Microbial Secondary Metabolites in Microbiomes 2024

3rd International Conference, 9 – 11 June 2024, Elsinore, Denmark

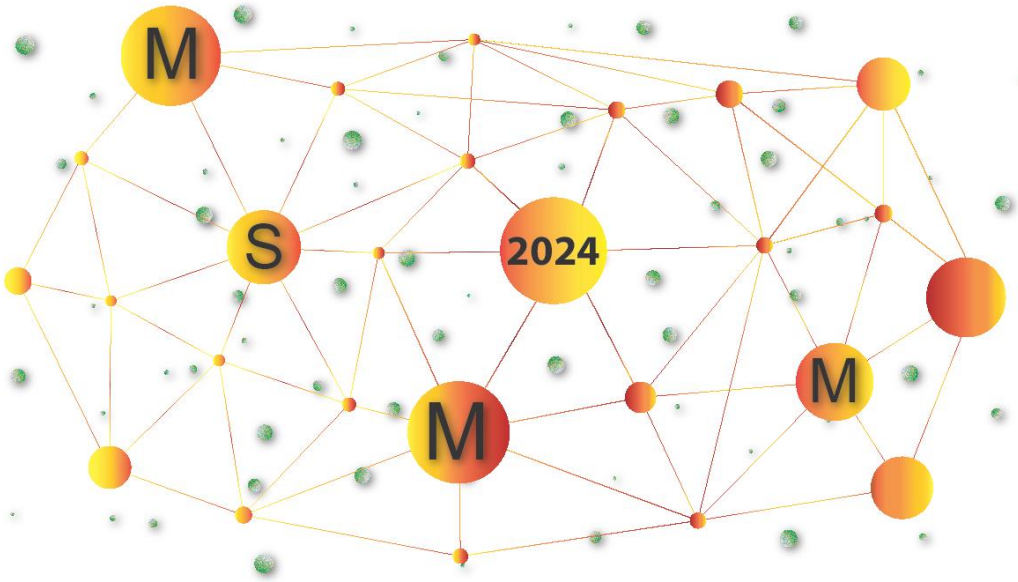
Synthesis and production of microbial secondary metabolites in microbiomes.

Molecular mechanisms and regulation of microbial secondary metabolites in microbiomes.

Biotransformation and interactions of/between microbial secondary metabolites in microbiomes.

Secondary metabolite mediated interactions in microbiomes and microbiome-host-systems.

Book of abstracts



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Invited Speakers

Mitja Zdouc

Postdoc
Wageningen University, The Netherlands

Pre-processing in MZMine, FERMO

BIO: Natural Product Drug Discovery with special emphasis on novel antibiotics. Cultivation and characterisation of Actinomycetes. Profiling of extracts by metabolomics. Identification and isolation of novel metabolites. Structural elucidation by NMR.

Trent Northern

Science Deputy at EGSB Division, and Chemist Senior Scientist at Environmental Genomics and Systems Biology, Lawrence Berkeley National Laboratory, Berkeley, US

The chemical underground: metabolomic approaches for connecting metabolites, plants, and microbes in soils

BIO: Dr. Trent Northen is Deputy Division Director and a Senior Scientist within the Environmental Genomics and Systems Biology Division at Berkeley Lab, Adjunct Professor of Comparative Biochemistry at UC Berkeley, the Metabolomics Program Lead at the DOE Joint Genome Institute, Laboratory Research Manager for the multi-institutional m-CAFEs SFA program, and Director of High Throughput Biochemistry at the Joint Genome Institute. Dr. Northen obtained his BS in Chemical Engineering at the University of California Santa Barbara, his PhD in Chemistry and Biochemistry from Arizona State University and performed Post-Doctoral Fellow at the Scripps Research Institute. He has received numerous awards including a DOE Early Career Award, two R&D100 awards, Berkeley Lab Inventor of the Year and was awarded a Presidential Award for Science and Engineering (PECASE) by President Obama. His research has resulted in over 25 patents/applications and >160 peer reviewed publications including numerous papers in influential, peer-reviewed journals.

Dr. Northen's laboratory focuses on understanding the role of exogenous small molecule metabolites in mediating microbial interactions with other microbes and plant hosts and how these processes impact soil carbon cycling. A long-term goal of the Northen lab is to help harness plants and

microbes for sustainable agriculture—including to restore soil carbon as a climate change mitigation strategy. Towards these goals the Northen lab has developed a range of metabolomic, cheminformatic, and bioinformatic capabilities for metabolite identification and analysis. Dr. Northen has also championed the development of fabricated ecosystems spanning scales and complexity. Most recently he has led a project at Berkeley Lab developing the 'EcoBOT' which automates plant-microbe-environment studies and integrates lab and field studies.

