the wastewater can favor the selection and growth of microbial populations resistant to antibiotics in irrigated soils. Given the importance of soil texture and water quality in shaping the soil bacterial community composition, we investigated how the shift from untreated to treated wastewater affected the abundance of ARGs and MGEs in soils irrigated over 100 years with untreated wastewater. To this end, three typical soils from the Mezquital Valley in Mexico (Leptosols, Vertisols and Phaeozems) were irrigated with untreated and treated Mexico City wastewater in an incubation experiment in microcosms. The soils were also irrigated with spiked untreated and treated wastewater fortified with antibiotics and disinfectants. The abundance of ARGs and MGEs in total community DNA was monitored over time using qPCR assays. Results obtained so far showed an increase in the relative abundance of sulfonamide resistance gene (sul1), plasmids belonging to IncP-1 (korB) and pSK1 (Staphylococcus) and class 1 integron-integrase genes after 4 days and 14 weeks in soil microcosms irrigated with spiked wastewater regardless of the soil type. Interestingly, the relative abundance of erythromycin and trimethroprim resistance genes (ermA and dfrD, dfrG) and Staphylococcus multiresistance PI258 family plasmids only increased in soil microcosms irrigated with unspiked wastewater and slightly decreased when spiked wastewater was applied. Multivariate analysis based on Euclidean distance matrices of ARGs and MGEs relative abundances showed no differences due to the soil type, but a clear and significant separation due to the spiking level. While soil microcosms irrigated with unspiked wastewater were mainly associated with trimethoprim and erythromycin resistance genes (dfrD, dfrG and ermA), which significantly and positively correlated with the presence of inc18 and pI258 plasmids, soil microcosms irrigated with spiked wastewater were associated with intI1, korB, pSK1-encoded and sul1 genes, which were significantly and positively intercorrelated. On the contrary, a negative correlation between the presence of pI258-asscociated and intI1, korB or pSK1-associated genes was found. Consequently, our preliminary results provide evidence that antibiotics and disinfectants can increase the abundance of specific ARGs in wastewater-irrigated soils and give insights into the co-selection of ARGs and MGEs. Further exogenous plasmid capturing experiments together with 16S rRNA amplicon sequencing data analysis will contribute to a better understanding of the role of plasmids and the soil type-dependent microbiome on the spread of ARGs.

[P136] EVAPORATION MODE OF DROPLETS PROMOTE CONJUGATION-MEDIATED PLASMID TRANSFER IN MICROBIAL POPULATIONS

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Conjugative plasmids bestow important traits to microbial communities, such as virulence, antibiotic resistance, pollutant biotransformation, and biotechnology-relevant functions. While the biological mechanisms and determinants of plasmid conjugation are well established, the underlying physical and ecological driving forces remain unclear. Microbial communities often inhabit unsaturated environments, such as soils and host surfaces (e.g., skin, teeth, leaves, roots), where water evaporation and associated small-scale hydrodynamic processes frequently occur at numerous air-water and solid-water interfaces. Here, we hypothesized that evaporation can induce water flows with profound effects on the spatial distribution and surface deposition of cells, and consequently on the extent of plasmid conjugation. Using droplet experiments with an antibiotic resistance-encoding plasmid, we show that evaporation-induced water flows reduce cell-cell distances and significantly increase the extent of plasmid conjugation. Counterintuitively, we found that evaporation results in lower expression levels of conjugation-related genes. This negative relationship between the extent of plasmid conjugation and the expression of conjugation-related genes could be attributed to increased conjugation efficiency during evaporation. This study provides new insights into the physical and ecological determinants of plasmid conjugation, with important implications for understanding the spread and proliferation of plasmid-encoded traits.

In addition, we quantified in our experiments how these patterns regulate the spread of antibiotic resistance-encoding plasmids in range expansion. We found that the spread of antibiotic resistance on plasmids is a function of the density of cells initially deposited at the periphery of the droplet, which is a manifestation of the coffee-ring effect. Using an individual-based model, we systematically relate how different initial cell deposition patterns caused by the coffee-ring effect and different intensities of Marangoni convection determine the extent of plasmid transfer during range expansion. Evaporation-induced hydrodynamic processes determine key ecological parameters of surface-associated microbial communities that alter the spread of antibiotic resistance.

[P137] SUB-LETHAL CONCENTRATION OF DIFFERENT ANTIBIOTICS AFFECTS NATURAL TRANSFORMATION OF ACINETOBACTER BAYLYI BD413 IN FRESHWATER CONDITIONS

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The diffusion of Antimicrobial Resistance (AMR) is a global health crisis, which affects 700,000 people every year. Globalization has changed the evolution and spread of AMR determinants in the environment, making a One-Health approach necessary to manage it. One of the problems related to AMR diffusion is that possible pathogens, derived from anthropogenic environment, could acquire antibiotic resistance genes (ARGs) originated from bacteria inhabiting the natural environment.
Freshwater bodies, in which treated wastewater are released, are considered an important route of AMR diffusion, especially in the context of water reuse for irrigation purpose. Despite some evidence suggests that the presence of antibiotics in the environment could influence the evolution of resistance and the spread of ARGs through Horizontal Genes Transfer (HGT), less is known about how natural transformation is influenced by the presence of different antibiotics. Hence, the aim of this study was to investigate how different antibiotics at sub-lethal concentrations could affect natural transformation frequency in microcosms mimicking environmental conditions (i.e., water composition, temperature) typical of freshwater bodies. Transformation frequency was evaluated in Acinetobacter bayliyi BD413, used as model strain of natural competent bacteria, measuring its ability to acquire the plasmid pZR80-GFP, in presence of different antibiotics. Sub-lethal concentrations of antibiotics have been firstly chosen determining the minimum inhibitory concentration (MIC) of the different antibiotics against A. baylyi BD413. Secondly, natural transformation has been studied in microcosms with A. baylyi BD413 and plasmid pZR80-GFP incubated in artificial lake water (ALW) added with antibiotics. Transformants were selected by plating the transformation mix on selective medium and checked by the expression of the GFP through epifluorescence microscopy. Results underlined that natural transformation frequency is influenced by the presence of sub-lethal concentration of antibiotics. In particular, natural transformation frequency can differently shift according to the supplemented antibiotic: being favoured by those molecules which resistance is carried on the plasmidic DNA, being unresponsive by those molecules which the bacteria is resistant or being unfavored by those molecules which resistance is not carried on the plasmidic DNA.
This study confirms that the presence of antibiotics in the environment influences transformation frequency, underlining how anthropogenic activity could enhance the spread of AMR determinants in environments, such as freshwater bodies, with possible consequences for food safety and human health.

[P138] EVALUATION OF THE EFFECT OF SULFAMOANOMETHOXINE ON FRESHWATER SEDIMENT BACTERIAL COMMUNITY

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Introduction: Antibiotic resistance (ABR) is a global public health threat. The environment (eg. Surface waters, sediments, waste treatment plants) serves as a hotspot for the emergence and spread of ABR. In these environments, antibiotics have been detected at concentrations below minimal selective concentration for bacterial species (ng/L- µg/L). The recognition of the environment as a hotspot for AMR provides a framework to help understand the AMR crisis and the role environmental microbial communities play in ABR. We aimed to evaluate the effect of sulfamonomethoxine at currently detected levels on sediment freshwater microbial community using culture-independent approaches.

Methods: Triplicate microcosms consisting of 15g sediment & 20 mL freshwater were set up using sediment and freshwater samples collected from Tarland Burn. Microcosms were amended with sulfamonomethoxine to final concentrations of 0.1 µg/L and 10 µg/L along with untreated controls and incubated at 25oC in the dark. Samples were collected from microcosms non-destructively before antibiotic amendment and on days 1, 7, 14, 29, and 63. The V4 hypervariable region of bacterial 16S rDNA was sequenced to evaluate changes in the microbial community in response to antibiotic exposure. Sequence data were analyzed with Quantitative Insights into Microbial Ecology 2.

Results: A significant increase in within-sample alpha diversity indices (Shannon, Pielou's evenness, and observed features) was observed in the microcosms treated with sulfamonomethoxine to a final concentration of 0.1 µg/L compared to 10 µg/L and untreated control microcosms (Kruskal Wallis: $p < 0.02$). PERMANOVA tests of between group beta diversity metrics (unweighted Unifrac distance) also showed significant differences in the same comparisons ($p < 0.05$). Differential abundance tests revealed bacterial species belonging to the families Methyloligellaceae, Nitrospiraceae, Planococcaceae, Bryobacteraceae, Spirochaetaceae, Erysipelotrichaceae, Xanthobacteraceae, and Nitrosomonadaceae were significantly enriched in microcosms treated with sulfamonomethoxine to a final concentration of 0.1 µg/L compared to 10 µg/L and untreated controls (ANCOM: $w > 5000$).

Discussion/Conclusions: Exposing freshwater sediment bacterial communities to a relatively low sulfamonomethoxine concentration increases the diversity of the bacterial community. The observed increase at such low levels is contributed to by the presence of moderately abundant taxa that can potentially degrade sulfamonomethoxine, act as host to ABR determinants, and provide nutrients to the rest of the community. Increased abundance of these taxa with their potential function at such low levels indicates that the current level of antibiotic pollution can alter bacterial community structure and possibly promote the selection and persistence of ABR in the natural environment.

[P139] X-FINDER, A NEW TOOL FOR UNDERSTANDING THE GENOME OF YOUR BACTERIUM

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Bacteria have many cell functions like pathogenicity, biodegradation, or biosynthesis of complex compounds, CRISPR, specialized cell structures, etc... These functions are critical for the interactions with other organisms and the environment and are critical for us to utilize or control those bacteria. Nowadays, we have exponentially increased number of sequenced bacterial genomes. But most of those sequences have no known cell functions associated. Here we developed a new platform, X-finder, for exploring novel cell functions from the genomes. Genes involved in a certain cell function are usually clustered together on bacterial genomes. Useful gene clusters always got spread among different hosts through horizontal gene transfer. As a result, we can identify them by looking for horizontally transferred, conserved gene clusters among different genomes. This strategy is independent of prior knowledge, thus can find new gene clusters even if they have no similarity to known ones. We finished 114,000 comparisons among 804 bacterial genomes within three days. 16185 gene clusters were predicated.

[P141] ASSOCIATION BETWEEN THE QUANTITY OF ANTIBIOTICS USE IN THE CLINICAL SETTINGS AND ANTIBIOTIC RESISTANCE GENES IN ENTEROBACTERALES HOSPITAL WASTEWATER ISOLATES

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Keywords: Antimicrobial resistance, Enterobacterales, Hospital wastewater, Indonesia

Background: The increasing of antimicrobial resistance prevalence might be associated with inappropriate use of antibiotics in hospital settings [1]. Hospital wastewater is regarded as a hotspot for antimicrobial resistance, allowing antibiotic resistance genes to be transferred horizontally between pathogens and commensal bacteria [2,3].

Aim: This study aimed to analyze the correlation between the quantity of antibiotic use in clinical settings and the prevalence of antibiotic resistance genes in Enterobacterales hospital wastewater isolates.

Methods: Twelve hospital wastewater samples were taken from the Dr. Saiful Anwar hospital's influent wastewater treatment plant from September until November 2021. Enterobacterales including Escherichia coli and Klebsiella pneumoniae were isolated from wastewater samples by culture-based methods, followed by bacterial DNA extraction from the isolates. The copy numbers of ESBL resistance genes (n=8), carbapenem resistance genes (n=7), aminoglycoside resistance genes (n=14), and fluoroquinolone resistance genes (n=7) were measured using a high-throughput real-time polymerization chain reaction (HT-qPCR) system. The HT-qPCR quantification results were analyzed and visualized using the ResistApp platform. Data on antibiotic use in the clinical setting was obtained from the Department of Pharmacy during the inlet wastewater sampling period.

Results: The results showed that ESBL resistance genes were the most prevalent found in Enterobacterales hospital wastewater isolates, but they were not statistically significant (p=0.456). Carbapenem resistance genes were not detected in the isolates. Cephalosporins and carbapenem, both beta-lactam antibiotics, were the most prescribed antibiotics in the clinical setting (p=0.024). The prevalence of antibiotic resistance genes in the Enterobacterales hospital wastewater isolates was not correlated with the quantity of antibiotics prescribed in the clinical setting (beta-lactam antibiotics, p=0.236; aminoglycosides, p=0.972; fluoroquinolones, p=0.683).

Conclusion: The quantity of antibiotics use in clinical settings was not associated with the prevalence of antibiotic resistance genes in Enterobacterales hospital wastewater isolates. Further study with more samples is needed to analyze the association between beta-lactam resistance genes in Enterobacter hospital wastewater isolates and the use of beta-lactam antibiotics in clinical settings.

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[P142] CHROMOBACTERIUM VIOLACEUM ACQUIRES RESISTANCE TO B-LACTAM ANTIBIOTICS BY HYPERPRODUCTION OF B-LACTAMASES CAUSED BY MUTATIONS IN THE AMPD GENE

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One of the biggest issues affecting public health is the rise of antibiotic resistance in pathogenic bacteria. Worldwide, there is a high prevalence of β-lactamase-mediated resistance to β-lactam antibiotics. Mutations in the ampD gene, which codes for an amidase that affects peptidoglycan recycling, can result in bacterial strains that overproduce β-lactamases. By producing two chromosomal β-lactamases, AmpC and CphA, the environmental opportunistic bacterium Chromobacterium violaceum is intrinsically resistant to β-lactam drugs. In this study, we isolated spontaneous mutants resistant to ceftazidime to elucidate how these β-lactamases are regulated. By antibiogram, we confirmed resistance to other β-lactams in 19 spontaneous mutants, and by β-galactosidase assay we observed that overexpression of two β-lactamase genes (ampC and cphA) was present in 63% of these mutants. Sequencing of ampD paralogous genes revealed mutations in a single gene (CV_0566) present in 92% spontaneous mutants that showed overexpression of the enzymes, while the other strains have their resistance linked to other components of the genome. Null mutants by allelic exchange of the amidase genes were obtained and the derepression of both β-lactamases was confirmed only in the ΔCV_0566 mutant. The MIC to ceftazidime was 3 times greater for ΔCV_0566 mutants than for the wild-type strain, according to agar dilution assays. Complementation of the null mutant and spontaneous mutants with mutation in CV_0566 allowed reversion to the wild-type phenotype. In conclusion, our data indicate that the occurrence of mutations in the CV_0566 gene that encodes an AmpD amidase causes overexpression of the β-lactamases AmpC and CphA and high resistance of C. violaceum to several β-lactam antibiotics.

[P143] "MICROBIOME & HEALTH" – AN EXCELLENCE-MOOC MAKING MICROBIOME RESEARCH ACCESSIBLE TO EVERYONE

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Microbiome research demonstrated the importance of balanced microbial communities for the entire planet. To raise awareness, we must communicate microbiome research results also to society. Therefore, we conducted an extensive literature research and translated all the information gathered in a way that both the public and the academic community can benefit from. The "Microbiome & Health" MOOC offers a knowledge platform for many disciplines e.g., medicine, agriculture, food science, biotechnology, and bioinformatics. The course is structured in six chapters: i) a definition of the microbiome, ii) an insight into techniques and methods, iii) latest findings in plant microbiome research, vi) facts about the human microbiome, v) knowledge about the resistome and the exposome, and vi) the potential of microbiome research for planetary health and the sustainable development goals. The prepared knowledge is conveyed in short, concise videos, underpinned by 3D visuals and animations. Currently, also a game is in development that will allow to learn about the development of the human microbiome from before birth to death. In scenarios, the player will make various choices that can affect the microbiome and thus learn about potential drivers affecting the human microbiome. With the excellence-MOOC "Microbiome & Health" we aim to disseminate knowledge, but also the importance of microbiome research, especially considering all challenges we are facing during the Anthropocene epoch. As an open educational resource, it is freely accessible and can be shared with everyone, thus ideally suited to reach the widest possible audience.

[P144] COMBINED ^{2}H-NANOSIMS AND POPULATION-RESOLVED METATRANSCRIPTOMICS ELUCIDATE RAPID AND COLLECTIVE REACTIVATION OF A BIOCRUST MICROBIAL COMMUNITY

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Lack of water might be the most basic and universal factor that can limit soil microbial activity, which is essential for healthy soil ecosystems. Mechanisms of microbial desiccation tolerance and resuscitation upon increased water availability have been mainly studied in few cultured organisms, not representative of the dominant taxa in soils. Thus, key information such as the dynamics and underlying physiologies of reactivation and the number of cells that can revive in soils is lacking.
Alternating phases of microbial activity and dormancy are most pronounced in desert soils, where microorganisms revive fast during rare rain events. We used biocrusts from the Negev Desert, Israel, to investigate changes in gene transcription of individual microbial populations and single-cell biomass generation during a controlled rehydration experiment. The biocrusts were rehydrated with heavy water, and deuterium incorporation (a general marker of anabolic activity) was measured by nano-scale secondary ion mass-spectrometry (NanoSIMS). With this approach, we detected biomass production in >90% of cells only few hours after rehydration, calculated biomass generation rates and estimated replication times of individuel cells. We used previously generated metagenome-assembled genomes (MAGs) to split and normalize the metatranscriptomes by populations and analyzed the individual transcriptional responses of different community members in a highly resolved time series spanning 15 minutes to 55 hours. Normalization of transcripts per population allowed to detect transcriptional patterns otherwise hidden in the bulk metatranscriptome data. Significant differential expression of genes as soon as 15 or 30 min upon hydration demonstrated rapid and collective reactivation of the microbial populations, independent of their taxonomy or encoded physiology. We observed distinct temporal phases of cellular processes, beginning with DNA repair powered by storage compound oxidation and only later followed by uptake of external carbon sources and resumption of main metabolism. Among many other insights, we were able to resolve distinct transcription patterns of two closely related hydrogenases, suggesting the potentially different roles of atmospheric hydrogen oxidation in different microbial populations.
By combining single-cell activity measurements and population-resolved metatranscriptomics, we obtained first detailed insights into resuscitation mechanisms and dynamics of typical abundant soil microbiota under in-situ conditions.

[P145] FEATURE EXTRACTION TECHNIQUES BASED ON DEEP LEARNING FRAMEWORK FOR ENHANCED CLASSIFICATION OF NON-CODING RNA

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The accurate identification and classification of non-coding RNA (ncRNA) are crucial for unraveling their functions and regulatory mechanisms in various biological processes, where understanding ncRNA roles can shed light on complex microbial interactions and their impact on ecosystems (Panni, S., Biochimica et Biophysica Acta (BBA)-Gene Regulatory Mechanisms, 2020). Traditional machine learning approaches have been employed for distinguishing ncRNA. However, these methods often necessitate extensive feature engineering and may be constrained by the accuracy of the selected features (Bonidia, R. P., Briefings in Bioinformatics, 2022). Recently, deep learning techniques have demonstrated considerable potential in improving classification performance for ncRNA (Chen, K., BMC bioinformatics, 2023).

In this study, we introduce a robust hybrid deep learning framework that integrates either Convolutional Neural Networks (CNN) or Long Short-Term Memory (LSTM) networks with external features to enhance classification accuracy. The framework employs a combination of one-hot encoding, k-mer embedding, and advanced feature extraction techniques to represent the input sequences. The features extracted are subsequently incorporated into the deep learning architecture, enabling the model to exploit both the spatial and sequential information inherent in the ncRNA sequences. The framework further benefits from using advanced training strategies, such as dropout and batch normalization, to mitigate overfitting and improve generalization.

To evaluate the performance of our proposed framework, we used both benchmark datasets and real-world laboratory RNA samples extracted with the Infernal tool (Nawrocki, E. P., Bioinformatics, 2009). The results demonstrated a high level of accuracy in classifying ncRNA, significantly outperforming existing methods. This indicates the effectiveness and robustness of our hybrid deep learning framework in handling complex ncRNA sequence data.

Our proposed framework paves the way for further investigation in the field of ncRNA classification and may contribute to a deeper understanding of the biological roles and functions of ncRNA. The successful integration of CNN or LSTM with external features offers a promising avenue for future research in the development of advanced models for ncRNA classification, ultimately enhancing our knowledge of the diverse roles that ncRNAs play in cellular processes and disease states.

[P146] SOIL FOOD WEB INTERACTIONS SHAPE MICROBIAL COMPOSITION AND ACTIVITY IN TEMPERATE DECIDUOUS FOREST

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Temperate forest soils play a crucial role in the biogeochemical cycling of nutrients, such as organic carbon. Decomposition of soil organic matter, a process mediated by microbial enzyme activities, is driven by various biotic and abiotic factors, as well as their complex interactions.
The aim of the study was to assess the effect of top-down (via predation by microbivore earthworms and predacious centipedes) and bottom-up (via inputs of organic and inorganic substrates) control of the soil food web on the structure of the microbial communities, their genetic potential and decomposition activities. Seven treatments in eight replicates were applied in mesocosms in oak-hornbeam deciduous forest soil in the Czech Karst (Czech Republic): additions of cellulose, nitrogen and phosphorus, earthworms (Lumbricus terrestris), centipedes (Lithobius forficatus) and selected combinations. Soil samples were collected 10, 28 and 115 days after the beginning of the experiment. In the samples, the extracellular activities of seven hydrolytic enzymes were measured using fluorogenic substrates. To determine a genetic pool of soil microorganisms after 10 days, shotgun metagenomic analysis was used. The metagenomic library was sequenced by the Illumina method, and the raw pair-end reads were filtered, assembled, and annotated to explore the taxonomic and functional features of each treatment.
Activities of most of the extracellular enzymes differed between treatments. Addition of earthworms/centipedes with or without nutrients stimulated activities of acid phosphatase, β-glucosidace, cellobiohydrolase, arylsulphatase and lipase at the beginning of the experiment; however, it had the opposite or insignificant effect after 115 days. On the contrary, the activity of chitinase was not altered after 10 or 28 days but was significantly reduced in the treatments with added macrofauna at the end. Analysis of soil metagenomes identified several taxonomic groups and genes which were enriched in individual treatments, suggesting their role in the soil communities during decomposition.
In conclusion, the findings contribute to the current understanding of complex relationships of the trophic cascade in the soil, emphasizing the importance of approaches investigating multiple factors.

[P147] RAMAN ASSISTED ACTIVE BACTERIAL CLUSTER ISOLATION

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Traditional microbiology relies heavily on isolation of pure cultures. However, this is only readily achievable for a small fraction of bacteria. Many keystone microbes are difficult or maybe even impossible to grow as pure culture. These microbes are involved in environmental processes, such as comammox bacteria, essential in potable water production via rapid sand filtration. Therefore, they are relegated to studies in enrichment cultures, which limit our ability to study their ecophysiology. The presented study explores the possibility of sorting microbial clusters using Raman spectroscopy in a viscoelastically focused stream of microbial clusters within a capillary. This method allows acquisition of some taxonomic information on the members of the clusters and/or on their activity via isotope labelling. Based on researcher defined criteria, microbial clusters can be subsequently captured and sent for further analysis or cultivation. This approach aims to enable selection of a microbe of interest together with a set of accompanying bacteria, forming a tight collaborome community. Study of such collaboromes takes another step beyond traditional pure culture based microbiology, which does not reflect the typical lifestyle of microbes, and enables broader exploration and application of microbial assemblies. We are presenting experimental data from deuterium activity measurement using Raman microspectroscopy in a flow focusing capillary system and the potential for broad application.

[P148] GENOME-MINING FOR BACTERIOCINS ALLOWS FOR QUICK IDENTIFICATION OF BIOPROTECTIVE LACTOBACILLUS STRAINS

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Ribosomally synthesized bacteriocins are a versatile group of peptides with antimicrobial properties. In food applications, bacteriocins produced by lactic acid bacteria are used to reduce food waste by protecting against spoilage agents and pathogens. However, identifying relevant strains with bioprotective abilities can be a time-consuming process, and as time is money, for industrial application, a quick assessment of the ability of various strains to produce functioning bacteriocins is desirable. Here, we explore an automated in silico genome-mining approach for characterizing the bacteriocin diversity in several Lactobacillus species. Different computational tools, namely Bagel4, AntiSmash 6.0 and Gecco, were tested for their capability to detect bacteriocin gene clusters. The resulting predictions were then correlated with screening results from in vitro inhibition assays for validation. Through pangenome-based clustering, it could be shown that the type of bacteriocin genes that were harbored by the strains strongly correlated with their taxonomy, as well as strain similarity. Strains that inhibited an indicator strain in vitro always had at least one bacteriocin predicted. However, the results suggested that in many cases there was no inhibition of the indicator strain, despite the prediction of bacteriocin gene clusters from the genome sequence. These findings highlight that, when screening only in vitro, strains harbouring a bacteriocin gene cluster might be missed, when the “right” conditions for bacteriocin gene expression are not met or a non-sensitive indicator strain is used. Possession of bacteriocin gene clusters was species-dependent, and the pangenome-based clustering showed that within a species, predictions of bacteriocin production were correlated with strain similarity. By combining the in-silico predictions with experimental assays, we lay the groundwork for quick identification of strains with bioprotective activity. Furthermore, by applying an automated genome-screening first, a more targeted in vitro screening could be realized in the future. As similar strains are expected to have similar in vitro screening results, it might not be necessary, to screen every strain, thus saving resources and energy.

[P149] OPTIMIZING TOTAL RNA APPROACH FOR ELUCIDATION OF THE ACTIVE MICROBIAL COMMUNITY OF AGRICULTURAL SOIL

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Bacteria, fungi and microeukaryotes live closely together in soil and rhizosphere. Thus, a holistic approach is needed in order to study the active microbial community within these samples. One such is the Total RNA approach, in which RNA converted to cDNA is sequenced. Following, reads are sorted as rRNA and mRNA, and then assembled into contigs and assigned taxonomy or function. Since specific primers are not used, this approach reveals the taxonomy of the active organisms across all kingdoms. However, the Total RNA approach first of all depends on getting RNA of sufficient quality and quantity from the samples. For rhizosphere, especially, sample volume may be low.

Aim: The aim of this study was therefore to optimizing the Total RNA metatranscriptomic approach in all its steps from RNA extraction, sequencing, and bioinformatics pipeline.

Methods: we tested four commercially available RNA extraction kits. For this, RNA was extracted from 0.2 g lyophilized soil using the Soil Total RNA Purification Kit (Norgen), AllPrep PowerFecal DNA/RNA Kit (Qiagen), FastRNA Pro Soil-Direct Kit (MP Biomedicals), and NucleoBond RNA Soil Kit (Macherey-Nagel) with five technical replicates each. Successful library building was done with the NEBNext RNA Library Prep Kit for Illumina (New England BioLabs) on RNA extracted with the Macherey-Nagel and Norgen kits. These were then sequenced on Illumina NextSeq platform. Further, the 'Macherey-Nagel' libraries were sequenced at different depths (yields 2, 5, 10 or 15 Gb) in order to investigate the depth required to cover the full diversity of the active microbial community within the samples. The sequenced reads were sorted with SortMeRNA. Following the SSU reads were assembled and annotated using MetaRib and CREST, while the non-rRNA reads were assembled and annotated using the CoMW pipeline.

Results: the RNA extraction kit from Macherey-Nagel was by far the most efficient, followed by the Norgen kit, while the Qiagen and MP Biomedicals yielded too little RNA to produce libraries for sequencing. The assembly and annotations showed that the app. 10Gb was the most optimal yielding the highest number of contigs and taxa, while 15Gb did not increase these values.

Conclusions: while Total RNA has been used to study the microbial diversity in a limited number of environments, this approach is still in its teens and as such in need of further development. Here we provide a much-needed optimization of the approach at all its steps from wet lab to dry lab.

[P150] HIGH-THROUGHPUT SINGLE-CELL METAGENOMIC SEQUENCING OF MICROBIAL COMMUNITY IN SEWAGE AND FECES

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Single cell sequencing from metagenomes can be a powerful complementary technique to shotgun metagenomics. It is an emerging technology that separates complex microbial communities into single individual bacterial cells and performs high-throughput sequencing of genetic material from the single bacterial cell within complex matrices. We developed and validated the use of microfluidics and semi-permeable capsule (SPC) technology (Droplet Genomics) to separate individual bacterial cells from sewage and pig feces [1]. Single bacterial DNA was extracted and amplified within individual SPC. Each SPC was then barcoded with split-pool method and used for Illumina sequencing. Single cell amplified genome (SAG) library was used to explore the bacterial community composition and distribution of associated antimicrobial resistance genes (ARGs) in such complex microbiomes in the single cell. In our study, we obtained 1738 SAGs library with at least 1e+5 reads per cell in deep sequencing and with 7971 SAGs library with at least 1e+4 reads per cell in shallow sequencing. For the 9709 SAGs library, bacterial species were identified using the Genome Taxonomy Database and acquired antimicrobial resistance genes were identified using the ResFinder database. Different computational methods and analyses were performed on the SAGs as a method for linking ARGs to their hosts directly from microbiome within the sewage and fecal samples. For the deep sequencing method with sewage sample, we are able to link 52 unique AMR genes within 47 species of bacteria out of 474 SAGs. These assembled genomes from deep sequencing in sewage were mostly belonged to species that were previously unsequenced or unidentified based on GTDB database. For the feces sample, one pooled SAG library with 129 Streptococcus alactolyticus SAGs was chosen to build clonal phylogenetic tree based on SNP differences between droplets showed the possibility of characterizing strain diversity within a species. Few challenges remain in the analysis and interpretation of single-cell meta genomics data due to the possible contamination with doublet cells, and the genome assembly quality of different samples that can be obtained through deep sequencing of fewer cells vs shallow sequencing of many cells within a sample.

[P151] THE MICROBIAL RESOURCE RESEARCH INFRASTRUCTURE - EUROPEAN RESEARCH INFRASTRUCTURE CONSORTIUM (MIRRI-ERIC) AND ITS POTENTIAL TO AID RESEARCH

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MIRRI-ERIC is the pan-European distributed Research Infrastructure for the preservation, systematic investigation, provision and valorizations of microbial resources and biodiversity. It brings together over 50 microbial Biological Resource Centres (mBRCs), culture collections and research institutes from ten European countries.
The mission of MIRRI-ERIC is to serve Bioscience and Bioindustry users by facilitating access to a broad range of high-quality bioresources and data in a legally compliant way. By offering access to human expertise and providing a collaborative platform for long-term sustainability of microbial biodiversity, MIRRI-ERIC will increase knowledge and promote professional development.
MIRRI-ERIC makes available a vast and diverse portfolio of high-quality services that are based on its partner organizations' facilities/equipment and expertise. MIRRI-ERIC's services and expertise can help researchers and bioindustries derive the maximum value and impact from their projects, technologies, and products. Services commonly provided by partner organizations include supply, deposit, or identification of microbial resources by gene sequencing, as well as advanced services such genomics, screening of metabolites, phylogenetic analysis, consultancy. MIRRI-ERIC is able to provide up to 95 services.
MIRRI-ERIC and its services thus present a unique opportunity for researchers in microbiology to advance and increase the value of their research.

[P152] MOLECULAR MECHANISMS FOR THE EVOLUTION OF GENE STRUCTURE AND ORGANIZATION: THE HISTIDINE CASE

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Aim: The origin and evolution of metabolic pathways represented a crucial event occurred during molecular and cellular evolution, rendering the primordial cells less dependent on the exogenous supply of abiotically formed molecules. The evolution of genes is the result of different molecular mechanisms, including point mutations, gene duplication, gene fusion, gene elongation, and horizontal transfer of external DNA. One of the most studied metabolic pathways is histidine biosynthesis, which shows a plethora of gene structures and organizations. Analyses of the structure of his genes revealed that these different molecular mechanisms played an important role in shaping this route. The aim of this work was to explore the molecular mechanisms that shaped metabolic pathways during evolution, using the histidine biosynthesis as a model.

Methods: A comparative analysis was performed to study the structure and organization of histidine biosynthetic genes in the Bacteroidota-Rhodothermota-Balneolota-Chlorobiota superphylum. The Bacterial Two-Hybrid (BACTH) system and super-resolution fluorescence microscopy were applied in Escherichia coli to investigate the possible physical interactions between histidine biosynthetic enzymes. The molecular mechanism of gene elongation was simulated using directed evolution experiments, subjecting E. coli cells to selective pressures.

Results: Data obtained from the comparative analysis highlighted a high variety of genes structures and organizations, allowing to suggest a possible model for the assembly of his genes in operons during bacterial evolution. The study of the compartmentalization of histidine biosynthetic enzymes demonstrated the in vivo interaction between HisF and HisH enzymes. Experiments performed in this work also allowed to mimic a possible gene elongation event occurred during the evolution of hisF gene. Finally, the HisF involvement in different cellular processes in the bacterial world was explored, suggesting its central role in cellular metabolism.

Conclusions: Results obtained from the proposed analyses could represent a further step towards the understanding of metabolic pathways evolution.

[P153] ROBUST AND VERSATILE ULTRA-HIGH-THROUGHPUT SINGLE-MICROBE GENOME SEQUENCING

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Whole-genome and targeted sequencing open a window to understanding the function of unculturable microorganisms. On one hand, metagenomic sequencing is attractive for its straightforward sequencing library preparation from bulk environmental samples but only offers limited resolution into individual species. On the other hand, single-microbe sequencing offers true single-clone resolution but can only meaningfully address the high biological diversity expected in environmental samples if performed on thousands of individual cells in parallel. To satisfy the need to study such large numbers of single-microbes per sample, well- and droplet-based approaches keep evolving in parallel to provide single-cell compartmentalization required during sequencing library preparation. However, these approaches suffer from a fundamental trade-off between throughput and versatility. Being individually addressable, microwells enable multi-step processing but are not scalable. Droplets offer a throughput of up to a million cells per experiment but only allow a limited number of processing steps to be performed. The latter limitation is severely felt went it comes to microbial research, as harsh conditions typically required for lysis are incompatible with downstream nucleic acid barcoding steps. Our Semi-Permeable Capsule (SPC) technology combines the throughput of droplets with the versatility of wells by enabling a virtually unlimited number of processing steps on genetic material from millions of individual microbes in parallel. We demonstrate the use of SPCs for barcoding >100,000 individual microbial genomes to obtain single-microbe whole genome sequencing data of unprecedented quality.

[P154] FULL-LENGTH 16S RRNA GENE SEQUENCING COMBINED WITH GTDB DATABASE IMPROVED THE DESCRIPTION OF MICROBIAL COMMUNITIES FROM MARINE POLAR ENVIRONMENTS

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The description of natural microbial communities by culture-independent methods is shifting from second- to third-generation sequencing. Even though third generation sequencing has been shown to provide near-perfect description at species level, this validation has been made in laboratory settings with simplified communities composed of well described species [1]. This resulted in a recent surge in studies comparing second- and third-generation sequencing approaches in natural environments. However, to the best of our knowledge, such methodological studies have not been performed in the north Atlantic/Arctic Ocean interface, which represents an underexplored environment, with novel diversity.

We hypothesized that full-length 16S rRNA gene sequencing, enabled by third-generation sequencing, can improve the effectiveness of taxonomic classification relative to short-regions of the same gene with second-generation sequencing. We also hypothesize that taxonomic database selection is relevant in the effectiveness of taxonomic classification of underexplored environments.

To test our hypothesis, we sequenced the V4-V5 region of the 16S rRNA gene with Illumina and the full-length 16S rRNA gene with circular consensus sequencing (CCS) PacBio, to characterize prokaryotic communities from Arctic seawater. Samples were collected at the transition between the north Atlantic and Arctic Ocean, during the Norwegian Polar Institute Monitoring Cruise and the Environmental Monitoring of Svalbard and Jan Mayen (MOSJ) campaign. Then, denoised, high-quality reads were classified with two different taxonomic databases – Silva and Genome Taxonomy Database (GTDB).

We found that combining full-length sequencing of 16S rRNA genes with GTDB resulted in highest taxonomic classification efficiency. In fact, more than 50% of amplicon sequence variants (ASVs) were classified down to species level. In contrast, the Silva database was able to classify less than 10% of ASVs down to species level, independently of sequencing strategy.

Alpha diversity was similar between combinations of sequencing strategy and taxonomic database, except for full-length 16S rRNA gene sequencing with GTDB, which resulted in an improved detection of the microbial rare biosphere. For beta diversity, we expected community structure to be shaped by water masses and region

(e.g. [2]), which was confirmed by all combinations tested, but more clearly with the full-length sequencing approach, together with the Silva database.

In conclusion, the most complete description of the microbiomes in this underexplored environment was obtained by combining full-length 16S rRNA gene sequencing with GTDB. However, at phylum level, all combinations were equivalent.

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[P155] BACTERIAL COMMUNITIES IN SURFACE SEDIMENTS FROM LAKE VILLARRICA IN SOUTHERN CHILE

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Lake Villarrica, one of the main freshwater ecosystems in Chile, was recently declared a nutrient-saturated lake due to increased nitrogen (N) and phosphorus (P) levels. Although a decontamination plan based on environmental parameters is being established, it does not consider microbial parameters. Here, we used high–throughput DNA sequencing (HTS) and quantitative polymerase chain reaction (qPCR) approaches to investigate the composition and functional genes involved in P and N cycling of bacterial communities in surface sediments with contrasting anthropogenic pressures from the nutrient-saturated Lake Villarrica. Analyses showed that Proteobacteria, Bacteroidetes, Acidobacteria, and Actinobacteria dominated the community. In this sense, members of these phyla have been proposed as suitable environmental indicators of anthropogenic impact on lakes. Alpha diversity analysis revealed a high bacterial richness and diversity in the more anthropogenized sediments. Principal coordinate (PCoA) and redundancy analysis (RDA) showed significant differences in bacterial communities between sampling sites according to their geographical closeness. Analyses of larger predicted functional activities revealed that N cycling functions (e.g., nitrification and denitrification) were significant. Studies have proposed that the microbial respiration reactions in sediments appear to be influenced by the trophic status of the lakes. The microbial co-occurrence networks analysis suggested Chitinophagaceae, Caldilineaceae, Planctomycetaceae, and Phycisphaerae families as keystone taxa. Bacterial functional genes related to N (nifH and nosZ) and P (phoC, phoD, and phoX) cycling were detected in all samples by qPCR. In addition, denitrifying gene (nosZ) was the most significant factor influencing the topological characteristics of co-occurrence networks and bacterial interactions. Denitrifying bacteria, as well as anammox bacteria, in sediment samples, were highly relevant in sediments of Lake Villarrica, as they not only to release N2 to the atmosphere but also are keystone taxa for microbe-microbe interactions. Our results represent one of a few approaches to elucidate the structure and role of bacterial communities in Chilean lake sediments, which might be helpful in conservation and decontamination plans.