Jacob Agerbo Rasmussen

Postdoc

GLOBE Institute, University of Copenhagen, Copenhagen, Denmark

Integrating microbiome omics, Anvi'o

BIO: Jacob is a molecular and evolutionary biologist with international experience. He has applied a range of different molecular tools to study the interactions between microbiome and its host, using novel molecular tools and analytical skills to combine multiple big datasets in a variety of omics, including genomics, metagenomics, and metabolomics, resulting in information used to develop new molecular based management tools.

Jacob is interested in deciphering molecular interactions, especially with the focus on holobionts and apply it to livestock productions, like aquaculture, to create a more sustainable production, by utilizing state of the art biotechnology such as metagenome sequencing to boost or modify healthy microbiomes in fish and other production animals.

Serena Robinson

Group Leader, Eawag, Department Environmental Microbiology, Überlandstrasse 133, CH-8600 Dübendorf, Germamny

Mind the gap: challenges and opportunities in paired 'omics

BIO: Serena studies 'specialized metabolism' and the pathways involved, including tightly-regulated enzymes often encoded by gene clusters for the synthesis, transformation, and degradation of bioactive compounds. Specifically, her group addresses how anthropogenic inputs affects the

specialized metabolism of aquatic microbes in freshwater ecosystems? And how specialized metabolites affect biodegradation rates in microbial consortia? And whether we can use machine learning to predict the function and substrate specificity of specialized microbial enzymes involved in pollutant degradation? Her group uses heterologous expression hosts and in vivo and in vitro assays to discover new enzymes and pathways from uncultivated microbes. They then use our experimental results to train machine learning models to learn patterns of substrate specificity and function of enzymes involved in secondary metabolite biosynthesis and pollutant biodegradation. In the long term, the group is interested in characterizing and engineering enzymes as biocatalysts for environmental biotechnology applications such as improving water quality. At a more fundamental level, the group is are also interested in the ecological roles of specialized enzymes and metabolites in microbial communities.

Roberto Kolter

Professor emeritus
Harvard Medical School, Boston, US

Garden-Variety Thoughts on Microbial Secondary Metabolites

BIO: Roberto Kolter is Professor Emeritus in the Department of Microbiology, Harvard Medical School. For 35 years his lab worked on antibiotic synthesis, bacterial starvation physiology, experimental evolution, bacterial biofilms, and chemical communication in the microbial world. He currently blogs at Small Things Considered.

Deniz Tasdemir

Professor
GEOMAR Helmholtz Centre for Ocean Research
Kiel, Germany

Computational metabolomics in discovery of bioactive natural products from cultivable marine mycobiomes

BIO: Deniz Tasdemir is a professor of marine natural product chemistry at GEOMAR Helmholtz Centre for Ocean Research Kiel (Germany), where she leads the Research Unit Marine Natural Product Chemistry and GEOMAR Centre for Marine Biotechnology (GEOMAR-Biotech). After earning her PhD in phytochemistry at ETH-Zurich, Deniz performed post-doctoral research on anticancer sponge metabolites at University of Utah. She has been studying chemical diversity of marine macro- and microorganisms sourced from tropical, temperate, polar and deep-sea environments for discovery of new bioactive/functional secondary metabolites. She integrates LC-MS/MS based untargeted metabolomics, spatial metabolomics (DESI imaging MS) and innovative microbial culture techniques with high performance chromatography and spectroscopy in her drug discovery and chemical ecology research. She is the recipient of many prestigious awards, such as the Pierre Fabre Prize (PSE), Egon-Stahl Silver Medal (GA) and Waters Award for Excellence in Natural Product Innovation.

Kathrine Duncan

Associate Professor
Newcastle University Biosciences Institute,
Newcastle, UK

Deep Ocean Biodiscovery - diving into abyss

BIO: It is widely accepted that 'omics methodologies have accelerated our understanding of life at molecular, cellular and organism levels. Our research combines 'omics methods to link biology (genes) to chemistry (antibiotics). We apply this approach to understand the 'chemical language' of microorganisms, and what influences it. This assessment of chemical space across biological parameters can enable informed biodiscovery of new antibiotics. You can find out more about the Duncan lab at: www.medicinesfromthesea.com.
This year Kate moved to the University of Newcastle (UK) and leads a team focussed on microbial metabolomics and antibiotic discovery. Prior to this Kate completed a Tenure-Track Chancellor's Fellowship at the University of Strathclyde (2016-2020) and was awarded full tenure to associate professor (2020-2023). Prior to starting her group, Kate completed two Postdoctoral Fellowships at Scripps Institution of Oceanography (California) in Marine Biomedicine and at The Scottish Marine Institute in Marine Biotechnology, a PhD in Biomedical Science (Canada) and a 5-year Masters in Chemistry (Aberdeen, Scotland) with International Placement (Florida). Kate is an advocate for community approaches to data sharing (ActinoBase, NPAtlas, MI-BiG, GNPS etc) and equality in STEMM.