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[P156] OCCURRENCE OF ANTIBIOTIC-SENSITIVE BACTERIA ON TROPICAL PEAT SWAMP SOIL

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Changes in natural ecosystems, such as land conversion for agricultural use including a residential area, resulted in the turning of microbial population dynamics. Many antibiotics are currently used for treating infections that are not only for humans but also for veterinary therapy. One of the large conversions of natural forest is the Indonesian tropical peat swamp area, Giam Siak Kecil – Bukit Batu (GSKBB) in Riau Province. This work indicated the existence of resistant bacteria to various antibiotics in that Indonesian biosphere reserve, due to some part of this area having been converted. The presence of antibiotic-resistance bacteria in peat swamp soils was investigated with microbial culture analyses for isolation and antimicrobial susceptibility test, and PCR methods to reveal the species and the presence of antibiotic-resistant genes (ARGs). Moreover, a prevalence rate of tetracycline associated with tropical peat swamp natural peat swamp soil microorganisms was identified using the next-generation sequencing (NGS) method. From antibiotic culture, media showed that natural forest has the highest diversity of culturable antibiotic-resistance bacteria. The molecular assessment showed that these culturable antibiotic-resistant bacteria were Escherichia sp. and Kocuria rhizophila (isolated from a natural forest), Paenibacillus terrigena (the community residence area), Roseomonas sp. (the restoration area), as well as Escherichia coli and Bacillus sp. (the rubber plantation). The phylogeny revealed that these isolates are genetically distinct and distributed in different clade. Further analyses for antimicrobial susceptibility test using MICTM strip (Himedia) revealed that the minimum inhibition concentration of these isolates among 6 – 16 µg/ml for amoxicillin, 0.38 – 6 µg/ml for tetracycline, and 0,38 – 2 µg/ml for vancomycin. PCR methods showed no indication the isolates had ARGs. Although the bacteria of peat swamp soils that isolated from antibiotic culture media, the MIC was still lower than CLSI guidelines and sensitive (S) criteria based on ECOFFs (EUCAST). Further investigation, the molecular assessment using NGS showed that there were changes in the microorganism community due to the disturbance of existing antibiotics tetracycline (5 mg/L – 20 mg/L). PCA analyses showed that three soil samples with and without tetracycline addition were clustered individually. UPGMA analyses revealed that there was the genus of Proteobacteria, Actinobacteria, Firmicutes, Acidobacteria, Euryarchaeota, Chloroflexi, Thaumarchaeota, WPS-2, Planctomycetes, Diapherotrites and others with different composition. We concluded that Indonesian tropical peat swamp soil microorganisms have MIC values lower than CLSI guidelines and sensitive criteria based on ECOFFs (EUCAST) and may be taught with antibiotics tetracycline.

[P157] HARNESSING THE INTRAGENOMIC VARIABILITY OF RRNA OPERONS TO IMPROVE DIFFERENTIATION OF VIBRIO SPECIES

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Background and Aim: Among the bacteria used as environmental indicators in monitoring marine and aquatic environments, species in the bacterial genus Vibrio are of particular interest due to their varied and important ecological roles and impacts. Although 16S rRNA gene is frequently used as a phylogenetic marker to analyze the microbial diversity in aquatic systems, the use of this marker in analysis of environmental DNA often fails to reveal individual species including those of the Vibrio genus. The aim of this study was to reveal the intrinsic weaknesses of 16S rRNA gene that limit its implication in the differentiation of Vibrio spp. and test the capacity of 23S rRNA gene to overcome these limitations.

Methods: Here we used the custom and public repositories of rRNA gene sequences to create a compilation of 16S and 23S rRNA gene sequences. After their alignment using MAFFT (Version 7.490) and curation using the full genome sequences previously retrieved from NCBI GenBank, some aligned sequences were manually trimmed in MEGA-X at their 5'- and 3' extremities to ensure uniform length of sequences flanking the conserved rRNA regions. These were then used to construct phylogenetic trees in IQTREE (Version 2.1.3).

Results: First, we construct a phylogenetic tree by using 16S rRNA sequences retrieved from 40 completely sequenced Vibrio genomes. We evaluate whether nucleotide polymorphism within 16S rRNA loci of single Vibrio species genomes might cause polyphyly and taxonomic ambiguity. Further, we identify how variation at particular nucleotide positions in 16S rRNA gene can drive polyphyly during phylogeny reconstruction and demonstrate the role of these positions in determining tree topology. Finally, we demonstrate that an increase in the number of informative nucleotide positions by concatenation of 16S and 23S rRNA gene sequences makes it possible to address many of the ambiguities in 16S rRNA-based phylogenetic reconstructions and improves the differentiation of Vibrio species.

Conclusions: Thus, our work (i) defines the key sequence features that limit the potential of 16S rRNA gene to serve as a phylogenetic marker in discrimination of Vibrio species and (ii) assesses the capacity of 23S rRNA to improve species resolution in phylogenetic analysis. Our findings will help to improve the detection and identification of Vibrio species in complex environmental samples, thereby facilitating Vibrio monitoring in aquatic ecosystems.

[P158] BMD-SRA: A BOOSTING MODEL FOR DIFFERENTIATING SEQUENCE READ ARCHIVE SEQUENCES

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The number of sequence files deposited in the Sequence Read Archive (NCBI-SRA) has been growing exponentially through the years, and with it, the number of incorrectly annotated types of sequences. The submitted sequences are then used for genomic, metagenomic, and taxonomic studies. This presents a need in the research community for a model that facilitates the collection of correctly annotated data. This study aimed to develop a boosting classification model called BMDSRA that classifies input sequences into four sequence types: 1) Metagenomes, 2) Amplicons, 3) Single-Amplified Genomes (SAGs), 4) Isolated-Genomes.

For developing the Machine Learning (ML) algorithm, we gathered 3000 test samples for each sequence type respectively. Test samples were used for supervised ML. Metagenomes were collected from various metagenome databases (DBs) (Kasmanas et al., Nucleic Acids Research, 2020) (750 samples from each), manually curated, and created by our team. Amplicon samples were gathered from the Joint Genome Institute portal based on their library strategy. The SAG samples were collected by manually inspecting published research papers, proving they were sequenced from a single cell. The Isolated-Genomes were gathered from SRA, searching for bacteria-type strain Genomes from different taxonomies. The BDMSRA reads a small portion of the sequence file using a sub-sampling approach (SRA Toolkit Development Team, https://trace.ncbi.nlm.nih.gov/Traces/sra/sra.cgi?view=software) and extracts statistical features generated based on Shannon entropy, Tsallis entropy, and Fourier z-curve. The extracted features were evaluated using the QPFS method (Soheili et al., Scientific Programming, 2020), and the reliability of training data was tested with an outlier analysis.

From the 119 generated features, we chose 38 with the highest importance for developing the model. The outlier analysis showed that the SAG and Amplicon data sets were the most reliable, with few outliers. The outliers from Metagenomes and Isolated-Genomes were subjected to further manual investigation. The model was created and evaluated by using 5-fold cross-validation. The confusion matrix showed an overall accuracy of 92% (96% for SAGs, 95% for Amplicons, 92% for Metagenomes, and 85% for Isolated-Genomes). The false negatives from Isolated-Genomes classified as Metagenome (7.6%) and SAGs (5.9 %) are likely due to the wrong classification in the SRA. The false negatives from Metagenomes classified as Isolated-Genomes (6.7%) are potentially due to downloading process from our Dbs.

BMDSRA can help researchers verify that the sequences they submit or collect from public repositories are correctly annotated. Further, our tool could also select samples for metastudies and determine if sequence projects are well performed.

[P159] LIVE-FISH-FACS-DROPLET CULTIVATION: A STRATEGY TO EXPLORE RECALCITRANT ACIDOBACTERIA

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Antibiotics from few culturable microorganisms have prevented millions of deaths over the past many decades. While initially effective, their efficacy has dramatically decreased due to the emergence and spread of antibiotic resistance. As antibiotic resistant microorganisms are predicted to cause 10 million deaths by 2050, we are in dire need of novel antimicrobial molecules. The potential of natural microbiomes to produce diverse secondary metabolites (SMs) with therapeutic properties is an attractive solution to mitigate this crisis, and the abundant acidobacterial phylum has proven to harbor a vast repertoire of biosynthetic gene clusters encoding potentially bioactive SMs. However, most clades of the Acidobacteria are recalcitrant to cultivation, which impedes our ability to harness the potential of these SMs. To circumvent this limitation, we present a strategy aiming at coupling Fluorescence in situ Hybridization within living bacteria (live-FISH) with Fluorescence-Activated Cell Sorting (FACS) and droplet cultivation to selectively isolate these bacteria in order to gain access to previously uncultured and biosynthetically talented acidobacterial strains. Employing DNA probes hybridizing to the 16S rRNA of Acidobacteria subdivision 1 in a conventional FISH experiment allowed us to identify members of this group, including the uncultivable Angelobacter clade, from soil samples. Following this, we utilized Acidobacterium capsulatum as a model organism to demonstrate the feasibility of a live-FISH methodology targeting Acidobacteria. Despite a severe reduction in cell viability, we were able to improve cell survival through a modified hybridization approach. Separate from the Live-FISH procedure, we encapsulated A. capsulatum cells in minuscule droplets, and after three days of incubation, we observed proliferation, demonstrating the capacity of A. capsulatum to thrive in 200 pL-droplets. Further refinement of the live-FISH method and its integration with droplet encapsulation and cultivation will lead to targeted isolation of previously uncultured acidobacterial strains, allowing us to tap into the biosynthetic repertoire of this promising phylum.

[P161] COMPARATIVE GENOMICS IN ACTINOMYCETES

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The lack of financial incentive from the pharmaceutical industry has led to a stagnation in discovering new antimicrobial compounds, creating a severe threat to human health from multi-resistant bacterial pathogens. Advances in cost-efficient sequencing equipment and bioinformatics tools enable the identification of biosynthetic gene clusters with desirable properties, leading to the discovery of new secondary metabolite compounds and producer strains from Actinomyces. Comparative genome-mining approaches aim to identify candidates for future antibiotics drug development and other bioactive metabolites from a database of 1000 newly generated whole genome sequenced strains.

Trans-AT polyketides are a group of polyketide compounds that are synthesized by modular polyketide synthases (PKSs) that unlike classical- or cis-PKSs, which are organized as colinear and multimodular enzymes, use separate modules that are brought together by trans-acting acyltransferase (AT) enzymes to form the final polyketide product, which allows for greater structural diversity and the potential for unique bioactive properties. By predicting the domains required for synthesizing these interesting trans-AT PKSs it is possible to isolate the BGCs from the producing strain and test the properties and underlying mechanisms of these very special compounds.

[P162] NEW STRATEGY FOR THE SIMULTANEOUS ANALYSIS OF BENTHIC MICROBIAL AND MEIOFAUNAL COMMUNITIES: EDNA EXTRACTION AND MOCK COMMUNITIES

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Benthic prokaryotic communities (organism size usually ranges from 0.02 to 1 µm) are of utmost importance for the functioning of wetlands and marine environments. They are influenced not only by physical-chemical parameters but also by their interactions with other benthic communities such as micro-eukaryotes (average organism size: 10 µm) and meiofaunal invertebrates (organism size between 45 µm and 1 mm). It is thus of paramount importance to adopt a holistic approach, analyzing all members of the benthic community, to fully understand their organization and functioning. However, meiofaunal representative environmental DNA (eDNA) is difficult to obtain because of their low abundance at micro scale, which is an important limitation to such holistic approaches. The aim of this study is to propose a new strategy for the simultaneous analysis of microbial (bacteria, archaea and micro-eukaryotes) and meiofaunal (eukaryotes) benthic communities, which includes DNA extraction and mock communities.

The developed strategy combines two DNA extractions: one for micro-organisms, using 0.25 g of sediments, and one for meiofauna, using 0.25 g of the sieving refluxes from 5 g of sediments. Mock communities were used to evaluate the DNA extraction strategy biases. These mock communities included organisms from the three domains of life: bacteria, archaea, and eukaryotes (micro-eukaryotes and meiofaunal invertebrates). The DNA extraction strategy was applied using three conditions for the mock communities: (i) varying eukaryotes abundance, (ii) salted or fresh meiofauna and (iii) the presence or not of sediments.

The DNA extraction strategy allows to detect most organisms present in the mock communities. The comparison of salted and fresh communities showed that meiofaunal microbiota affect microbial community composition. Finally, comparing the mock communities with and without sediments demonstrated that sieving does not significantly affect both microbial and meiofaunal communities.

The use of mock communities has allowed to validate the developed DNA extraction strategy showing that it can be used to successfully analyze microbial and meiofaunal communities' eDNA from a unique sample. The use of this strategy on environmental samples will be helpful to further understand the interactions of meiofaunal invertebrates and microbes in benthic environments and thus the functioning of these ecosystems.

[P163] SOUTHERN OCEAN MICROBIAL ECOGENOMICS: A GENOMIC ATLAS HIGHLIGHTING SOUTHERN OCEAN'S DIVERSITY AND SINGULARITY

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The Southern Ocean (SO) plays a major role in global climate regulation. It is submitted to extreme seasonality and temperatures, and hosts some of the most productive marine biomes. However, most SO planktonic ecosystems remain largely uncharacterized and existing surveys indicate the existence of most novel genes compared to other marine provinces. The high susceptibility of the SO to climate change pushes towards the need for a reference atlas of these planktonic ecosystems and their genomic content. Here, we present insights from the Antarctic Circumnavigation Expedition: the largest metagenomics sampling effort ever achieved in the Southern Ocean, filling a major gap in the global ocean microbiome analysis. We characterized the SO specificity through the analysis of its microbial diversity and function at genome-level.

We analyzed 218 metagenomes sampled at 30 stations from the Antarctic Circumnavigation Expedition (2016-2017 austral summer), at sampling depth ranging from 4 to 3460m and filtered for attached and free-living prokaryotes. We evaluated metagenome-assembled-genomes (MAGs) content against recently published marine prokaryote reports to highlight the SO unicity and the functional novelty of this planktonic life. We identified 175 million genes and reconstructed 11,938 prokaryotic MAGs representing 1478 different species and analyzed their distribution in the SO to define ecoregions and identify driving abiotic and biotic parameters. We found that SO water masses, latitude and water-depth were shaping the microbial community's distribution at a genomic and functional level. This unprecedented catalogue of Southern Ocean genomes and genes was annotated using state-of-the-art functional and metabolic databases and showed a potential of 661 new species and 77% novel genes. In particular, we studied the genomic adaptations to the SO of lineages of interest, such as the Polaribacter genus, and through functions of interest, such as iron acquisition. This wealth of new genomic diversity from a circum-Antarctic track constitutes an important contribution to advance our knowledge on marine microbial life and can now be used both as a reservoir of novel enzymes sequences for biotechnological applications and as a reference to evaluate future climate related biodiversity changes in this highly sensitive region.

[P164] OVERCOMING OBSTACLES IN MICROBIOME ANALYSIS: LESSONS FROM FECAL AND SPUTUM STUDIES

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Technological advancements have greatly accelerated the study of the microbiome, enabling us to investigate correlations between it and its host. However, collection and/or isolation methodology and analysis pipelines can significantly skew results. Accordingly, it is essential to use models that can account for relevant covariates to be able to achieve global generalization of associations found.

In one study, we investigated the effects of different storage and DNA extraction methods on the composition of the gut microbiome. We compared it to true biological signals such as intraindividual variability from diet, health, and demographics. In another study, we examined the sputum microbiome of 200 adults stratified by HIV and COPD, comparing it with a similar cohort from the UK to assess the generalizability of findings.

Our results highlighted the importance of explicitly analyzing and accounting for confounding factors in microbiome studies. We demonstrated that DNA extraction methods strongly and significantly impact observed microbiome variability (Wilcoxon sum test, $P < 0.001$). Even more interesting, when analyzing low biomass sputum samples, which can be contaminated with host reads. These amplified host DNA sequences were clustered and falsely assigned to bacterial taxa during the preprocessing, leading to false microbiome signatures of various health and nutritional factors.
Moreover, we could demonstrate, by systematic confounder analysis, that it is possible to disentangle the effects of HIV status, antiviral treatment, medication and lifestyle on the sputum microbiome, giving us the tools to understand the effects of treatment, disease and environment on the microbiome as contrasted to those of batch and individual idiosyncrasy.
In summary, our studies demonstrate the need to consistently consider potential pitfalls in microbiome analysis, including the impact of methodology, sample type, and confounding factors. We highlight the importance of using sufficiently complex models to account for covariates and achieve global results generalization. Our findings also emphasize the significant impact of DNA extraction methods on observed microbiome variability and the influence of misamplification with host DNA on microbiome signatures underscoring the need for careful consideration and standardization of technical factors in microbiome research.

[P165] GSPREADCOMP, OPEN-SOURCE USER-FRIENDLY WORKFLOW, SHOWED PLASMID-MEDIATED HORIZONTAL TRANSFER ENRICHMENT OF ANTIMICROBIAL RESISTANCE IN VEGANS AND HIGHER VIRULENCE IN KETOGENIC-RELATED BACTERIA

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Plasmid-mediated conjugation (PMC) is one of the most impactful mechanisms of Horizontal gene transfer (HGT) (Johnston, Nature Reviews Microbiology 2014). Specifically, PMC can make pathogenic bacteria resistant to antimicrobials limiting treatment options. We developed gSpreadComp to detect PMC in entire microbiomes without reference genomes reducing the high-cost barrier of comparative genomics for non-bioinformaticians looking for ecological insights in HGT.
The gSpreadComp is a modular workflow integrating genome quality estimation, taxonomic assignment, plasmid and pathogen bacteria identification, virulence factors (VF) annotations, gene spread, and plasmid-mediated HGT analysis. Optionally, antimicrobial resistance genes (ARGs) annotation is also in the workflow. gSpreadComp configures all dependencies and databases during installation. We use weighted average gene prevalence (WAP) and normalized mutual information to estimate spread. The Smillie (Nature, 2011) heuristic was used to identify HGT. We applied gSpreadComp to ARGs in human gut metagenomes with different diets (17 ketogenic, 158 omnivores, 10 vegans, and 44 vegetarians) and 45 ancient samples (1300-5300 years old) from the AncientMetagenomeDir (Yates, Scientific Data, 2021). We recovered metagenome-assembled genomes (MAGs, % completeness – 5* % contamination > 50) using MuDoGeR (Rocha, bioRxiv 2022) and used them as input for gSpreadComp. The workflow directly produces the metrics and figures to analyse the data.
Our dataset yielded 3659 MAGs dereplicated into 400 Operational Taxonomic Units. We annotated 356 unique ARGs from 24 resistance types. Glycopeptide and multidrug resistance are spread ubiquitously (WAP > 0.7) in all diet types. However, bacitracin resistance is statistically more prevalent ($p < 0.05$) in Vegans than in Ketogenic or Omnivores. Fosmidomycin is less prevalent in Vegans than in all other diets. In addition, phenicol resistance was more prevalent in ancient samples. Following, Bacteroidota phylum showed more VF in Ketogenic diet. Moreover, pathogens found in ancient samples seem to have less VF than in modern diets. Plasmid-mediated HGT at Family level from ARGs happens more often in Vegans, 24.3% of all potential plasmid-mediated HGT events, while Ketogenic had 11.7% and Ancient had the lowest (1.8%). Finally, modern diets had an average of 39.2 unique ARGs in highly pathogenic species and 12.1 in other species. Our study indicates potential enrichment of specific ARGs in different diets and a concerning high resistance level from pathogens. It shows a higher risk of ARGs plasmid-mediated HGT in Vegans and higher virulence in ketogenic-related bacteria. The gSpreadComp can be used in any other system and help design strategies to control plasmid-mediated transmission.

[P166] "EXPLORING THE ORIGINS AND EVOLUTION OF LIVESTOCK PATHOGENS THROUGH METAGENOMIC ANALYSIS OF ANCIENT DNA FROM SOUTHWEST ASIA

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In recent years, progress in Next Generation Sequencing (NGS) and DNA extraction techniques has enabled the retrieval of numerous ancient human pathogen genomes from archaeological remains. However, the recovery of ancient livestock pathogen genomes has been limited. The domestication of these livestock during the Neolithic era in Southwest Asia led to increased human-animal interactions and increased the risk of zoonotic disease transmission between animal herds and humans. Our research aims to obtain pathogen and animal metagenomic ancient DNA from various livestock species and timelines, with a particular focus on 10,000-year-old sheep and goat material from the Zagros Mountains in Iran, a region associated with some of the earliest evidence of goat herding and zoonotic brucellosis in humans. Here, we present a metagenomic approach to recover pathogens and apply it to screening data from ancient livestock, along with preliminary evidence of co-infection in ancient sheep metagenomes. Recovering livestock pathogens from the earliest stages of domestication could lead to understanding their impact on the evolution and spread of zoonoses within livestock populations and across species barriers.

[P167] SPATIALLY RESOLVED MULTI-OMIC LANDSCAPE OF THE ANIMAL GUT MICROBIOME

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Spatial organization is a pervasive feature of natural ecosystems, and the animal gut is no exception. Hidden within animals is a collection of bacteria associated with the intestinal tract, organized in a complex structure that changes alongside variations in the gut habitat. While much has been done to explore the large-scale diversity of the gut microbiota, the fine-scale spatial organization of this complex ecosystem remains largely unexplored. Current approaches used to study the microbiota from fecal material or intestinal contents require homogenizing the biological material. In consequence, these methods are unable to reveal the gut microbial biogeography and fine-scale spatial structure of microbial communities. Due to this lack of resolution, we are missing relevant information, which could contribute to understanding the mechanistic underpinnings of microbial interactions.

In the emerging field of spatial omics, 3D'omics aims to generate spatially resolved multi-omic information by embedding and processing samples in ways that preserve their 3D relationship, structure and biomolecular integrity. Here, we use a monogastric terrestrial model, namely poultry, to analyze the effect of pathogen colonization in the shaping of the gut microbiota and carry out multi-omic analyses on decreasing sample sizes to find the lower limit of detection.

Salmonella infantis is the most common pathogen isolated from broiler chickens and is difficult to eradicate from myriad farms. As such, understanding how S. infantis impacts the poultry gut landscape is pertinent. To apply the 3D'omics pipeline to this question, intestinal samples from healthy vs Salmonella infantis infected chickens were sub-segmented and cryosectioned longitudinally (proximal to distal), and microdissected across the lateral axes using laser capture microscopy to obtain spatially referenced microsamples for downstream analyses. We compared the diversity of bacteria using 16S rRNA gene sequencing in these gut environments and directly observed the depletion of specific taxa during S. infantis colonization. We also employ fluorescence in situ hybridization (FISH) and confocal microscopy to visualize bacterial taxa at a single-cell level as well as metabolomics using LCMS to associate microbes and metabolites to gain further insights into structuring of gut communities. Synthesizing the information generated here will allow us to move towards spatially referenced metagenomics and metatranscriptomics to probe the effect of Salmonella infections on the 3D'omic landscape in the chicken gut. Lastly, such a pipeline is not restricted to just studying poultry microbiota. Future aims of the project involve the expansion of 3D'omic techniques to study larger livestock such as swine.

[P168] RECORDING MICROBIAL SIGNALS IN SOIL MICROBIOMES

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Background: Microbes reside in complex microbiomes, where secreted specialized metabolites play a major role in interspecies communication. However, investigating the role of specialized metabolites in microbial signaling is currently limited to methods that are disruptive and affected by detection limits.
Aim: The aim of our research is to engineer soil-dwelling microbes capable of recording the presence of bioavailable specialized metabolites in situ. This approach provides an ultrasensitive, non-disruptive alternative to existing methods, which additionally allow for both spatial and temporal investigations of signals in situ. The sensors function by inferring an irreversible genetic switch in the presence of the metabolite in question, which is catalyzed by a tightly controlled integrase.
Methods: Our whole-cell biosensors are constructed using state-of-the-art cloning techniques and tested for their sensitivity and specificity using flow cytometry. Escherichia coli is used as host organism for testing purposes and successful constructs are subsequently transferred to their final host destination, Pseudomonas putida KT2440.
Results: We have developed a handful of different sensors responding to pico-nanomolar concentrations of ecologically relevant metabolites. Each sensor has been tuned to avoid leaky expression in the absence of inducing metabolite in E. coli. Sensor sensitivity and responsiveness was analyzed with flow cytometry to generate response curves. Additionally, we have transferred one of our sensors to P. putida KT2440 and determined its effectiveness.
Conclusion: Whole-cell biosensors hold an immense potential to detect and record signals in natural environments. They are highly tunable genetic devices that serve as a promising alternative method to in situ investigations of natural products.

[P170] REMICULT: A BIOLOGICAL, VECTOR-BASED METAGENOME ENGINEERING APPROACH TO CULTURE ENVIRONMENTAL MICROBIAL DARK MATTER

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Microbiologists have only obtained <1% of Bacteria (and Archaea) species in pure cultures while the remaining species have aptly been coined as the 'Microbial Dark Matter' (MDM). Evidently, accessing MDM culturability will pave the way for new avenues in microbial ecology and biotechnology, and we believe one reason to this unculturability is generation of toxic reactive oxygen species (ROS) at laboratory conditions. Many environmental communities experience low fluxes of electron donors, thus generating low amounts of ROS. By contrast, laboratory conditions present high fluxes of electron donors, leading to high generation of ROS, while simultaneously isolating members from one another. We hypothesize that some environmental community members lack ROS defenses. Thus, any attempt to culture said members fails at standard conditions as ROS accumulates and eventually hampers growth.

AIM: The aim of the Remicult project is to develop a biological, gene delivery system for metagenome engineering of environmental communities. Through application of the system, we intend to remediate culturability of members that lack ROS defenses. This delivery system employs vector constructs that harbor genes for ROS-scavenging (H_2O_2) and a fluorescent marker (gfp) for cell sorting. As a first step, we set out to design and construct vectors, and evaluate the phenotypic effects in model strains.

METHOD: First, we set up a model system to determine the minimal inhibitory concentrations (MIC) of H_2O_2 to four E. coli MG1655 strains: (i) a wild type (wt), (ii) a catalase HPI deficient mutant (ΔkatG), (iii) a catalase HPII deficient mutant (ΔkatE), and (iv) a double mutant (ΔkatE/ΔkatG). Then, shuttle vectors were constructed, harboring an operon insert with a gfp gene fused to a katG gene, controlled by a lac promoter. Control vectors lacking katG were likewise constructed. Inserts were subsequently transposed onto a broad-host vector backbone via a Tn7 transposon system, and the phenotype of vector-carrying strains were evaluated with respect to transconjugation efficiencies and recovered growth at H_2O_2 MIC concentrations.

RESULTS: We have now verified the DNA sequence of the broad host katG vector and control vector. Furthermore, we have observed recovered growth along fluorescent signal in complementation experiments of mutants harboring the katG vector at H_2O_2 MIC concentrations. In E. coli-to-E. coli filtermating experiments, followed by fluorescent cell sorting, we also observe comparable transfer efficiency between katG vector and control vector.

CONCLUSION: The RemiCult project is thus mature enough to venture in to remediating culturability of the proposed (unculturable) MDM species from various complex natural communities.

[P171] CLUSTERING, DECONTAMINATION AND ASSEMBLY OF NON-TARGETED SINGLE-CELL SEQUENCING DATA FROM ENVIRONMENTAL SAMPLES FOR STUDYING THE SPREAD OF MOBILE GENETIC ELEMENTS

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The study of microbial interactions with the aim of tracing the spread of mobile genetic elements in highly complex environmental samples, such as sewage, could benefit from taking a high-throughput and non-targeted approach to single-cell genome sequencing. With this approach, we encapsulate all cells and particles from the environmental sample into droplets, and amplify, barcode and sequence all the extracted DNA [1], yielding partial genomes from thousands of droplets. However, the downstream data analysis becomes muddled by the unknown microbes present in the sample and requires a novel workflow to be able to co-assemble sequencing reads from the individual droplets sharing the same taxonomy. We created a set of in silico methods for 1) the read-based taxonomical annotation of droplets, 2) removing presumptive doublets and clustering the remaining droplets via network-analysis of their pairwise average-nucleotide identity (ANI) and 3) co-assembling, quality checking and annotating the draft genomes. We single-cell sequenced particles from a sewage sample from Bangladesh collected in 2018. After demultiplexing, 799 droplets had enough sequencing reads for the downstream analysis. Using the Genome Taxonomy Database, 50 different bacterial species were identified in the sample, with the most abundant members of the community belonging to the species Exiguobacterium profundum_A, Acinetobacter junii, A. harbinensis, Chryseomicrobium excrementi and Rhodococcus erythropolis. 127 droplets could not be typed and thus have the potential to be members of novel species. The ANI-based clustering formed 28 clusters, of which 10 could be co-assembled into draft genomes, in some case, from as few as six droplets. The completeness of the draft genomes ranged between 9.56% to 100%, with contamination estimated to be less than 1%. Of these, six mid- and two high-quality draft genomes could be used to examine the population structure of their species. Moreover, antimicrobial resistance genes (ARGs) were predicted in contigs from plasmids in different clusters and could be traced back to their host cells. This allows the study of the dynamics of spread of ARGs between bacterial sub-populations.

1. Ling et al, “High-throughput Single-Cell metagenomic sequencing of Microbial Community in Sewage and Feces”, Conference abstract, 16th Symposium on Bacterial genetics and ecology, Copenhagen, 2023

[P172] NATIONAL RESEARCH DATA INFRASTRUCTURE FOR THE RESEARCH OF MICROBIOTA (NFDI4MICROBIOTA) PROVIDING INFRASTRUCTURE, TOOLS AND METHODOLOGIES

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Introduction: Recent technologies like high-throughput molecular sequencing led to the generation of large data. However, the use and re-use of this data has failed to exploit its potential. The NFDI (National Research Data Infrastructure) wants to change this by developing a comprehensive research data management, encompassing different consortia. NFDI4Microbiota aims to facilitate the digital transformation in the microbiological community (bacteriology, mycology, virology, and parasitology). Providing access to data, analysis services, training and standards.

Aims: Central for the NFDI4Microbiota consortium is the development and provision of the computational infrastructure and analytical workflows required to store, access, process, and interpret various microbiome- and parasitology-related data types. NFDI4Microbiota works on developing and implementing software and standardized workflows for users to analyze their own datasets (i.e., for quality control, data processing, statistical analyses, and visualizations of different data types and results).

Methods: The German microbial research will be engaged through training and community building activities, and by creating a cloud-based system that will make the storage, integration, and analysis of microbial data, especially omics data, consistent, reproducible, and accessible. So, NFDI4Microbiota will promote the FAIR (Findable, Accessible, Interoperable and Re-usable) principles and Open Science.

Results: NFDI4Microbiota consists of ten well-established partner institutions, is supported by five professional societies and more than 50 participants. Several workshops and training events for the community have already taken place and more will follow. Moreover, the consortium launched an ambassador program to connect with the participants, thereby helping to identify the needs of their local community. Technical solutions are developed, tested and refined in several use cases from different fields of microbiology. All relevant information and specific services are made available via the web portal.

Conclusion: Producers and users of data will benefit from FAIR data (Findable, Accessible, Interoperable and Re-usable) more likely to be cited and integrated into a wider microbial inquiry. The current data parasitism would shift to a future data mutualism benefiting all partners. The NFDI4Microbiota will support the parasitology community through this process with an elaborate training program.

[P173] EVOLUTIONARY MODELLING OF STOCHASTIC GENE REGULATION

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Gene expression in individual cells is often noisy and dynamic, generating phenotypic heterogeneity in genetically homogeneous bacterial colonies. This heterogeneity can be beneficial, allowing a fraction of cells to be prepared for stress in non-stress conditions, or enabling cell differentiation during biofilm formation. However, the functional benefit of noisy gene expression depends on its amplification and propagation by the gene regulatory network (GRN) to reliably generate specific adaptive phenotypes. Examples of noise-amplifying circuits have been found in Bacillus subtilis, where they drive biofilm differentiation, and in other bacteria, but it remains unclear what range of possible evolutionary outcomes rely on gene expression noise and in which genomic, cellular, and environmental contexts noise-amplification is expected to evolve.

To tackle these questions, we developed a computational model of an evolvable gene regulatory network that explicitly accounts for stochastic gene expression. The model represents a population of bacteria exposed to a fluctuating environment-representing variable resources or stress signals. Each different environment requires the expression of a different combination of transcription factors (TFs) for growth and replication. Bacteria encode a gene regulatory network in their genome, consisting of genes for TFs and promoter regions. TFs bind to the promoter regions to activate or repress downstream TFs. The expression of TFs is dynamically updated with a stochastic algorithm (Gillespie algorithm). During replication, genomic mutations can duplicate and delete genes, as well as change the types of TFs bound by binding sites, their binding strength and their activating or repressing effect. In summary, the model captures the observation that bacteria must adapt against three forms of stochastic perturbations: genetic (mutations), regulatory (gene expression noise) and environmental (resource fluctuations).

We find that bacteria evolve a wide range of gene regulatory strategies, with novel strategies evolving from older ones through systems drift over long evolutionary time scales. When we impose a large metabolic load on genome maintenance, we observe the evolution of gene regulatory networks that rely on stochastic activation of upstream genes, and near-deterministic activation/inhibition of downstream targets. This results in gene expression bursts reminiscent of B. subtilis noise-amplifying gene circuits. With smaller genomic cost, instead, we find that GRNs with multiple weakly activating connections evolve to respond to environmental fluctuations. Towards using the model as a GRN inference tool, we demonstrate that the evolutionary optimization process can incorporate additional constraints to generate robust, if at times surprising, solutions.

[P174] PRACTICAL APPROACH FOR EVALUATING THE ECOLOGICAL DISTURBANCE OF THE POTABLE GROUNDWATER USING FLOW CYTOMETRIC PHENOTYPING AND MACHINE LEARNING

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Microbial cells exhibit immediate physiological responses to environmental fluctuations. These changes can be analyzed using single cell phenotypic information from flow-cytometer (FCM) and can also be applied to measure ecological disturbance. To broaden the practical application of this technique, we collected FCM fingerprints from 292 potable groundwater, including 171 high-quality and 141 potentially-risk groundwater. The microbiome of potentially-risk groundwater had significantly higher phenotypic alpha-diversity than that of high-quality groundwater. Although the distinction between the two quality groups was significant on the non-metric dimensional scaling plot, clusters were highly overlapped, indicating that no FCM phenotype is indicative of potentially-risk status. This could be due to the fact that 46.8% of the potentially-risk samples were diagnosed based on multiple factors, and even samples diagnosed as potentially-risk based on a single parameter had various causes. We introduced a machine-learning classifier trained with FCM fingerprints (986 phenotypes) data with addition of the chemical items (pH, turbidity, nitrate, and chloride). The Random Forest model could classify the potentially-risk groundwater from the high-quality groundwater with 88.9% accuracy. Nitrate, turbidity, and chloride, as well as 14 FCM phenotypes exhibiting higher nucleic acid contents or high cell complexity, were identified as important variables for potentially-risk prediction, whereas low nucleic acid contents and less cell complexity phenotypes were important variables for high-quality prediction. Our study highlights the potential of using FCM phenotyping combined with machine learning analysis as a reliable tool for assessing groundwater quality in a practical setting.

[P175] A SUITCASE LABORATORY FOR MOLECULAR MICROBIOLOGY

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To help achieve Sustainable Global Development Goal 6 (SGD6) 'Clean Water and Sanitation for All', it is vital to deploy molecular diagnostic tools and scientific advances to understand and aid the fight against the 'permanent pandemic' of waterborne disease. With environmental DNA (eDNA) based methods, faecal and other potentially hazardous bacteria in water can be screened rapidly and comprehensively to save lives. During the COVID-19 pandemic, we developed an affordable suitcase laboratory that enables eDNA based water quality testing anywhere in the world. Our suitcase laboratory contains all the equipment items needed for biomass concentration and isolation from water, DNA extraction, PCR and qPCR amplification, sequencing library preparation and sequencing. In the United Kingdom, we have deployed this suitcase laboratory for onsite water testing at a sewage treatment plant, at a farm and in the back of a mobile laboratory ('lab in a van'). We have also successfully applied the suitcase laboratory for fieldwork in Ethiopia, Tanzania, Nepal, Cambodia and Thailand. This presentation will report on the use of our suitcase laboratory at the Kality Wastewater Treatment Plant (KWWTP) in Addis Ababa, Ethiopia, to support the training of 37 water researchers and professionals from 10 African countries in molecular microbiology. For this training, water samples were collected from the Little Akaki River in the i) upstream rural catchment and ii) downstream of Addis Ababa, and from the iii) inlet and iv) outlet of the KWWTP. Under the guidance of skilled trainers from Newcastle University UK, Newcastle University Medicine Malaysia, and Ardhi University Tanzania, trainees gained hands-on experience in analyzing these samples via plate counting, qPCR and 16S rRNA gene amplicon sequencing methods. Within the week of the workshop, it was established that the downstream sample contained high concentrations of Faecal Coliforms and Faecal Streptococci from the plate counting results. According to qPCR assays, the downstream sample displayed notably higher concentrations of total bacteria (16S rRNA) and human host Bacteroides (HF183) compared to the upstream sample. However, there is no significant difference between the downstream and KWWTP inlet samples. From the sequencing results, we identified a high prevalence of (Ali)Arcobacter butzleri, a still poorly understood waterborne hazard that can cause watery diarrhea.