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radley S. Moore

Distinguished Professor,
Scripps Institution of Oceanography & the Skaggs School of Pharmacy and Pharmaceutical Sciences,
University of California San Diego, US

Biosynthesis and molecular monitoring of harmful algal bloom toxins

BIO: Bradley Moore is a Distinguished Professor at the University of California, San Diego and Director of the Center for Marine Biotechnology and Biomedicine at the Scripps Institution of Oceanography. He is a biosynthetic chemist who specializes in reading and writing the genetic code of marine microbes, algae, and sessile animals to develop new ocean-based medicines and molecular tools to improve human health. Moore has published over 260 peer-reviewed articles, trained nearly 100 graduate students and postdoctoral fellows, and has been recognized with awards and honors by the National Institutes of Health, the American Chemical Society, the American Academy of Microbiology, the American Society for Pharmacognosy, and the Royal Society of Chemistry.

Elisabeth Shank

Associate Professor
Department of Systems Biology, UMass Chan Medical School, Worcester, Massachusetts, US

Metabolic interactions in the soil microbiome

BIO: I am a microbiologist whose lab studies the chemical and physical interactions of bacteria and fungi with each other and their plant and human hosts. We use microbiology, fluorescence imaging, mass spectrometry, and computational approaches to answer outstanding questions in microbial ecology and apply this knowledge to environmental and clinical challenges. The diverse research in my lab has been supported by funds from the National Institutes of Health, National Science Foundation, and Department of Energy. I started my independent laboratory at UNC-Chapel Hill in 2013. In 2020 I relocated my group to the University of Massachusetts Chan Medical School, where I am now an Associate Professor in the Department of Systems Biology. In addition to research, I am dedicated to mentoring trainees and increasing STEM diversity. I actively work to improve my own mentoring skills and participate in activities that encourage best practices among my colleagues to enact institutional change. I also serve the microbiology community by acting as an editor at the American Society for Microbiology (ASM) Journal of Bacteriology; as the Leader of the ASM Council on Microbial Sciences Molecular Biology and Physiology Community; and as a member of the Directorate Advisory Committee for the Earth and Biological Sciences Directorate at the Pacific Northwest National Laboratory.

Alex Hall

Professor
Eidgenössische Technische Hochschule (ETH)
Zurich, Switzerland

Impact of antibiotic-degrading resistance on microbial community structure

BIO: Some antibiotic-resistance mechanisms degrade antibiotics in the local environment. This can protect sensitive cells from antibiotic inhibition, but it is unclear how such effects play out in multi-species communities. We study the impact of clinically important carbapenemase-mediated resistance in experimental multispecies communities of bacteria. Carriage of a carbapenemase-encoding plasmid by one community member can reduce the local antibiotic concentration, but we found that some sensitive species benefit from this more than others. We identified phenotypic traits of component species that help to explain this variability, and therefore to predict changes in community structure caused by antibiotic-degrading resistance mechanisms. These effects can also be visualised in communities distributed across structured environments (on agar surfaces). In ongoing work, we are testing how sensitive the community-wide impacts of resistance are to species identity (which species carry resistance genes) and networks of horizontal resistance-gene transfer.

Laura Sanchez

Associate Professor
University of California Santa Cruz, Santa Cruz, US

Visualizing specialized metabolite production in microbes

BIO: She started her independent lab in the Fall of 2015 at the University of Illinois at Chicago in the Department of Pharmaceutical sciences. The lab relocated to the University of California, Santa Cruz Department of Chemistry and Biochemistry in January 2021. Her team specializes in using and adapting imaging mass spectrometry and tandem mass spectrometry for small molecule analyses in complex systems. She was a K12 BIRCWH Scholar (2016-2017) which supported the translation of the techniques to women's health

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Jos Raaijmarkers

Head of the department of Microbial Ecology,
Netherlands Institute of Ecology (NIOO)
Professor, Leiden University

Back to the Roots

BIO: Jos Raaijmakers is head of the department of Microbial Ecology at the Netherlands Institute of Ecology (NIOO) of the Dutch Academy of Sciences (KNAW) and Professor of Microbial Ecology at Leiden University. His research program focuses on the diversity, dynamics and natural functions of microorganisms associated with plants. His group pioneered plant-microbiome research by elucidating microbial consortia and molecular mechanisms involved in disease-suppressive soils. To validate the role and functions of specific plant-associated microbial genera, they contributed to the development of novel bioinformatic tools and discovered several new biosynthetic gene clusters and metabolites involved in microbe-plant, microbe-microbe and microbe-protozoa interactions. In their Back-To-Roots research program, they collaborate with various national and international universities and research institutes in South America, Africa and Asia to elucidate how plant domestication has impacted on microbiome assembly and activity. Current projects focus on the molecular mechanisms of plant microbiome assembly and how plants benefit from the large genomic, genetic and metabolic potential of the microbiome. The functions of the plant microbiome that are studied in detail are microbial protection of plants against biotic and abiotic stress, in particular fungal diseases, root parasitic weeds and drought. Understanding the influence of plant genotype and environmental conditions on the dynamics, assembly and activities of microbiomes will help to design new strategies to minimize fertilizer and pesticide input in crop production and to maximize the beneficial effects of microbiomes on plant productivity and food quality.