[P177] GENOME ARCHITECTURE OF STREPTOMYCES AND OTHER LESSONS FROM GENERATING THE WORLD'S LARGEST COLLECTION OF COMPLETE GENOMES FROM FILAMENTOUS ACTINOBACTERIAL ISOLATES

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The microbial isolate is the rock on which microbiology as a field is build. To this day, microbial isolates and the complete genomes assembled from them are crucial for understanding the basic biology of bacteria, as functional characterization of cellular components like genes, proteins, and secondary metabolites exclusively is being deciphered through studies performed in pure isolates or using information from pure isolates. Here, we present a large study of newly isolated filamentous Actinobacteria and dive into their biology through comparing the ca 1000 chromosome resolved genomes which we have assembled. We analyze their genomic architecture through the presence and genomic location of core genes and biosynthetic gene clusters (BGCs). With this dataset, with >500 new species, and which more than triples the number of high-quality genomes from the important genus Streptomyces in public databases, we show a highly conserved location of >100 core genes present in all the genomes and more than 30,000 BGCs of >50 classes. A prominent example is the replication initiation gene DnaA, which we document is always found very close to the mathematical center of the linear Streptomyces chromosome. Other core genes, such as many of the ribosomal genes, are found 1-2 Mbases from the chromosome center, and BGCs, especially classes of BGCs often involved in production of bioactive compounds like T1PKS, T2PKS, NRPS, and transAT-PKS's, are generally found towards the highly dynamic ends of the linear chromosomes. This is the first study to document the generalized chromosome organization in Streptomyces on a large set of genomes. Our results are useful both in biotechnological engineering where the understanding of genomic architecture can guide strain design, and to researchers who are using reference-based methods to analyze single genomes, metagenomes, or pangenomes.

[P178] PLANT STAGE AND CROPPING HISTORY SHAPE SOIL AND ROOT-ASSOCIATED BACTERIA IN CONTINUOUS WHEAT

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Wheat is a crucial staple and cash crop subjected to yield decline and pathogen susceptibility due to continuous cultivation and increased climate change. This study aimed to determine whether changes in the soil and rhizosphere bacterial and archaeal communities induced by successive wheat cultivation are responsible for the yield decline. The study was designed to investigate the influence of cropping history, time, and space on the soil and rhizosphere bacterial and archaeal communities. Samples were taken from different plant developmental stages and microhabitats at two locations in Germany in the years 2020 and 2021. The samples were analyzed using 16S rRNA gene amplicon sequencing, and dominant culturable rhizosphere and rhizoplane colonizers were identified using culturing methods. The results showed no significant effects on biodiversity due to cropping history. At the same time, microhabitats strongly impacted community structure and composition, followed by plant developmental stages and cropping history. The most significant effect on communities was observed in the rhizoplane, while the effect of cropping history was most extensive in the rhizosphere and bulk soil. The dominant bacteria in the rhizoplane were Pseudarthrobacter, Sphingomonas, Nocardioides, Devosia, and Bradyrhizobium, with changes in relative abundance over the plant developmental stage and cropping history. The study speculated that plant genotype might shape the bacterial community composition in the rhizoplane and override cropping history effects. The study also employed culturing methods to determine the functional properties of soil and root-associated microbiota. The results showed that the highest proportion of bacteria with antagonistic potential was detected in the rhizoplane of continuous wheat rotations at both locations, indicating the recruitment or selection of functionally active (beneficial) bacteria by plant roots. In conclusion, the study observed that wheat's soil and rhizosphere bacterial and archaeal community is highly dynamic and influenced by various factors such as the growing season, developmental stage, microhabitat, and field site, and to a minor extent by the rotational position. The study suggests that further analyses are required to understand how rotation-dependent changes in the bacterial and archaeal communities contribute to yield decline and how to use beneficial inoculants for microbiome manipulation or microbe-assisted soil amelioration to mitigate yield decline in intensive wheat production.

[P179] EFFECTS OF MARINE MICROPLASTICS ON FATE AND ENRICHMENT OF ANTIBIOTIC RESISTANCE GENES ACCORDING TO EXPOSURE TIME AND POLYMER TYPES

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As a significant emerging environmental concern, many studies have focused on identifying and quantitatively analyzing suspended plastics in surface and sub-surface seawater. Recently, microplastics (MPs) are becoming new carriers for antibiotic resistance genes (ARGs) in marine environments. Specific biofilms on MP called plastisphere can provide an ideal niche to increase ARG spread through horizontal gene transfer (HGT), increasing the risk to ecosystems and human health. However, microbial communities formed on different plastic types, and ARG abundance has remained unclear. In this study, four types of MPs (polyethylene (PE), polypropylene (PP), polystyrene (PS), and polyvinyl chloride (PVC)) were periodically cultivated in the marine environment (46, 63, and 102 days) to investigate microbial communities and abundance of ARGs. The α-diversity and microbial compositions differed significantly from seawater ($P < 0.05$). β-diversity analysis indicated that the microbial communities were temporally changed by the cultivated period to the marine environment rather than polymer types of MPs (ANOSIM, P=0.0003, R=0.9699). Flavobacteriaceae and Rhodobacteraceae (approximately 21.36 ± 6.32 % of total abundance) were relatively high abundances of MPs. Additionally, qPCR results showed MPs selectively enriched ARGs. Especially, sul1, tetA, tetQ, and qnrS genes had relatively high abundances in PVC types of MPs. The abundances of intl1, mobile genetic elements (MGEs), were consistently identified as high levels compared to other MGEs during all periods (46, 63, and 102 days). Moreover, ARG profiles were a relatively good-fit correlation with bacterial compositions. The findings of this study suggest that the accumulation of ARGs and the intl1 gene in marine MPs can potentially accelerate transmission through HGT in the plastisphere.

[P180] AN ANAEROBIC BIOREACTOR FOR STUDYING BAGECO IN COMPLEX MICROBIAL COMMUNITIES

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How does the structure and function of gut microbial communities change with time and how do we study this in the lab? The gut habitat is characterized by anaerobic conditions and continuous flow with coupled inputs of growth substrates. This presents experimental challenges and is the reason for why we created an affordable anaerobic bioreactor in which we can maintain a stable but simplified gut model community where we could test the impact of different environmental factors such as variable pH, H2 (g), CO2 (g), and antibiotics.

To create a diverse microbial model community, we used either a complete fecal slurry or the supernatant thereof. With both inocula there was an initial expected decline of bacterial richness in the bioreactors, but after 5 days a stable community of 100-200 ASVs, depending on initial fecal donor, had established. The main 4 phyla of the gut, Bacteroidetes, Firmicutes, Actinobacteria and Proteobacteria were all present in the bioreactors. We observed an increase in the relative abundance of Bacteroidetes over time, and a concomitant decrease in Actinobacteria. Multi-dimensional feature selection (MDFS) showed that the most important ASVs all were obligate anaerobes, confirming the anaerobic status. Further variations in the environment, such as acidification of one of the reactors at day 4 lead to a significant decrease in Bacteroidetes and significant increase in Firmicutes. Interestingly, one day later the numbers were back the original ratio. Also, the gas used to maintain a microaerophilic environment had a major influence on the bacterial communities. After a switch from CO2 to N2, after 8 days of operation, there was a decrease in Firmicutes relative abundance from 40% to 30%, most likely due to decrease of Blautia spp. MDFS here showed that even though the total Firmicutes relative abundance decreased, Lachnospiraceae increased after the introduction of N2 (g). The results of perturbation with metronidazole, a broad spectrum antibiotic towards anaerobic bacteria, was in agreement with previous findings where Firmicutes decreased and Proteobacteria increased.
In summary we established bioreactors with a model community containing the major phyla of the gut microbiota where we could test the impacts of different environment factors. The system can be used to assess effects of antibiotics on communities, colonization resistance, plasmid transfer between species, and create predictive models for the functioning of their gut microbiome.

[P183] SURFACTIN FACILITATES THE ESTABLISHMENT OF BACILLUS SUBTILIS IN BACTERIAL SYNTHETIC COMMUNITIES

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Soil bacteria are prolific producers of a myriad of biologically active secondary metabolites. These natural products are cornerstone in modern society, since they have been used as
therapy against diseases and cancer, food additives, and an alternative for chemical pesticides. Secondary metabolites have been extensively studied under in vitro conditions that revealed inhibition towards other microbes, impact on motility and niche colonization, signaling and cellular differentiation. Despite the growing body of knowledge on their mode of action, biosynthesis, and regulation, we still do not fully understand the role of these compounds on the ecology of the producers and resident communities in situ. Here, we specifically examine the influence of Bacillus subtilis-produced cyclic lipopeptides (LPs) during bacterial synthetic community (SynCom) assembly, and simultaneously, exploring the impact of LPs on B. subtilis establishment success in a SynCom propagated in artificial soil microcosm.We found that surfactin production facilitates B. subtilis establishment success within the SynCom. Surprisingly, while neither the wild type nor the LP non-producer strains had major impact on the SynCom composition over time, the B. subtilis and the SynCom metabolomes are both altered during co-cultivation. Overall, our work expands the knowledge on the role of surfactin production in microbial communities, suggesting a broad spectrum of action of this natural product.

[P184] GENOME-CENTRIC ANALYSES OF METAGENOMES RECOVERED FROM BIOMETHANATION REACTORS SHOWED MORE DNA PHAGES INTEGRATED INTO MICROBIAL GENOMES AFTER STARVATION

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Viruses are known to shape microbial communities driving methanogenesis in Power-to-Gas applications. Understanding the behaviour of prokaryotes and viruses during hydrogen biomethanation may be essential for improving process efficiency and reducing costs. We hypothesized that viruses influence the resilience of hydrogenotrophic communities during intermittent starvation. To test our hypothesis, we evaluated how H2 and CO2 starvation affect the dynamics of prokaryotes and viruses in biomethanation reactors. We operated three controls and three treatment bioreactors in parallel for approximately 100 days. Samples for metagenomics were collected in parallel at nine-time points in three experimental phases: stabilization, starvation, and recovery. Using MuDoGeR (da Rocha U. N. et al. Biorxiv. (2022)), we recovered metagenome-assembled genomes (MAGs) and uncultivated viral genomes (UViGs) from the 54 metagenomes. All UViGs were dereplicated and reassessed using VIBRANT. Recovered MAGs and UViGs were dereplicated at an average nucleotide identity (ANI) of 0.95 (a proxy for species). Subsequently, the MAGs in each cluster were manually curated and defined as operational taxonomic units (pOTUs, proxy for strain level), and dereplicated complete and high-quality UViGs were defined as viral operational taxonomic units (vOTUs). We also assigned the virus-host pairs using WiSH.

From the 54 metagenomes, we recovered 877 MAGs dereplicated into 49 pOTUs. Although Firmicutes A encompassed ~45% of the observed pOTUs, Methanobacterium spp. (Archaea) showed the highest relative abundance in all libraries (81-95%). We identified 12 vOTUs (8 Caudoviricetes and 4 unclassified viruses). Lifestyle analyses indicated six Caudoviricetes vOTUs and one unclassified vOTU as a temperate virus. Host prediction analysis using WISH indicated three temperate vOTUs that may infect Bacteria and Archaea. WiSH predicted PTOGUVIG_KN11 (Caudoviricetes) and PTOGUVIG_KN12 (Unclassified virus) may infect Methanobacterium spp. (Archaea, p=0.041), Aminobacterium colombiense (Bacteria, p=0.032) and eight species from Clostridia, including Clostridium V ultunense (Bacteria, p=0.023).

Beta diversity analysis revealed similar clustering of samples by experimental phases in the pOTUs and vOTUs. Statistical analysis (t-test) indicated that the number of MAGs with prophages was significantly higher in treated bioreactors than in the parallel controls during the starvation and recovery phase ($p < 0.05$).

Our analyses suggest viruses can switch from the lytic to the lysogenic cycle under starvation in bioreactor systems. This switch may be a survival strategy when Prokaryotes are depleted of nutrients and energy limits virus replication. Understanding how to control these survival strategies may open new doors for bioreactor engineers by the targeted introduction of novel functions into specific Prokaryotes using viruses.

[P185] DEVELOPMENT OF IN VITRO BIOASSAYS FOR ASSESSING PESTICIDES TOXICITY ON SOIL NITRIFYING MICROORGANISMS

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Pesticides are major environmental pollutants of terrestrial and marine ecosystems. To date single-species tests are routinely used as a highly conservative step to determine pesticides toxicity on aquatic and terrestrial organisms. This has not been explored for soil microorganisms due to the lack of identified microbial indicators, and the lack of standardization of in vitro bioassays. Recent benchmarking research has pointed to ammonia-oxidizing microbes (AOM), which perform the first and typically rate-limiting step of nitrification, as ideal microbial indicators of the effects of agrochemicals on the soil microbial community due to their key functional role, their sensitivity to external perturbations, and the availability of established tools to measure their activity and abundance in both culture and in situ.

Our main objective is to develop and standardize in vitro assays for the systematic assessment of the toxicity of pesticides on AOM. Here, to identify the most sensitive strain per group of nitrifiers, we assayed the toxicity of selected pesticides of all major classes (herbicides, insecticides, fungicides) with a range of phenotypically and ecologically distinct strains of ammonia-oxidizing bacteria (AOB) (Nitrosomonas europaea, Nitrosomonas communis, Nitrosomonas ureae, Nitrosospira multiformis, and Nitrosospira briensis), ammonia-oxidizing archaea (AOA) (Ca. Nitrosocosmicus franklandianus, Nitrososphaera viennensis, Ca. Nitrosotalea sinensis,) and nitrite-oxidizing bacteria (NOB) (Nitrobacter sp. NHB1, Nitrobacter winogradskyi, Nitrospira defluvii) representing globally distributed lineages found in soil.

Toxicity was assessed at the functional level by measuring nitrite production or consumption in liquid cultures of AOB/AOA and NOB respectively, amended with a broad range of pesticides concentrations, and relevant toxicity endpoints (EC50s) were calculated. The stability of pesticides during laboratory incubation was determined in parallel.

All pesticides affected at least one non-target nitrifier with the different strains exhibiting various levels of sensitivity. Ca. N. sinensis and N. ureae, followed by N. briensis were the most sensitive AOA and AOB strains respectively. NOB showed lower sensitivity to pesticides than their ammonia-oxidizing counterparts, with Nitrobacter sp. NHB1 strain being the most sensitive. Categorization of pesticides according to their use revealed contrasting toxicity patterns, with fungicides like pyraclostrobin and etridiazole being highly toxic to all nitrifying strains, while insecticides (e.g., chlorpyrifos) and herbicides (e.g., metsulfuron-methyl) imposing greater effects on AOA and AOB strains, respectively. Our work is expected to benchmark the development of novel ecotoxicity tools for characterizing the impacts of pesticides on non-target soil microbes.

[P186] DIVERSITY OF STREPTOMYCES AND THEIR ANTIMICROBIAL PROPERTIES AMONG GREEK ECOSYSTEMS.

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Members of the genus Streptomyces are known to undergo a complex morphological differentiation and life cycle, but their most important feature for humans is that they produce a wide range of valuable secondary metabolites. Recent literature indicates that the geomorphological and climate conditions of Greece have resulted in soil reservoirs with a wide variety of multi-producer Streptomyces strains. The streptomycete diversity in Greek environments remains unexplored and represents a promising pool of novel pharmaceuticals. We aimed to investigate the abundance and diversity of streptomycetes in various habitats throughout Greece, and to examine whether there is a relationship between their antimicrobial profile and the characteristics of the sampling site. A total of 3014 strains of Streptomyces were isolated from 55 different sampling sites. 1387 strains were tested for their antimicrobial activity, using a diffusion method against 6 indicator type strains, including 2 Gram negative bacteria, 2 Gram positive and 2 yeasts. The 16S rRNA gene sequences of the isolated strains were used to determine their phylogenetic relationships. Over 25% of the isolates from the rhizosphere of evergreen sclerophyllous plants showed growth-inhibiting properties against at least one indicator microorganism. Streptomycetes isolated from the rhizosphere of certain trees and shrubs (olive, carob, Ebenus, Cistus) exhibited high antimicrobial capacity, with over 50% of them inhibiting the growth of three or more indicator strains, regardless of their isolation site. 20% to 50% of isolates from rhizosphere of herbaceous and aromatic plants exhibited antimicrobial properties that didn't vary based on the sampling site. 65% of the isolates from sites contaminated with fertilizers and pesticides had a higher percentage of streptomycete populations with antimicrobial properties, with 63% of them effectively inhibiting the growth of three or more indicator microorganisms. The antimicrobial potential of the isolates from NATURA protected areas was lower in comparison. Fewer Streptomyces strains were isolated from hot springs and volcanos, but over 26% of them exhibited growth-inhibiting properties against at least one indicator strain. Based on our phylogenetic analysis, 4 clusters of Streptomyces isolates were most widely distributed among examined habitats, while other clusters were present in fewer than three habitats. In conclusion it appears that the antimicrobial capacity of our Streptomyces isolates was influenced either by their microniches (e.g., rhizosphere of Ebenus sibthorpii shrub), or by plant-derived polyphenols (e.g., olive tree) or by soil contamination (e.g. agricultural areas). Therefore, Greek environments host diverse streptomycetes that could be a valuable source for novel pharmaceuticals.

[P187] SCREENING OF GREEK ACTINOBACTERIA FOR ANTIAGING COMPOUND PRODUCERS

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The Athens University Bacterial & Archaea Culture Collection (ATHUBA) contains many actinobacterial isolates from Greek environments with potential industrial utility. 1000 strains of the ATHUBA culture collection were screened for antiaging activity, more specifically for bacteria producing secondary metabolites capable of inhibiting elastase (which breaks down skin collagen and can cause wrinkles) and tyrosinase (which produces melanin and can cause liver spots). The strains were grown in liquid culture and their secondary metabolites were extracted twice, first with ethyl acetate and then with methanol. The ethyl acetate and the methanol extracts were then tested for inhibition of elastase and tyrosinase using in vitro enzymatic assays. The screening data demonstrated that 1.4% of strains produced elastase inhibitors and 26.4% produced tyrosinase inhibitors and that ethyl acetate is a more efficient solvent for extraction of molecules of interest than methanol. The 70 most active of these extracts, were then tested for cytotoxicity on the BJ human fibloblast cell line and the HaCaT human keratinocyte cell line. 30 extracts were found to be non-cytotoxic, and they were then tested for tyrosinase inhibition on the B16F10 murine melanoma cell line and for elastase inhibition on the BJ human fibloblast cell line. 3 extracts were found to inhibit tyrosinase and 1 extract was found to inhibit elastase in living cells. These 4 extracts are undergoing fractionation and mass spectrometry in order to identify the active molecules and the most suitable compounds will be used in the manufacture of antiaging skin creams by our industrial partner.