Mônica T. Pupo

Professor, School of Pharmaceutical Sciences of
Ribeirão Preto, University of São Paulo, Brazil

Specialized metabolites play functional and defensive roles in stingless bees' microbiomes

BIO: Mônica T. Pupo is a Full Professor at School of Pharmaceutical Sciences of Ribeirão Preto (FCFRP), University of Sao Paulo (USP), Brazil, where she currently serves as deputy director. She is a natural product chemist whose research has focused on understanding the role of specialized

metabolites in interspecies interactions in plant and insect microbiomes, and also in the discovery of natural products hits for infectious diseases. She graduated in Pharmacy (1990) from USP, and then earned her PhD in Chemistry from Federal University of São Carlos (1997). She was a postdoctoral researcher at São Carlos Institute of Physics, USP, for one year and then she joined FCFRP-USP in 1998 as an assistant professor. She was appointed as associated professor in 2009 and full professor in 2019. She was a visiting scholar at Jon Clardy's group at Harvard Medical School (2006-2007), and has coordinated different international collaborative research grants in the fields of Chemical Ecology and Natural Products Discovery.

Speaker abstracts

Opening lecture

9 June 2024

18:00 - 19:00

Garden-Variety Thoughts on Microbial Secondary Metabolites

Roberto Kolter Professor emeritus, Harvard Medical School, US

Microbial communities are both ubiquitous and fundamental for ecosystem function. Invariably, the constituent microbes interact through the production, release and response to secondary metabolites. In this presentation I will go over what we already know regarding the ecological functions – and speculate on what we might learn in the future – of these crucial molecules that make "everything depend on everything else."

Session 1: Synthesis and production of secondary metabolites in microbiomes

10 June 2024

9.15- 9.45

Computational metabolomics in discovery of bioactive natural products from cultivable marine mycobiomes

Deniz Tasdemir Professor, GEOMAR Helmholtz Centre for Ocean Research Kiel, Germany

Fungi are found in all marine habitats and at all depths. They make a significant contribution to marine environments, by feeding on decaying organic matter (e.g., dead animals), but also as parasites, pathogens or symbionts (epiphytic an endophytic), as a consequence of the evolution of fungal cell biology and feeding strategies. Marine macrophytes are regarded as holobionts with a rich mycobiome that enhances their tolerance to abiotic and biotic stresses. Culture-based studies on the other hand indicate the endless biosynthetic potential of marine fungi as source of novel secondary metabolites with high pharmaceutical relevance. A prominent example is Plinabulin®, a synthetic derivative of the diketopiperazine halimide obtained from the algicolous fungus Aspergillus sp. that is undergoing phase III clinical trials against lung cancer.1,2 Natural product research is going through a breakthrough with the introduction of Molecular Networking strategy, empowered by the GNPS platform, for massive, untargeted metabolomics studies. Particularly Feature-Based Molecular Networking (FBMN)3 finds substantial applications because it also allows detection of isomers and semi-quantification of analytes. Our marine microbial drug discovery efforts heavily employ MN and additional bioinformatics approaches for i) assessment of the impact of culture conditions (e.g., OSMAC or co-cultivation approaches), ii) prioritization of marine fungal extracts with high chemical novelty for downstream work and iii) bioactivity prediction and purification of new and highly bioactive fungal natural products in a targeted manner. In this presentation, I will highlight successful application of various molecular networking tools for accelerated discovery of marine fungal natural products with pharmaceutical potential.

References:

1. Fenical et al. US Patent US6358957B1, 2000.
2. Cimino et al. Biomed. Rep. 2019, 10, 218-224.
3. Nothias et al. Nat. Methods. 2020, 17, 905-908.

9.15- 9.45

Deep Ocean Biodiscovery - diving into abyss

Kathrine Duncan Associate Professor, Newcastle University Biosciences Institute, Newcastle, UK

The extreme variety of deep-sea habitats, encompassing ancient abyssal plains some of which date to over 180mya, to high-temperature hydrothermal vents, makes the deep-sea the perfect evolutionary test-lab for natural product research. Often, we think of deep-sea as void of life, but a recent study has uncovered 5,000 new species in Pacific deep-sea mining regions (Rabone, 2023) and we know that understanding diversity-ecosystem function is of primary importance in the face of biodiversity loss. These ecosystems have remained largely unexplored in terms of biodiversity and biomedical potential, yet over recent years they have gained significant interest due to the potential of deep-sea mining for metals such as cobalt and nickel. Here we aim to profile the biodiversity and biomedical (antibiotic) potential of deep-sea microorganisms. Microbial drug discovery in the 'omics era relies on three key datasets, biosynthetic, chemical, and biological (activity). Yet, to integrate and interrogate these large and complex datasets remains a challenge and results in the low-throughput prioritization of only a few strains based on observed antibiotic activity. Despite this wealth of genomic and metabolomic data, linking metabolites to the BGC responsible for their production and to observed bioactivity is limited, slow (manual) and challenging. Here, approaches to combine data sets consisting of bacterialt genomes (and their predicted BGCs), the chemical products of these same strains and their bioactivity profiles will be discussed. Several datasets of Actinobacteria genomes have been mined for BGCs and these strains cultured to generate metabolite extracts for comparative metabolomics (high resolution tandem mass spectrometry / molecular networking) and antibiotic screening (against a panel of clinically relevant pathogens). Driven by biological questions, tools have been developed to establish patterns across strains and learn relationships between BGC, spectral features and bioactivity – here we will discuss experimental data considerations and how ‘omics information is informing biodiscovery from the deep-sea.

10.45-11.05

Exploring biosynthetic potential & microbial biodiversity in Southern California’s deep ocean

Johanna Gutleben1*, Gabriel Castro-Falcón1, Alexander Chase2, Lisa Levin1, and Paul Jensen1
1. Scripps Institution of Oceanography, University of California, San Diego, La Jolla, California 92093, United States
2. Department of Earth Sciences, Southern Methodist University, Dallas, Texas 75275, United States
*Presenting Author: Johanna Gutleben, jgutleben@ucsd.edu

The deep-sea environment off the coast of California harbors valuable natural resources, such as phosphorite minerals and ferromanganese crusts. Such minerals are potentially subject to future exploitation throughout the global oceans as deep-sea mining becomes economically feasible. However, exploitation of these natural resources risks a loss of biodiversity, long-term ecological destruction, and degradation of invaluable ecosystem services such as bioprospecting. Currently, the Southern California Borderland (SCB) is poorly explored, and generally the animal and microbial communities associated to such mineral-rich substrates and their applications are mostly unknown.

In a mission to explore the SCB and collect baseline information prior to exploitation of such environments, we surveyed slopes, ridges, and seamounts by ROV to collect 134 samples of animals, minerals, and sediments at depths ranging from 300 – 2800m. Across sample types, we discovered an astounding amount of microbial diversity associated with mineral surfaces and animal types. This distinct microbial diversity translates also to high biopharmaceutical potential, as assessed by amplification of polyketide synthase subunits that encode a large diversity of natural products. For instance, analyses of 15 Cnidaria and 18 Porifera genera exhibited high host-specificity in their microbiomes and biosynthetic potential that suggest insights into the ecological roles of these molecules. Consequently, we characterized a bright yellow pigment from a glass sponge, which is structurally related to the bioactive microbial metabolite α-lipomycin, and putatively identified the biosynthetic gene cluster responsible for its biosynthesis within a dominant alphaproteobacterial symbiont.

In summary, these unpublished results underscore the importance of characterizing unknown biodiversity for potential applications. Beyond providing a valuable starting point for characterizing the pharmaceutical potential of the deep-sea, these results may provide a basis for future decision making and impact assessments should such mineral resources be considered for exploitation.

11.05-11.25

Biosynthetic potential of deep-sea sponge microbiomes

Maria Dell, Alena Streiff, Alessandro Lotti, Christoph Meier, Taro Shiraishi, Jackson K. B. Cahn, Christopher Field, Masato Kogawa, Eike Peters, Christian Rückert, Michelle Schorn, Kentaro Takada, Hiromi Yokoyama, Yuito Yamada, Jörn Kalinowski, Tomohisa Kuzuyama, Detmer Sipkema, Shinichi Sunagawa, Haruko Takeyama, Toshiyuki Wakimoto, Jörn Piel
Eidgenössische Technische Hochschule (ETH) Zurich, Zürich, Switzerland

Natural products serve as an inspiration for structural scaffolds in widely used pharmaceutically active compounds. To keep the flow of new lead structures into drug development pipelines, it is important to tap new sources of natural product diversity. Marine organisms are an exceptionally abundant source of compounds with unprecedented chemical entities exhibiting significant and potent biological activities, such as anticancer, neurotoxic, anti-inflammatory, and anti-infective properties. Especially sponges have repeatedly proven to harbour diverse natural products with a high prevalence for unprecedented and as-yet unique compounds. However, it is challenging to bring sponge-associated metabolites into the development pipeline for therapeutical use. This is due to the ecological and economic challenge of isolating sufficient biological material even for preclinical studies. The development of metagenomic sequencing has changed this prospective and enabled the identification of symbiotic bacteria as the true producers of these sponge-associated compounds. This creates exciting opportunities to characterize and produce novel specialized metabolites via engineered bacterial production platforms. This is exemplified by bacteria of the genus *Entotheonella,* which have been shown to be key representatives of the *Theonella swinhoei* microbiome and the main producers of compounds characterized from this sponge.