[P188] SEARCHING FOR MEANING FOR MICROBIOME STUDIES ON HERITAGE

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Microbiomes study has been slowly growing when related to heritage and conservation and restoration cases studies. The application of metagenomics has been driven by the decreasing price for external sequencing services that are now easily available and both the sampling and prior laboratory work can be done in standard microbiology laboratories. However, the amount of data that is gather with metagenomic methodologies is still not totally useful as meaning to the presence of different species on the surfaces must be attributed and its relation to biodeterioration is many times missing. This research paper will discuss study cases related to heritage in Portugal and compare it with international case studies allowing the discussion on the validity, advantages, disadvantages, and usefulness of microbiome studies in heritage specially as related to impacts of climate change on heritage and on biocolonization of outdoor works of art.

The authors acknowledge the financial help from project HAC4CG- Heritage, Art, Creation for Climate change. Living the city: catalyzing spaces for learning, creation, and action towards climate change. NORTE-45-2020-75. SISTEMA DE APOIO À INVESTIGAÇÃO CIENTÍFICA E TECNOLÓGICA- “PROJETOS ESTRUTURADOS DE I&D&I” HORIZONTE EUROPA.

[P189] ORGANIC FARMING CHANGES THE SOIL MICROBIAL COMMUNITY, IMPROVING THE SOIL ORGANIC MATTER STORAGE IN AGROECOSYSTEM

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The terrestrial ecosystem, a large reservoir of reactive carbon, moderates the global and regional biogeochemical carbon cycle. Sustainable soil management (e.g., organic farming) in agroecosystem could contribute to climate change mitigation via increasing soil organic matter storage. Here, we investigated the effect of the duration of organic farming on soil microbial community and examined how these changes are associated with soil carbon use efficiency (CUE) and soil organic matter stocks using structural equation modeling. We found that organic farming increased the microbial diversity and altered the structure of the soil microbial community when compared with the agricultural soil managed with synthetic fertilization. Microbial diversity was positively correlated with CUE only where organic farming was maintained for longer than 10 years. We also found that community compositions, which increased with the duration of organic farming, were a significant predictor of CUE. As the duration of organic farming increased, the direct CUE-microbial link was more influential than the indirect CUE-microbial-abiotic (e.g., water contents, micro- and macronutrients) links. This study provides evidence that long-term period organic farming can improve the soil organic matter storage through changes in microbial community, which in turn contribute to climate change mitigation.

[P190] BIOMINING SYNERGY: CAN INDIGENOUS BACTERIA WORK TOGETHER TO DECALCIFY MAGNESITE ORES?

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Magnesium is one of the most used metals with growing interest to process low-grade Mg ores and Mg-containing by-products. However, they contain a high level of impurities, mostly CaCO3, silicon and Fe, that limits metal recovery. By producing organic acids dissolving CaCO3, bacteria are innovative biotechnological tools for the pre-treatment of these raw materials for improved Mg recovery. In this study, we aim to investigate how decreasing sampling size for enrichment can help us access specific micro-niches among endogenous communities and retrieve microbial consortia with bioming potential. We have made enrichments from 4 different magnesite residues using sequential dilution ranging from 100mg to 1µg starting material and monitored both bacterial community composition and organic acids production ability, addressing the following questions. What is the bacterial diversity (alpha and beta) of enriched bacterial assemblages and specifically those producing organic acids at the different sampling scales? Can we detect “new” taxa? What microbes are co-occurring with potential synergetic effect?

[P191] BACTERIA-ACTIVATED MICROCARRIERS FOR HEAVY METAL REMOVAL FROM INDUSTRIAL WASTEWATERS

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Heavy metals in water bodies represent a serious environmental and human health threat. Heavy metals are used in industrial processes (industrial welding, dyes and pigments manufacturing, electroplating processes, leather tanning, wood preservation). Since bacteria interact with metals by passive adsorption processes and active enzymatic reactions, they can be proposed in water bioremediation strategies. The biological activation of adsorbing biomaterials (i.e., agro-wastes, biochar, activated carbon, lignite) with specific metal-removing bacterial strains is under study in order to obtain microporous microcarriers activated with bacterial biofilms. These systems are characterized by selective heavy metal extraction while providing cells with higher resistance to environmental stress [Priyadarshanee & Das 2021 J.Env.Chem.Eng].
The present study investigates the feasibility of EPS producing Serratia plymuthica strains SC3I(2) and As3-5a(5) and Rhodococcus quingshengii strain SC26 to be used in removal of N(II), Cu(II) and Cr(VI) from industrial wastewaters. S. plymuthica strain SC3I(2) was able to remove 89.4% of Ni(II) from a 50 mg/L solution, and showed maximum biosorption capacity of 33.5 mg/g in non-proliferating planktonic cell condition. S. plymuthica strain As3-5a(5) removed up to 91.5% of Cu(II) from a 200 mg/L solution, yielding maximum biosorption capacity of 80.5 mg/g. R. quingshengii strain SC26 reduced 51.14 mg/L Cr(VI) to Cr(III) in active growing-cell condition. Cu and Ni biosorption was assessed on real electroplating wastewaters: strains As3-5a(5) and SC3I(2) removed 8.89 and 2.37 mg/L of Cu(II) from Cu contaminated wastewaters and 222.23 and 116.16 mg/L of Ni(II) from Ni contaminated wastewater, respectively. The biosorption capacity of strains As3-5a(5) and SC3I(2) were respectively of 0.12 and 0.16 mg/g d.w. of Cu(II) and 3.24 and 8.13 mg/g d.w. of Ni(II). The activity of bacterial strains was monitored in mini-column experiments by inoculation of different microporous microcarriers. Once colonization occurred, cell-activated microcarriers were subjected to Ni(II), Cu(II) and Cr(VI) solutions and real contaminated electroplating wastewater flow, demonstrating their activity also in high specific surface biofilm-based system.
This study provides details regarding heavy metal bioremoval by EPS-producing bacteria in a solid phase bioremediation system, which could be implemented either for heavy metal recovery and for further biocatalysis purposes.

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[P192] BIOSTIMULATION OF MICROBIAL ORGANOHALIDE RESPIRATION IN CHLORINATED ETHENE-CONTAMINATED GROUNDWATER BY USE OF FOOD WASTES

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Chloroethenes (tetrachloroethene, PCE, trichloroethene, TCE, dichloroethene, DCE, and vinyl chloride, VC) are major causes of groundwater contamination due to their massive use as industrial solvents. They are dechlorinated in anaerobic and reductive conditions through organohalide respiration (OHR) pathway by organohalide respiring bacteria (OHRB): chloroethenes are used as electron acceptors and hydrogen as electron donor leading to substitution of each chlorine atom with hydrogen forming DCE, VC and ethene. Keystone OHRB are Dehalococcoides and Dehalogenimonas belonging to Chloroflexi, and only few strains (Dehalococcoides mccartyi strains BTF08 and 195 and Candidatus Dehalogenimonas etheniformans) are able conduct complete dechlorination to ethene. For this reason, accumulation of lower chlorinated ethenes (DCE and VC) is a common issue in contaminated sites. Biostimulation by addition of reducing substrates to groundwater can be conducted as a bioremediation strategy that allows to feed anaerobic food web. The increased concentrations of hydrogen and organic acids fuel OHRB thus improving bioremediation efficiency. Different reducing substrates such as lactate and formate have been tested, and substrates derived from agricultural and food processes are under study in order to increment waste circularity. In this work, molasse from sugar beet, tomato extract from lycopene biorefinery and whey were tested for their ability to enhance OHR activity in groundwater. Microcosms were set up with samples from an aquifer affected by 150-300 mg/L chlorinated ethenes and added of substrates to provide a final COD value of 200 O2 mg/L. As determined by GC-MS analyses after 4-month incubation, PCE and TCE were dechlorinated to DCE and VC also in unamended microcosms, demonstrating that natural attenuation occurred. The presence of substrates increased dechlorination rate, resulting in a less accumulation of VC. OHR biomarkers for TCE, DCE and VC reductases (tceA, vcrA) and for Dehalococcoides genus (dhc) were detected in the range of 10exp4-10exp7 gene copies/mL. 16S rRNA Illumina libraries are under analyses.
In conclusion, food wastes proved to be efficient in the biostimulation of OHR activity thus envisaging an increased sustainability of bioremediation intervention in a circular economy frame.

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[P194] (PAN)GENOMIC ANALYSIS OF NEWLY DISCOVERED RHODOCOCCUS PSEUDOKOREENSIS AND ITS IMPACT AS BIOINOCULANT ON APPLE REPLANT SOIL

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Apple replant disease (ARD) is a well-studied phenomenon which occurs after repeated planting of apple trees in the same soil, nevertheless, development and causal agents of this disease are still strongly debated [1]. As affected trees grow significantly worse and have lower fruit yield and quality, ARD denotes great economic losses for tree nurseries and apple orchards worldwide. The BonaRes ORDIAmur project (www.ordiamur.de) is developing solutions to mitigate ARD and searching for ecological and applicable methods for farmers, like microbial biostimulants. Biphenyl and dibenzofuran phytoalexins are an important aspect of apple root defense reactions [2]. However, these aromatic compounds in root exudates also have been hypothesized to be involved in ARD etiology [3]. In metagenomic data of ARD affected soil, we could identify a reduced genetic potential for degradation of aromatic compounds, as well as reduced abundances of certain groups of Actinobacteria [4]. After isolation and selection according to criteria related to mitigation of ARD symptoms, the most promising candidate identified as a bioinoculum was a newly described type strain of Rhodococcus pseudokoreensis R79 [5]. Genome analysis of R79 and pangenomic comparison to other members of the genus Rhodococcus indicated the presence of genes important for the degradation of aromatic compounds and revealed the different mechanisms for acquisition of these genes in the genus. Over 5 % of identified protein features of R79 predicted by RAST (Rapid Annotation using Subsystems Technology) were assigned to the metabolism of aromatic compounds. Specifically, we found gene clusters coding for the degradation of biphenyl. In a greenhouse experiment, we tested R79 in different concentrations as bioinoculum to enhance apple plant growth in ARD affected soil. Although plant parameters like growth were not significantly enhanced compared to the uninoculated control, inoculating the plants with R79 shifted the bacterial community composition the same way for all concentrations used, and significantly enhanced rhizosphere biodiversity as compared to the uninoculated control. These findings provide insight into the mechanisms of bioinoculation with R. pseudokoreensis R79 and possibly help fighting ARD in the future through the mechanism of biphenyl degradation.

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[5] Kämpfer et al. (2022) https://doi.org/10.1007/s00203-022-03079-2

[P195] GENOME-CENTRIC ANALYSIS OF AN AROMATICS-DEGRADING SULFATE-REDUCING MICROBIAL COMMUNITY

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Microbial consortia can syntrophically mineralize monoaromatics like BTEX (benzene-toluene-ethylbenzene-xylenes) through a complex network of interactions. We aimed to describe the taxonomic and functional diversity of a consortium that degrades aromatics by using sulfate as a terminal electron acceptor (TEA).
We extracted DNA from a laboratory microcosm's liquid and solid phases fed with benzene under sulfate-reducing conditions, containing coarse sand and an anoxic mineral salt medium for several years. The coarse sand was originally taken from an on-site reactor flushed with anoxic sulfidic groundwater at a BTEX-contaminated field site. We recovered metagenome-assembled genomes (MAGs) using MuDoGer (Nunes da Rocha et al., bioRxiv 2022) from two metagenomes (64.2 and 35.3 Mio reads) and measured their completeness and quality using CheckM. MAG taxonomy was assigned using GTDB-tk. We further defined operational taxonomic units (OTUs, proxy for species) based on an Average Nucleotide Identity of 0.95. To perform functional analysis, we mined enzymes participating in aromatics degradation under sulfate-reducing conditions from KEGG, UniProt and NCBI, and we used StandENA to generate a standardized matrix of presence/absence from the annotated genes in our MAG set.
Our analysis yielded 214 bins, 204 Bacteria and 10 Archaea, from which 125 were MAGs. MAGs affiliated with Bacteria were distributed in 22 Phyla, three of which were hitherto unclassified. We observed Desulfobacterota, Chloroflexota and Patescibacteria as the most abundant phyla. The archaeal MAGs belonged to four phyla, and methanogenesis genes were found in MAGs assigned to Candidatus Methanofastidiosum. After clustering, we obtained 48 bacterial OTUs. We identified the genes encoding three enzymes (sulfate adenylyltransferase, adenylyl-sulfate reductase and dissimilatory sulfite reductase) necessary for dissimilatory sulfate reduction in two phyla, Desulfobacterota and Firmicutes. Further, Desulfobacterota was the predominant phylum possessing some of the genes' coding for enzymes involved in ATP-independent aromatic ring reduction of benzoyl-CoA, an intermediate compound in anaerobic aromatics degradation and subsequent ring cleavage. Our analysis also revealed the absence of the benzene carboxylase abcA gene known to activate the benzene ring in Peptococcaceae members, suggesting that other degraders are involved in benzene activation in this community.
We identified the genes encoding the respective enzymes involved in benzoyl-CoA degradation. Our study revealed a diverse community involved in the degradation of aromatics with sulfate as TEA and that the Desulfobacterota phylum plays a key role in respiration under sulfate-reducing conditions. Further studies must be performed to uncover the mechanisms and species transforming benzene and other aromatics to benzoyl-CoA.

[P196] THE CONVERSION OF TROPICAL PEATLANDS INCREASES MICROORGANISM ACTIVITY AND THE ABUNDANCE OF CERTAIN BACTERIAL PHYLA

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Most of Indonesia's tropical peatlands have degraded. It is estimated that 6.2 million hectares of peatland on Kalimantan Island and 6.44 million hectares of peatland on Sumatra Island's eastern coast, including the provinces of Riau, Jambi, and South Sumatra, have been degraded. The peatlands of Riau, in particular, have been converted into agricultural fields, plantations, and settlement areas. Anthropogenic activities such as peatland conversion, drainage, illegal logging, and fires are the primary causes of peatland degradation, resulting in dry and flammable peat and increased carbon emissions. This activity is thought to have an impact on peat microbial diversity, which in turn has an impact on the climate and environment. The purpose of this research is to determine and compare soil respiration rates, fluorescein diacetate (FDA) hydrolysis activity, and the diversity and abundance of bacteria in secondary peat forests and converted peatlands. Peat soil samples were collected from the Giam Siak Kecil-Bukit Batu Biosphere Reserve in Riau Province's Bengkalis Regency. Secondary forests (SF), burnt land (BA), oil palm plantations (OPP), rubber plantations (RP), restoration area (RES), and acacia plantations are among the sampling sites (AP). The alkaline trap method was used to determine the rate of soil respiration. FDA activity was colorimetrically determined using FDA as a substrate. Bacterial diversity was determined molecularly by using the Illumina HiSeq platform to sequence the V3-V4 hypervariable region. The conversion of peatlands causes an increase in CO2 flux, as evidenced by the fact that the cumulative respiration of both glucose-free and glucose-enriched soils in converted areas was significantly higher than that of SF. FDA's hydrolytic activity in converted land was also significantly higher than SF's. From the six sampling areas, the four phyla with the highest abundance are Acidobacteria, Proteobacteria, Actinobacteria, and Firmicutes. Bacterial abundance analysis at the genus level revealed that Alicyclobacillus, Burkholderia, and Sulfobacillus were abundant in BA, while Acidothermus from the phylum Actinobacteria was abundant in OPP, RP, and AP. The SF is dominated by unidentified chloroplast bacteria. The pattern of alpha diversity indicates that bacteria in converted peatlands are more diverse. Meanwhile, according to the beta diversity index, there were no significant differences in bacterial diversity between the non-plantation (SF, RES, BA) and plantation groups (OPP, RP, AP). According to PCA analysis, vegetation has a positive correlation with bacterial diversity and abundance. The findings revealed that the conversion of peatlands significantly increases microorganism activity and bacterial abundance.

[P197] DICHLOROMETHANE BIODEGRADATION IN GROUNDWATER IS MAINLY IMPACTED BY CHANGES IN OXYGEN TENSION CAUSED BY WATER TABLE FLUCTUATIONS

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The observed increase in extreme precipitation regimes such as heavy rainfall or drought events driven by climate change can have a massive impact on prevailing physico-chemical conditions in aquifers. In particular, during water table fluctuations, variables related to matrix grain size, such as water content and redox potential of contaminated aquifers, show large variations [1]. Dichloromethane (DCM, CH_2Cl_2) is a persistent and toxic industrial solvent frequently detected in groundwater. Its degradation by microorganisms depends on both abiotic and biotic variables. In previous work with lab-scale aquifers, we found that DCM biodegradation was more pronounced when fluctuations in water level occurred [2].
Here, we further investigated the impact of water content, redox conditions, and matrix granulometry on DCM biodegradation and bacterial community composition. Laboratory microcosms with matrices of either sand or calibrated glass beads were inoculated with contaminated groundwater at different water content (33%, 66% or 100%) and regimes of oxygen tension (oxic, anoxic or alternating). In sand matrix microcosms, DCM biodegradation increased under water-saturated and oxic conditions. Upon repeated DCM exposure, however, similar and complete degradation of DCM was observed under all conditions. A PCR-based analysis of DNA extracted from the sand matrices was carried out for two distinct biomarkers of DCM biodegradation potential. New variants of the DCM dehalogenase gene dcmA and of the recently proposed mecEF genes of an alternative anaerobic pathway for DCM degradation [3] were detected. The final community composition in microcosms varied mainly as a function of oxygen tension regimes, as well as the initial oxygen status. No significant differences were observed depending on water content.

Taken together, our results for sand matrix microcosms suggest that dissolved oxygen concentration may play a more significant role than water content in defining DCM biodegradation. Ongoing experiments with glass bead matrix microcosms will help to assess the role of matrix granulometry, and to examine how changes and fluctuations in groundwater recharge may affect DCM transformation and the associated bacterial community.

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[P198] SALINITY STRESS MITIGATION IN TOMATO THROUGH MICROBIOME-BASED RHIZOSPHERE ENGINEERING

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Salinity stress is one of the major causes for agricultural crop loss. It decreases soil fertility and impairs plant development and their physiology. The traditional approach for mitigating salinity stress includes the application of inorganic compounds which may conversely lead to more soil salinization and causes soil degradation. An eco-friendly approach is the application of plant beneficial microbes as bioinoculants. But it has some inherent challenges viz. competition with indigenous soil microflora thereby leading to a reduction in survival and efficacy of bioinoculants. To overcome these limitations, one of the novel sustainable approaches is microbiome-based rhizosphere engineering, which has the potential to not just enhance plant growth, but also ameliorate stresses encountered by plants. The aim of the current study was to mitigate salinity stress in tomato plants by a top-down approach through acclimatization of the microbiome of rhizosphere to salinity stress through successive plant growth cycles. The inoculum was prepared from the rhizosphere microbiome of the best performing plant for succeeding plant growth cycles. Acclimatization of rhizosphere microbiome to salt stress has been done across ten plant growth cycles. The results in salt-stressed plants treated with acclimatized soil microbiome showed an increase in root length, shoot length, dry weight, fresh weight, membrane stability index, chlorophyll, and carotenoid contents as well as gradual decrease in the levels of stress indicators like malondialdehyde, proline relative to control plants. The dynamics of rhizosphere microbiome was also characterized across the passages. This work highlights the efficacy of the multi-passaging method of acclimatizing the microbiome as a promising sustainable approach for alleviating salinity stress in plants.