Here, we performed an extensive analysis of 9 sponge metagenomes containing filamentous bacteria of the *Entotheonella* genus, demonstrating them to be a rich source of unique biosynthetic gene

clusters and novel natural product potential. Several of these biosynthetic gene clusters have been linked to known sponge-associated natural products, setting the stage for attempts at heterologous gene expression attempts. Intriguingly, a significant number of the uncovered biosynthetic gene clusters remain unexplored in their encoded biosynthetic enzymes and associated natural products. We hypothesize that bacteria of the genus *Entotheonella* can be seen as super producer organisms. Our research seeks to uncover unprecedented enzymology and natural product chemistry hidden in the biosynthetic dark matter of these symbiotic bacteria, expanding our knowledge of complex sponge-associated natural product biosynthesis, and providing access to new potent bioactive metabolites to fuel drug discovery efforts.

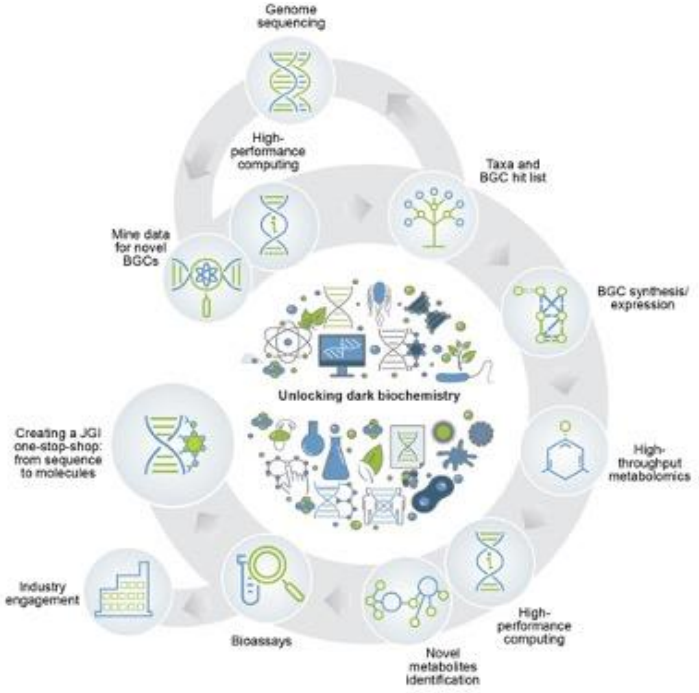
11.25-11.45

Leveraging scale to accelerate natural product discovery

Nigel J. Mouncey, Drew Doering, Jan-Fang Chen, Sylvia Kunakom, Hiroshi Otani, Daniel Udwary, Tanja Woyke, and Yasuo Yoshikuni
US Department of Energy, Joint Genome Institute (JGI), Berkeley, CA, USA
nmouncey@lbl.govJoint

The galaxy of natural products comprises a large family of diverse and complex chemical entities that have roles in both primary and secondary metabolism, and today >23,000 natural products have been

characterized. We are seeing a resurgence of activity in exploring secondary metabolites for a wide range of applications, due to not only increasing antibiotic resistance, but the advent of next-generation genome sequencing and new technologies to investigate natural product biosynthesis. There remains a treasure trove of natural products that remains to be unlocked. At the JGI, we are developing new tools and processes for identification of novel biosynthetic gene clusters from isolate genomes and metagenomes and complementing these with a suite of new experimental platforms to access the products of these clusters. We have built a new secondary metabolite biosynthetic cluster prediction pipeline and a data portal, the Secondary Metabolism Collaboratory, as a new community-centric resource. Furthermore, we have developed capabilities to understand how secondary metabolite biosynthesis is regulated, expression systems in native and heterologous hosts to access unexplored chemistry and used these to explore secondary metabolites from a range of organisms. I will share our recent developments in unlocking the Earth's Secondary Metabolome.



The Earth's Secondary Metabolome Initiative

Session 2: Molecular mechanisms and regulation of microbial secondary metabolites in microbiomes.

10 June 2024

13.00-13.30

Biosynthesis and molecular monitoring of harmful algal bloom toxins

Bradley S Moore UC San Diego & Scripps Institution of Oceanography, University of California San Diego, La Jolla, US

Freshwater and oceanic blooms of cyanobacteria and planktonic microalgae globally produce diverse toxins harmful to the environment and human health. Despite decades of research, the molecular basis for many microeukaryotic toxins had been unknown until only recently. This developing knowledge is beginning to provide new research opportunities to rapidly monitor and forecast the production of environmental toxins using the genetic basis of toxin biosynthesis. In this presentation, I will share some of our recent basic and applied work on marine (cyano)bacterial toxins such as the neurotoxin guanitoxin and the flame-retardant contaminant PBDE as well as microeukaryotic toxins such as the amnesic shellfish toxin domoic acid and the hemolytic fish toxin prymnesin. With harmful algal blooms intensifying on a global scale in frequency and severity in association with anthropogenic and climatic changes, our work is already beginning to impact how stakeholders monitor and forecast aquatic toxins.

13.30-14.00

Metabolic interactions in the soil microbiome

Elisabeth Shank Associate Professor, Department of Systems Biology, UMass Chan Medical School, Worcester, US

The activities of microbial communities have profound impacts on their hosts as well as on ecosystem-level processes. How microbes interact within these natural communities, however, remains largely unknown. The Shank lab addresses this gap by investigating microbial interspecies interactions mediated by specialized metabolites using a mix of traditional microbiology and genetics, coculture screening, bioinformatics, fluorescence microscopy, and mass spectrometry. We focus on how specialized metabolites impact microbial ecology by acting as cell-cell communication molecules as well as their applications to human health be acting as drugs such as antibiotics. Our overarching goal is to understand how microbial communities and their metabolite signals intersect to generate functional biological systems and to identify bioactive compounds to manipulate microbial communities to improve host health and the environment.

14.30-14.50

Coupling of secondary metabolite production in Bacillus subtilis

Caja Dinesen1,2, Carlos N. Lozano-Andrade1, Manca Vertot1, Scott A. Jarmusc1, Aaron J.C. Andersen1, Ákos T. Kovács1,2
1 DTU Bioengineering, Technical University of Denmark
2 Institute of Biology, Leiden University, 2333BE Leiden, The Netherlands

Bacillus subtilis produce a wide array of secondary metabolites (SMs) that aid its establishment in ecological niches such as plant roots. However, the regulation of biosynthetic gene clusters, including the interconnections between production of different SMs is unexplored. Here, we describe an inverse correlation between the relative levels of the lipopeptides, surfactin and the bacteriocin subtilosin A. These two SMs harbor vastly different properties, and therefore potentially different ecological roles. To explore this coupling, multiple assays were used to study the expression of the gene for subtilosin A synthesis (*sboA*) and production of the two SMs in various knockout mutants. Reporter assays revealed that *sboA* transcription is repressed in the presence of surfactin. Liquid chromatography-mass spectrometry and MALDI Mass Spectrometry Imaging further depicted that the production of subtilosin A is repressed in a mutant disrupted for surfactin production adjacent to a wild type colony, as a result of diffused surfactin from the wild type strain. Transcriptomics revealed the genes up-or down-regulated in the presence of surfactin.
Finally, we identified that the transcriptional regulator Rok influence the inverse production of surfactin and subtilosin A in *B. subtilis*. Our results reveal an intricate genetic regulation of natural product biosynthesis that possibly contribute to the ecological success of *B. subtilis*.

14.50-15.10

A metabolomics approach to uncover natural products from human-derived streptomycetes

Nicola U. Thome1,2, Joleen Masschelein2, Gilles P. van Wezel1
1 Department of Molecular Biotechnology, Institute of Biology, Leiden University, The Netherlands
2 Laboratory for Biomolecular Discovery & Engineering, VIB-KU Leuven Center for Microbiology, KU Leuven, Belgium
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The growing incidence of diseases caused by multidrug-resistant pathogens is a major threat to human health and necessitates the discovery of novel antibiotics and treatments. Herein, the human microbiome attracts interest as source of natural products with promising bioactivities and *in vivo* compatibility. We currently focus on an outstanding collection of streptomycetes isolated from the human lung.[1] Actinobacteria, especially *Streptomyces* spp., are well-known antibiotic producers, and yet their genomes harbor numerous not yet characterized biosynthetic gene clusters (BGCs), which correspond to so far undiscovered natural products.

As many BGCs are silent under routine laboratory growth conditions, we also grow the bacteria in different conditions, and then screen for changes in the production of specialized metabolites,

similar to the high-throughput elicitor screening approach.[2] In particular, we want to provide insights into how those human-derived strains respond to a range of human-associated molecules and hormones, which we study with comparative metabolomics. We use molecular networks built from untargeted LC-MS/MS data[3] to explore the chemical space of these human-derived isolates and to dereplicate known metabolites. In a first step to test the hypothesis that those specialized metabolites are also produced in the lung, we grow the bacteria in mucic acid-containing media.

With this ecology-inspired approach, we aim to identify compounds that activate the biosynthetic potential of Actinobacteria and apply them to find new antibiotics. The findings may furthermore indicate mechanisms that play a role in other microbiome-host interactions, too, and they may facilitate the development of drug-producing probiotics.