[P199] ARTIFICIAL SELECTION OF EFFICIENT CHITIN-DEGRADING BACTERIAL COMMUNITIES FROM NATURAL MARINE HABITATS

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Chitin is the second most abundant biopolymer in the world next to cellulose. Every year, millions of tons of crab, shrimp, and lobster chitinous shell waste are produced worldwide. Most of the time, such waste is thrown away into the sea, in landfills or is incinerated. In order to improve disposal of chitin and its conversion into valuable compounds for industrial applications (chito-oligosaccharides (COS), chitosan) in the future, we enriched marine bacterial consortia through an artificial selection procedure for chitin-degrading bacteria. To do so, different marine environments known/ expected to be hotspots of chitin degradation were sampled in 2020 in the North of France: marine sponges, sediment, and seawater. Bacteria were separated from living tissue/mineral particles and grown at 20°C in a liquid culture medium containing chitin as a sole source of carbon and nitrogen. Then, 3 subculturing steps were performed (every 7 days) in order to select for specialized bacterial consortia with reduced richness, containing species that contribute the most to chitin degradation in laboratory conditions. During the artificial selection process, we followed chitin degradation (assessed by measuring the settled remaining chitin in the culture medium (quantitative) and by characterizing the chitin degradation products (average molecular size given by Size Exclusion Chromatography)) and taxonomic composition (assessed by MiSeq Illumina sequencing of 16S rDNA). Metagenomics analyses were also performed on selected communities using shotgun Illumina sequencing, with a focus on the carbohydrate-active enZYmes (CAZymes) profile of the assembled metagenomes. Several target genes directly or indirectly involved in chitinoclastic / chitinolytic processes were screened such as those underlying the biosynthesis of endochitinases, exochitinase, chitin LPMOs, chitin deacetylase… In this study, it was shown that efficient partners at degrading chitin are selected quickly during the artificial selection process (already in the first culture). Also, the majority of the selected cultures were more efficient at degrading chitin than the initial ones. Moreover, chitin degradation was positively correlated to the relative abundance of the endochitinase-encoding genes in the metagenomes. Furthermore, in several enriched consortia, the dominant taxa were poorly characterized ones, totally unknown for their ability to degrade chitin, such as Arcobacter nitrofigilis, Motilimonas cestriensis, Marinomonas atlantica, or Poseidonibacter lekithochrous. Also, several minor taxa with an unknown function remained throughout the enrichment procedure. These experiments demonstrate the high potential for discovering novel chitin-degrading microbes in marine environments.

[P200] COUPLING OF SECONDARY METABOLITE PRODUCTION IN BACILLUS SUBTILIS

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Production of a diverse array of antimicrobial secondary metabolites (SMs) by the Gram-positive, soil-dwelling bacterium, Bacillus subtilis, makes this microorganism an appealing candidate as a biocontrol agent. Even though the synthesis of these SMs is described, the regulation of biosynthetic gene clusters, including the interconnections between the production of different SMs is unexplored. Our former characterization and systematic mutagenesis of a B. subtilis isolate library for lipopeptide production revealed the natural product chemistry of respective lipopeptide gene knock out mutant strains [1]. Intriguingly, we noticed an inverse correlation between the relative levels of the lipopeptide, surfactin and the bacteriocin, subtilosin A. To explore the coupling between the two SMs, a subtilosin A reporter (sboA promoter coupled to GFP) was introduced into various knockout mutants related to lipopeptide production or known regulators of subtilosin A and surfactin.

The expression of sboA was evaluated using a plate reader detecting the GFP signal in the presence or absence of surfactin and the production of both subtilosin A and surfactin was further verified using liquid chromatography-mass spectrometry. Absence of surfactin resulted in increased expression/production of subtilosin A. On the contrary, externally supplied surfactin to the srfAC mutant decreased subtilosin A levels to that of the WT. Pairwise inoculation of WT and surfactin mutant on an agar medium lowered subtilosin A level in the mutant colony adjacent to the WT, as a result of diffused surfactin from the WT. MALDI Mass Spectrometry Imaging is being performed to confirm these results.

Finally, to reveal the molecular mechanism behind the inverse correlation of surfactin and sboA expression, knockout mutants of known regulators of sboA was investigated. Our first results revealed that a mutant of the transcriptional repressor Rok harbors increased production of both SMs, suggesting a role of this regulator on the inverse production of surfactin and subtilosin A in B. subtilis.

Our results revealed an intricate genetic regulation of natural product biosynthesis that possibly contribute to the ecological success of B. subtilis.

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[P201] ALGAL MICROBIOMES AS POTENTIAL PROBIOTICS IN MARINE AQUACULTURE

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Bacterial infectious disease outbreaks among fish are a significant challenge in aquaculture, and pathogens are often introduced through live feed for the susceptible fish larvae. Although some fish species can be vaccinated, the extensive use of antibiotics to control bacterial pathogens largely contributes to antimicrobial resistance, posing a threat to public health. To find sustainable solutions for bacterial infections in the aquaculture sector, this project as part of the EU H2020 project MARBLES aims to identify microalgae-associated bacteria and complex microbiomes that can suppress fish pathogens and act as probiotics. To identify beneficial microbiomes, a high-throughput screening method relying on fluorescence to monitor the growth of GFP-tagged fish pathogens in complex microbial mixtures has been developed and subsequently used to identify microbial communities with pathogen suppression. This, we hypothesize can lead to defining a “minimal inhibitory microbiome”. The GFP-tagged Vibrio anguillarum (NB10_pNQFlaC4-gfp27) was co-cultured with 10-fold serial dilutions of axenic and non-axenic cultures of two marine microalgae, Isochrysis galbana and Tetraselmis suecica. We found that high concentrations of non-axenic cultures of both species can inhibit V. anguillarum, and that the I. galbana microbiome is more potent at suppressing the pathogen. Neither the axenic algal cultures, nor the sterile filtered samples of the cultures, where both the algae and the microbiome had been removed showed inhibition, indicating that it is indeed the algal microbiomes that suppress V. anguillarum. In parallel, culturable bacterial strains have been isolated from all inhibitory microbiomes, and current work involves identification via 16S rRNA sequencing and testing the antagonism of isolates against several bacterial fish pathogens. Genomic DNA of the inhibitory microbiomes has been extracted and 16S rRNA gene amplicon sequencing is currently ongoing to elucidate the microbiome composition and identify presumed key community members. Once key community members have been identified, a synthetic community can be assembled and screened for pathogen suppression. Furthermore, current efforts involve GFP-tagging of seven chosen fish pathogens based on their relevance in the aquaculture sector and screening the inhibitory microbiomes against these. The ultimate goal is to find a “minimal inhibitory microbiome” to be used as a probiotic solution for effective and sustainable control of bacterial infectious disease outbreaks in fish aquaculture.

[P202] EFFECT OF SALINITY ON THE VOLATILE COMPOUND EMISSION OF THREE HALOPHILIC STRAINS ISOLATED FROM NORTHERN GREENLAND

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In the current scientific and political climate, the transition to a green economy has become a priority, which requires great innovation and new technologies. To this end, we have turned to extremophiles, organisms that have evolved to thrive in extreme environments, in search of novel enzymes, bioactive compounds and other relevant products. In this work, three halophilic bacterial strains, Nesterenkonia aurantiaca strain CMS1.6, N. halotolerans strain CF4.12, and Oceanobacillus sp. Strain CF4.6, were selected from a collection of strains isolated from Peary Land (Northern Greenland), and then their production of Biogenic Volatile Organic Compounds (BVOCs) under different sodium chloride concentrations (5, 10 and 15% w/v) was analyzed. The BVOCs were sampled from euxenic cultures in a dynamic headspace setup using Tenax tubes, which were afterwards thermally desorbed and coupled to GC-MS. The resulting data was analyzed with PARADISe (1). Several compounds were found to be produced; among them, 2- and 3-methylbutan-1-ol stand out for being produced in largest amounts by both Nesterenkonia strains. These compounds have been proposed as biofuel (2). This work highlights the importance of extremophiles in the transition to a green economy and confirms the potential role of the genus Nesterenkonia as a biofuel producer, as previously described (3). These results can also be applied to the field of astrobiology, as the strains were isolated from a terrestrial analogue of extraterrestrial conditions. The identified volatiles could be used as biomarkers for future space missions and telescope observations.

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[P203] NOVEL ANTIMICROBIAL ACTIVITY AGAINST MULTI-DRUG RESISTANT PATHOGENS FROM AN INDIGENOUS STREPTOMYCES ISOLATE.

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Microbial drug resistance has been alarmingly rising over the past decades, becoming one of the most critical health concerns globally. Misuse and overuse of antimicrobial drugs have led to the prevalence of resistant pathogens, leading to increased healthcare costs, treatment failure, and mortality. In this context, discovering new bioactive compounds is of major importance. Actinobacteria represent the most prominent group of bacteria able to produce bioactive compounds with antimicrobial activity. Greek habitats, mainly due to the plethora of distinct microclimatic conditions, harbor a plethora of actinobacteria, including members of the genus Streptomyces, with antimicrobial potential. This work aims to explore the metabolic repertoire of Greek actinobacterial strains to address the emerging problems of multiple antibiotic resistance. For this purpose, actinobacterial isolates from the Athens University Bacterial & Archaea Culture Collection (ATHUBA) some of which were isolated from unique environments (caverns, volcanoes, thermal springs, etc.) were studied for their antimicrobial potential. As targets, we selected multi-drug resistant pathogens isolated from clinical samples, that are prioritized as emerging hazards for public health, both at national and global level and used them to screen 100 actinobacterial strains. Streptomyces sp. strain M18 showed strong inhibition to multi-drug resistant Klebsiella pneumoniae and Candida auris when cultivated antagonistically in solid cultures. Antimicrobial activity-guided fractionation using semi-preparative HPLC analysis coupled with diode array UV-VIS detection was performed for the isolation of the corresponding bioactive compounds in culture supernatants. Concurrently, in silico analysis of secondary metabolite biosynthetic gene clusters in whole genome sequence of the selected strain led to the identification of 29 clusters related to secondary metabolite biosynthesis, many of which have not yet been correlated to antimicrobial activity against Klebsiella pneumoniae and Candida auris. These results suggest that Streptomyces sp. strain M18 can produce novel bioactive compounds that could be used as part of the arsenal to combat the urgent threat of multiple antibiotic resistance.

[P204] ENHANCING SALT STRESS TOLERANCE IN VIGNA RADIATA THROUGH PLANT-ASSISTED SELECTION OF ACCLIMATIZED MICROBIOME

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The use of chemical fertilizers and pesticides in agriculture, combined with rising temperatures worldwide, leads to salt accumulation in the soil, negatively impacting plant physiology and crop yield. To address this issue, scientists have turned to eco-friendly approaches of utilizing beneficial microbes residing in the rhizosphere. However, using single strains or consortia of strains often proves ineffective due to issues concerning their root colonization and competition due to the native soil microbiome. To combat this challenge, a plant-assisted microbiome selection strategy was employed, whereby the host plant was adapted to select a microbiome that is beneficial for its growth and development under salt-stress conditions.

A novel approach utilizing multiple passaging was employed to condition the microbiome to help the Vigna radiata plant withstand salinity stress. Through successive cycles of ramping up the salinity stress, the plant was able to adapt and ultimately exhibit improved tolerance to the stress. Results showed an increased plant's resistance to salinity stress, as evidenced by an enhancement in root and shoot length and decreased stress marker levels/indicators such as proline, MDA, and MSI. Amplicon sequencing, employing the 16S rRNA and ITS as genetic markers, revealed significant changes in the microbial community inhabiting the rhizosphere of Vigna radiata under salinity stress. A significant increase in the relative abundance of dominant bacterial phyla such as Proteobacteria, Actinobacteria, and Firmicutes, coupled with a concomitant decrease in the abundance of the fungal phylum Ascomycota, was observed in the acclimatized microbiome during successive passages. Moreover, a decline in the abundance of Mortierellomycota and Chitridiomycota was explicitly noted during the 5th and 13th passages, respectively.

To overcome the limitations associated with the storage of acclimatized microbiome, synthetic microbial communities (SynComs) were generated that can perform at par with the stress-acclimatized microbiome while mimicking its functionality in mitigating salinity stress in crop plants grown in arable lands. The salinity stress mitigation potential of these SynComs was assessed under control and field conditions. These findings provide novel insights into the promising prospects of rhizosphere engineering in improving crop productivity and promoting sustainable agriculture in arable lands.

[P205] BACTERIA ASSOCIATED WITH WILD COLD-ADAPTED ALPINE PLANTS CAN ENHANCE COLD TOLERANCE IN TOMATO SEEDLINGS

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Cold stress is one of the major abiotic stresses that negatively affect crop production worldwide. Climate change is causing warmer and shorter winters, increasing the risks of plant exposure to late spring frosts with consequent severe economic losses, especially for crops that are not naturally cold-adapted, such as tomato. Microbial communities associated with plants growing in cold environments can promote plant growth at low temperatures and improve plant tolerance to cold stress. Thus, the development of microbial inoculants based on psychrotolerant endophytic bacteria could be a promising approach to protect crops from cold stress. This work aims to investigate the effect of bacterial endophytes from cold-adapted alpine plants on the cold tolerance of tomato seedlings. A total of 47 bacterial isolates collected from the roots of three cold-adapted Rosaceae plants were tested for their growth-promoting ability on tomato plants (Solanum lycopersicum var. Moneymaker). Seedlings were grown at 25°C under controlled conditions, and the most promising isolates were selected according to the increase of dry and fresh plant biomass. The ten best-performing plant growth-promoting bacteria were then tested for their ability to promote tomato growth at 10°C. Seedlings were grown for two weeks at 25°C and then exposed to cold stress for two weeks. Three isolates, belonging to the Pseudomonas, Chryseobacterium, and Paenibacillus genera, were able to improve tomato growth under cold stress. Biochemical assays were performed, such as auxin production, phosphate solubilization, nitrogen fixation, siderophore production, 1-aminocyclopropane-1-carboxylate deaminase activity, and ammonia production to assess the plant growth-promoting traits. The so-found bacteria could represent a valid approach to mitigate cold stress on tomato plants. Further analyses are needed to clarify the plant physiological changes associated with the bacterium inoculation.

[P206] VARIABILITY IN NITRIFIER ABUNDANCE AND COMMUNITY STRUCTURE BETWEEN RICE GENOTYPES IS REGULATED BY BIOLOGICAL NITRIFICATION INHIBITION EFFICIENCY.

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In fertilized soils, nitrification largely contributes to global nitrogen (N) fertilizer loss from agricultural systems, including rice cultivation, Asia's largest fertilizer consumer. One mitigation strategy involves nitrification inhibition by plant-derived biological nitrification inhibitors (BNIs). This study aims to demonstrate that the physiological variability within different rice genotypes induces variable levels of inhibition of ammonia oxidizers. We found that the BNI activity is higher in the rhizosphere compared to the surrounding soil. Moreover, the BNI effect significantly alters the ammonia-oxidizing archaeal community within the rhizosphere compared to the surrounding soil. In addition, the ammonia-oxidizing archaeal community structuring within the rhizosphere depends on the genotypic BNI variability of rice. We demonstrate this variability is due to the variability within the exudation profile and mass of the root exudates, including BNIs of different efficiencies. We used an improved hybrid soil-hydroponic system to collect root exudates, obtaining a root exudate composition similar to soil systems. In addition, we demonstrated that testing root exudates against a consortium of ammonia oxidizers instead of a single model strain better represents the BNI capability, demonstrating similar efficiency to that observed in soil. A new BNI compound, N-butyl dodecane 1-amine (NBDA), was identified, and AOA were found to be more sensitive to this compound than AOB. Our study adds to the knowledge of the influence of plants on ammonia oxidizing microbial community activity, ecology, and BNI efficiency. This study will further benefit in designing relevant exudate collection systems and culture bioassays aimed at assessing the potential BNI efficiencies of plants in soil.

[P207] ADAPTIVE EVOLUTION FOR OPTIMIZATION OF AN INDUSTRIALLY-RELEVANT MICROALGA

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Introduction: Fish oil is currently the main source of omega-3-polyunsaturated fatty acids (ω-3-PUFAs) for the increasingly growing aquaculture feed market. However, the majority of wild fish stocks are already exploited at maximal sustainable levels. Thraustochytrids are heterotrophic unicellular marine protists, very well known for their ability to accumulate a high content of ω-3-PUFAs and could therefore serve as an alternative source of these nutrients.
Considering the lack of genomic insights in thraustochytrids, rational genetic engineering of these microorganisms is limited. Therefore, Adaptive Laboratory Evolution (ALE) serves as an effective tool to study the molecular-level response of microorganisms to stress and adaptation.

Aim: To enhance the phenotype of a thraustochytrid strain using a combined two-stage ALE, and to identify the possible molecular mechanisms underlying such an adaptation.

Methodology: Various experimental cultivation systems and conditions of propagation were tested in order to establish the starting stress parameters for ALE. Cell growth curves of the original strain were determined my measuring cell density and cell dry weight. UV-C mutation was first applied to the cells at logarithmic phase to increase the genotypic diversity of strain, and the first ALE experiment was conducted for 30 cycles based on a long-term serial transfer procedure.

De novo whole genome assembly: Long-read Nanopore MinION sequencing was used for the whole genome sequencing in conjunction with Illumina NovaSeq 2x150 bp sequencing. Final de novo genome assembly was conducted using a hybrid approach combining high depth Illumina short reads with Nanopore long reads.

Results and Discussion: A two-stage ALE approach was designed and starting stress parameters for running the first ALE were determined. The Nanopore MinION sequencing run produced around 20.3 Gb of data, altogether generating an approximate 450x coverage of the genome. An additional 6.8 Gb of paired end 2x150 bp Illumina reads were generated, representing approximate genome coverage of 150x. The final Illumina-polished assembly generated with Flye showed improved quality and completeness with >95% sequence identity, 91.09% of complete BUSCO genes and greatly reduced indels to 26.10 per 100 kbp.

Conclusions: A high-quality genome assembly was generated that will serve as a reference for the subsequent mutant analysis. Analysing mutants from ALE experiment using omics-based analysis including transcriptomics, proteomics and metabolomics will allow for deciphering the connection between genotype and phenotype in the evolved strain compared to the original strain.

[P208] DYNAMIC OF SOIL MICROBIAL COMMUNITIES IN RESPONSE TO LONG-TERM REPEATED ORGANIC OR INORGANIC FERTILIZATIONS

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Within the soil biodiversity reservoir, the microbial community is essential for ecosystem functioning and resilience. Applications of organic and inorganic products in agriculture could improve soil microbial quality and increase crop productivity. However, little is known about the dynamic of the soil microbial communities after several years of repeated fertilizer inputs. In this study, we take advantage of a long-term field experiment in Rennes (SOERE PRO – EFELE), Brittany region (France). The EFELE experiment was set up in 2012 and has been cropped with wheat-maize rotation since the beginning of the trial. A mineral fertilizer (MIN), and three different organic amendments: cattle manure (CM), pig slurry (PS), and anaerobic digestate (DIG), were applied once a year from 2013 to the present. These treatments were compared to a control (CON) that has not received any organic or inorganic input. To monitor soil microbial community changes throughout 10 years of repeated fertilization practices, every two years (on wheat culture), soil samples were collected twelve months after the last organic or inorganic material application. We used high-throughput sequencing targeting 16S and 18S ribosomal RNA genes to describe the evolution of microbial soil communities. DNA extractions of all soil samples were performed in 2022. Results showed that the soil molecular biomass remained stable over 10 years of repeated fertilization practices, except for the CON treatment where a decrease (~58%) was observed. Interestingly, regardless of the sampling time considered, soils treated with CM displayed 20% higher soil molecular biomass compared to those that received DIG. The richness diversity index based on operational taxonomic units (OTUs) remained stable for both prokaryotic and fungal communities over time for all treatments. The Non-Metric multiDimensional Scaling approach (NMDS) showed that the structure of the prokaryotic community changed over time depending on the treatment. Regarding the fungal community structure, a time-dependent effect was also observed, although the discrimination between different treatments seemed less pronounced than the one observed for the prokaryotic structure. To conclude, the present study highlights that the soil microbial community could be lastingly modified after 10 years of repeated fertilization practices. These changes depend on the nature of the treatment applied.

Keywords: Organic and inorganic fertilization, high-throughput sequencing, soil microbial community.

[P209] HOW CULTIVAR, PARENTAL LINE AND ACCELERATED AGING AFFECT THE BRASSICA NAPUS SEED MICROBIOTA

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The seed microbiome, as the initial microbial inoculum to the plant, showed high potential for improving crop performance, such as the influence on germination and plant growth. However, the factors determining the seed microbial community structure and function are still not fully understood. Here, we analyzed both plant-related and environmental factors on the endophytic seed microbiome based on 16S rRNA gene amplicon sequencing of ten oilseed rape (Brassica napus L.) cultivars derived from 26 different field sites across Europe.
The endophytic seed microbiome was mainly influenced by the cultivar regarding bacterial diversity, abundance and composition. Nevertheless, all ten cultivars were dominated by six genera, namely Enterobacter, Pantoea, Pseudomonas, Ralstonia, Serratia and Sphingomonas. Bacterial biomarkers were identified to distinguish between host plant's characteristics, for example Sphingomonas for improved germination and Brevundimonas for resistance towards phytopathogens. Application of a Bayesian community approach suggested a vertical transmission of the seed endophytes, with the paternal breeding line having a higher contribution compared to the maternal breeding line. The results assume that the paternal parent might even determine the germination performance of the offspring.
Germination performance and seed vigor are highly associated with seed storage conditions. High temperature and moisture are known to accelerate the seed aging process. Experiments concentrated on accelerated aging tests simulating the seed aging process, which were conducted on four different oilseed rape genotypes derived from two different field sites in the same year of harvest. The results will help to further understand the important role of the seed microbiome on seed vigor, which is of special interest in regard of climate change. Summarized, the study provides valuable insights into the structure and function of the oilseed rape seed microbiome and highlights its potential for improving seed quality.

[P210] EXOPOLYSACCHARIDE SUPPLEMENTATION FOR IMPROVED SHELF LIFE AND EFFICACY OF BIOFORMULATION OF PLANT GROWTH-PROMOTING BACTERIA

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Increasing soil salinization, temperature, water limitation, and diseases are major challenges to crop productivity. Amongst these, soil salinization affects approximately 20% of arable land and is predicted to reach up to 50% by 2050. Hence modern agriculture is facing the huge challenge of feeding the ever-growing population with the constraint of deteriorating arable land. In view of this, there is an urgent demand for increased food production. Persistent use of chemical fertilizers and pesticides has tremendously fortified agricultural production, but it comes with numerous noxious effects on the environment. Beneficial microbes with multifarious plant growth-promoting traits have emerged as a powerful tool for agricultural intensification. But their successful application is associated with a few challenges including their viability, efficacy, and persistence in soil. Currently, beneficial microorganisms and their metabolites are gaining importance for stress management in plants. Exopolysaccharides (EPS) producing microbes have a profound effect on plant growth under saline conditions. The structural versatility, non-toxic, biocompatible, biodegradable, and nutrient-rich nature of EPS can make it an important substitute in bioformulations for improved shelf-life and efficacy. To exploit the vast potential of EPS of microbial origin in the agriculture system, the objective of present work was to develop bioformulation supplemented with EPS. For this, different bacterial strains were screened, among which Bacillus sp. showed higher EPS production and tolerance under salinity stress. It exhibited increased biofilm formation and plant growth-promoting traits including auxin production and phosphate solubilization under saline conditions. The presence of hydroxyl, ether, and carbonyl groups in EPS was confirmed through Fourier transform infrared spectroscopy. EPS exhibited high-temperature stability and water-holding capacity. Interaction of the isolate with Cajanus cajan (pigeon pea) under saline conditions improved the physiological and biometric parameters of the plants. Moreover, reduced level of stress markers including proline, malondialdehyde, and electrolyte leakage was evident in plants treated with inoculants. The amendment of EPS promoted the shelf life of bioformulation and its functional efficacy. The study developed a new strategy for the development of robust next generation bioformulation to enhance the quality and efficacy of bioformulation manifold and for stress management in plants in the future.

[P211] INOCULATION-INDUCED MODULATION OF BACTERIAL COMMUNITIES IN THE RHIZOSPHERE OF WINTER RYE REMAINS PRESENT FOLLOWING WINTER DORMANCY.

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The inoculation with plant-beneficial microorganisms (BMs) can increase the performance of important crops. However, it is unknown whether BMs can survive in a sufficient density over the winter period and retain their activity, despite the harsh conditions that occur during winter dormancy. Additionally, farming practices could affect the establishment and efficacy of applied BMs in the rhizosphere of a winter crop. In the present work, we tested whether the BMs sufficiently colonize the rhizosphere of winter rye, thereby affecting the composition of the rhizosphere bacterial communities and the plant growth performance. Specifically, winter rye was treated with BMs in a long-term field trial where fields have been subjected either to organic or integrated farming systems. The BMs inoculum included two bacterial strains (Bacillus Abi03 and Pseudomonas RU47), applied by drenching two weeks after winter rye seedling emergence, and one fungal strain (Trichoderma harzianum OMG16), which was incorporated directly in the soil before sowing the seeds. Samples were taken before and after the winter dormancy (autumn and spring samplings) and rhizosphere bacterial communities were analyzed in total community DNA with high throughput 16S rRNA gene amplicon sequencing. We found that BMs established in the rhizosphere over the winter period and increased the growth performance of crops, especially when applied under organic farming practice, and that BMs inoculation increased the crop nutrient acquisition. The farming practice and BMs inoculation significantly affected the β-diversity of the bacterial communities in the rhizosphere, with farming practice explaining higher variance of β-diversity in bacterial communities than BMs inoculation. In addition, the BMs significantly altered the composition of bacterial communities in the rhizosphere, in both samplings, but no interaction effect was observed. Meanwhile, several ASVs responded to the farming practices and to a lesser extent to the BMs inoculation, before and after winter dormancy. Only a few ASVs were positively or negatively associated with the increased effect of BMs inoculation on the growth of crops under organic farming practice. The combination of molecular- and culture-based approaches indicated that the organic farming practice improved the survival of Pseudomonas RU47. In conclusion, the BMs survive in the rhizosphere of winter rye and alter the bacterial community composition in the rhizosphere, likely contributing to the observed increased growth of winter rye at early plant developmental stage under organic farming practice.

[P212] DELINEATING THE IMPACT OF REVERSE TRANSSULFURATION ENZYMES ON THE DESULFURIZATION ACTIVITY OF RHODOCOCCUS QINGSHENGII IGTS8

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Environmental regulations pose increasingly lower limits on the sulfur content of transportation fuels. While the current hydrodesulfurization technology is quite successful in reducing the aliphatic sulfur content of oil products, it becomes prohibitively costly and energy intensive when S-contents lower than 100 ppm are sought, that concern the recalcitrant dibenzothiophenic aromatic compounds. Biodesulfurization is a process that selectively removes sulfur from dibenzothiophene and its derivatives (DBTs) by employing microbial biocatalysts that utilize DBTs as sulfur source. This is achieved through the action of the Dsz enzyme group that desulfurizes DBT to 2-hydroxybiphenyl (2-HBP). Several natural biocatalysts harboring the highly conserved desulfurization operon dszABC have been isolated. The expression of the corresponding enzymes is known to be significantly repressed by methionine, cysteine, and inorganic sulfate, however, the available information on the metabolic regulation of gene expression is still limited. The biosynthesis of the repressing amino acids (methionine and cysteine) is mediated through the reverse transsulfuration enzymes, namely, cystathionine β-synthase (Cbs) and cystathionine γ-lyase (MetB). Aiming towards a better understanding of the regulatory aspects of sulfur assimilation, scarless knockouts of the reverse transsulfuration pathway enzyme genes cbs and metB were constructed in the model desulfurizing biocatalyst Rhodococcus sp. strain IGTS8. We provide sequence analyses, establish the presence of an intact functional reverse transsulfuration pathway and report the enzymes' involvement in the sulfate- and methionine-dependent repression of biodesulfurization activity. Sulfate addition in the bacterial culture did not repress the desulfurization activity of the cbsΔ strain, whereas deletion of metB promoted a significant biodesulfurization activity for sulfate-based growth and an even higher desulfurization activity for methionine-grown cells. In contrast, growth on cysteine completely repressed the desulfurization activity of all strains. Transcript level comparison uncovered a positive effect of cbs and metB gene deletions on dsz gene expression in the presence of sulfate and methionine, but not cysteine, offering insights into a critical role of CbS and MetB in desulfurization activity regulation.

[P213] STRATEGICALLY DESIGNED SYNTHETIC MICROBIAL COMMUNITY FOR BIOTIC STRESS MITIGATION IN AGRICULTURE

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The vulnerability of agricultural system to various biotic stresses is responsible for decline in global crop productivity by nearly 20-40%. These pathogens generally invade plant tissues disturbing the structural integrity and metabolism of cells and, tissues of the host plant. Cajanus cajan (pigeon pea), constituting 10% of total pulse production, has enormous significance because of its high nutritional value and protein content. The stagnancy in the average yield of the crop due to wilt disease caused by fungal pathogen, Fusarium udum, is responsible for 15-30% of plant mortality. The commonly used chemical pesticides disrupt environmental health and are toxic to mankind. Moreover, the conventional bioinoculants used so far have not been effective in the long run due to reduced persistence and survivability under in vivo conditions. Microbiome-assisted rhizosphere engineering aims to re-structure the rhizospheric microbiome that benefits the plant by stress mitigation and growth promotion. The present study proposes a strategy based on rhizosphere engineering to combat biotic stress Fusarium udum, in Cajanus cajan by generating a synthetic microbial community (SMC). This is done by comparative microbiome analysis in Fusarium-infested and pathogen-free soil samples using Illumina MiSeq sequencing to decipher the changes in bacterial diversity in response to the pathogen. Then, bacterial strains with biocontrol potential against Fusarium were isolated from rhizospheric soil samples of Cajanus cajan from different geographical zones. The isolates were categorized into different metabolic bins based on their plant-growth-promoting (PGP) and biocontrol properties thus, generating a culture bank of antagonistic strains exhibiting functional redundancy. Next, to facilitate the quantitative estimation of desired PGP and biocontrol traits expressed by bacterial strains present in a community, a novel approach of iterative deconvolution was adopted. Using this approach, various possible combinations of compatible strains generated from the bank were assessed to establish the best performing SMCs and their efficacy was tested by seed germination assays followed by in planta assays. Thus, a robust, synthetic microbial community with multi-trait PGP strains for sustainable mitigation of biotic stress (Fusarium) with proven efficacy in host (Cajanus cajan) was generated. This new approach of strategic designing of SMCs can be extended for other applications as well.

[P214] MINERAL FERTILISATION INCREASES ABUNDANCE OF AMMONIUM OXIDISING BACTERIA AND NITROUS OXIDE EMISSIONS IN AGRICULTURAL SOIL

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Aim:
The aim of this study was to determine the effects of tillage and fertilization on the soil microbial community, particularly on the abundance of N cycling guilds, and to link them with the measured nitrous oxide (N_2O) emissions.

Methods: The study was conducted during the 2021 maize vegetation period on a long-term combined tillage (no-till (NT) vs. conventional plough tillage (CT)) and fertilization (unfertilized, mineral and compost) field trial, established in 1999. Gas samples were collected throughout the growing season using a static chamber approach, and a composite soil sample was collected at 10 cm depth to perform physicochemical analyses and quantification of microbial genes by qPCR (16S, ITS, nirS, nirK, nosZI, nosZII, nrfA, bacterial and archaeal amoA).

Results: Total bacterial, fungal and archaeal abundances were clearly increased in NT system with respect to increased soil organic carbon content (Govednik et al., 2023). Long-term mineral fertilization clearly increased the percentage of bacterial ammonium oxidizers (AOB) in the total bacterial community (AOB/16S) which also coincided with observed highest cumulative emissions in mineral fertilization. No-till increased the proportion of denitrifiers as described by (nirK+nirS)/16S ratio which was already observed before (Wang and Zou, 2020), but contrary to the expectations, did not increase the cumulative N_2O emissions. Interestingly, combination of no-till with compost fertilization showed comparable cumulative N_2O emissions with unfertilized control, despite receiving N at the dose of 120 kg N/ha. This might mean higher rate of complete denitrification to dinitrogen gas in no-till combined with compost treatment as indicated by overall lower (nirK+nirS)/(nosZ+nosZII) ratio compared to other fertilization treatments within no-till.

Conclusions: Both tillage and fertilization influenced the composition of the N-cycling community. Even though physico-chemical conditions influenced emission patterns over the growing season, our results show possible links between microbial community composition and N_2O emissions. However, this needs to be further examined and verified on other molecular levels. Understanding this link could provide novel insights and strategies for steering microbiome responsible for N_2O emissions.

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[P215] MICROBIAL COMMUNITIES FROM DEEP ROOT MICROHABITATS IN WHEAT CROPPING SYSTEMS: YIELD AND SUSTAINABILITY IN THE CONTEXT OF A CHANGING CLIMATE

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Healthy root systems help plants maximize available nutrients and moisture utilization. The composition and interaction of microbiota with roots below the topsoil are particularly critical during dry field seasons when the root's ability to uptake water and nutrients is limited. In this study, we investigated the soil microbial communities associated with distinct root microhabitats at various depths in an experimental field site, focusing on beneficial and detrimental microbiota that could explain yield decline in wheat grown after wheat. Soil cores were obtained from two cropping histories of wheat grown in fields with a six-year crop rotation (WR, wheat after oilseed rape and after winter wheat) and long-term wheat monoculture (WM, 15 years). Each core was separated into five depth layers, and each resulting soil sample was divided into three microhabitats. Amplicon sequencing of 16S rRNA gene fragments showed that the WR and WM soil microbial communities did not differ in species diversity but in composition. Significant dissimilarity of microbial composition was observed from different microhabitats and soil depths between and within cropping histories based on Bray-Curtis analysis. The rhizoplane was found to have the most dissimilar microbial composition and contained the highest taxa with differential abundance between WR and WM. The relative abundances of Shinella, Altererythrobacter, and Novosphingobium were significantly higher in WR, whereas Bradyrhizobium, Actinobacteria MB-A2-108, Phyllobacterium, and Nitrospira were significantly higher in WM. These taxa were present at all soil depths, indicating that their recruitment by lateral and longitudinal roots may be advantageous in dealing with nutrient and water shortages, even in deeper soil layers. This study provides vital information for designing management strategies to promote a healthy and diversified microbial community in wheat cropping systems. The results emphasize the need to address soil and roots from different depths and microhabitats as critical determinants of these communities, especially under upcoming climate scenarios, where water might be a limiting factor for plant growth. Although we cannot draw conclusions regarding the production reduction in various wheat cropping systems, these bacteria may be advantageous for more efficient utilization of nutrients (C and N) in rotation versus monoculture.

[P216] MARINE SPONGE AND OCTOCORAL-ASSOCIATED BACTERIA SHOW VERSATILE SECONDARY METABOLITE BIOSYNTHESIS POTENTIAL AND ANTIMICROBIAL ACTIVITIES AGAINST HUMAN PATHOGENS

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The microbiomes of marine animals, such as sponges (Porifera) and octocorals (Cnidaria, Anthozoa, Octocorallia), are prolific sources of bioactive natural products of potential pharmaceutical value. Multi-drug resistant microbes pose a worldwide threat, wherefore new drugs and a greater structural variety of natural products are urgently required to combat the antimicrobial resistance crisis. This study inspected two culture collections comprising 919 host-associated marine bacteria belonging to 55 genera and several thus-far unclassified lineages to identify isolates with potentially rich secondary metabolism and antimicrobial activities [1]. Altogether, the two collections encompassed four phyla, six classes, 17 orders, 33 families and 55 described genera, in addition to 30 isolates that were unclassifiable at either genus (19 strains), family (7 strains), or order (4 strains) level, and most likely represent novel taxa. Employment of low-carbon, customized marine media, prolonged incubation times and lower-than-usual incubation temperatures enabled the isolation of difficult-to-cultivate marine taxa with limited numbers of known species and genomes available, such as Anderseniella, Lentilitoribacter, and iconic symbionts of marine animals such as Endozoicomonas and Parendozoicomonas.

Seventy representative isolates from both culture collections had their genomes sequenced and mined for secondary metabolite biosynthetic gene clusters (BGCs). In total, 466 SM-BGCs were identified, with antimicrobial peptide- and polyketide synthase-related BGCs being frequently detected. Isolates of the Aquimarina genus and closely related Flavobacteriaceae strains were particularly enriched for polyketide BGCs, while Gammaproteobacteria strains of the genera Grimontia, Enterovibrio and Thalassomonas were found to be rich in non-ribosomal peptide BGCs. Only 38 of the 466 BGCs had similarities greater than 70% to BGCs encoding known compounds, highlighting the potential biosynthetic novelty encoded by these genomes. The 70 genome-sequenced strains were further screened for antimicrobial activities against four canonical human-pathogenic bacteria and five pathogenic Candida strains. Cross-streak assays showed that 33 of the 70 genome-sequenced isolates were active against at least one Candida species, while 44 isolates showed activity against at least one bacterial pathogen. Taxon-specific differences in antimicrobial activity among isolates suggested distinct molecules involved in antagonism against bacterial versus Candida pathogens. Several Flavobacteriaceae isolates and a Halomonas (order Oceanospirillales) strain stood out as the most promising producers of antifungal and antibacterial compounds. The here reported culture collections and genome-sequenced isolates constitute a valuable resource of understudied marine bacteria. Their antimicrobial activities and potential for the biosynthesis of novel secondary metabolites holds promise for a future sustainable production of marine drug leads.

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