References:

[1] L. Kotrbova, A. C. Lara, E. Corretto, J. Scharfen, V. Ulmann, K. Petrickova, A. Chronakova, *Sci Rep* **2022**, *12*,9353.
[2] S. R. Lee, M. R. Seyedsayamdost, *Angew Chem Int Ed Engl* **2022**, *61*, e202204519.
[3] M. Wang, J. J. Carver, V. V. Phelan, L. M. Sanchez, N. Garg, Y. Peng, D. Duy Nguyen et al., *Nat. Biotechnol* **2016**, *34*, 828.

15.10-15.30

A bottom-up approach to unravel the role of quorum sensing in particle-associated marine microbial communities

Rachel Gregor, Rachel E. Szabo, Mel Nie, Otto X. Cordero
Department of Civil and Environmental Engineering, Massachusetts Institute of Technology (MIT), Cambridge, MA, US
Presenting author: Rachel Gregor rgregor@mit.edu

Subject headline: Secondary metabolite mediated interactions in microbiomes and microbiome-host-systems

Small molecules are the currency of the microbial world, as their production, consumption, and exchange underpin the most basic processes of microbial existence. While the importance of trophic interactions (e.g. cross-feeding of simple sugars and acids) in structuring microbial communities is well-established, the roles of specialized metabolites such as antibiotics and communication signals remain unclear. For example, quorum sensing (QS) signals have been studied extensively in model organisms and are predicted to be prevalent in marine bacteria based on genomes. However, studying QS in marine bacterial communities has proven challenging, with seawater microcosm experiments often yielding inconsistent results.

Here, we take a bottom-up approach to characterize QS signals in coastal marine bacterial communities that degrade particulate organic matter, a key crossroads in the microbial loop of the marine carbon cycle. We focus on a collection of 150 particle-associated bacterial isolates from these

communities, 60 of which have putative N-acyl homoserine lactone systems in their genomes. The putative QS-active isolates were screened for QS signal production using four fluorescent biosensors for short-chain, medium-chain, and aromatic signals, with hits verified by mass spectrometry. We further examine the potential for QS signal crosstalk between two functional groups within these communities, polysaccharide degraders and cross-feeders.

Session 3: Biotransformation and interactions of/between microbial secondary metabolites in microbiomes

11 June 2024

9.30- 10.00

Impact of antibiotic-degrading resistance on microbial community structure

Alex Hall Professor, Eidgenössische Technische Hochschule (ETH) Zurich, Switzerland

Some antibiotic-resistance mechanisms degrade antibiotics in the local environment. This can protect sensitive cells from antibiotic inhibition, but it is unclear how such effects play out in multi-species communities. We study the impact of clinically important carbapenemase-mediated resistance in experimental multispecies communities of bacteria. Carriage of a carbapenemase-encoding plasmid by one community member can reduce the local antibiotic concentration, but we found that some sensitive species benefit from this more than others. We identified phenotypic traits of component species that help to explain this variability, and therefore to predict changes in community structure caused by antibiotic-degrading resistance mechanisms. These effects can also be visualised in communities distributed across structured environments (on agar surfaces). In ongoing work, we are testing how sensitive the community-wide impacts of resistance are to species identity (which species carry resistance genes) and networks of horizontal resistance-gene transfer.

10.00-10.30

Visualizing specialized metabolite production in microbes

Laura Sanchez Associate Professor, University of California Santa Cruz, Santa Cruz, US

In nature, small molecules are often produced by macro- and microorganisms in order to facilitate communication and drive biological processes to the benefit (or detriment) of the community as a whole. Chemical gradients and chemical cues via the production of small molecules are ubiquitous across biological systems and my lab has used imaging mass spectrometry (IMS) to study these cues and gradients in biofilm forming Gram negative microbes and host-microbe interactions. In order to study the chemistry in specific microenvironments, we adapt IMS platforms to visualize the molecule maps that small molecules occupy in microbial cultures or host tissues. IMS has previously been used to create small molecule maps in fresh frozen tissue sections and spheroids. We have also begun to adapt the platform to incorporate trapped ion mobility spectrometry (tims) and MS/MS directly from the IMS samples themselves. I will discuss how we've applied our MS based tools to study cyclic-di-GMP in Vibrio cholerae, biofilm formation in *Pseudomonas aeruginosa*, and colonization of the light organ of Euprymna scolopes by *Vibrio fischeri*.

11.00-11.20

Cyclic-lipopeptides produced by Pseudomonas spp. are degraded by bacterial competitors

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The production of specialized secondary metabolites often governs bacterial interactions. In this regard, several species of bacteria secrete bioactive small molecules that can interfere with the growth and development of competing species. In response, competing bacteria may evolve mechanisms to cope with the effects of these antagonistic molecules. Here, we explored the interplay of these counteracting forces in nine Pseudomonas strains that produce structurally distinct cyclic-lipopeptides (CLPs) against five bacterial competitors. CLPs are a multifaceted family of natural products crucial in Pseudomonas ecology. They can act as antimicrobials, antagonizing other organisms but may also be required for motility, surface colonization, and signaling processes. By combining bioassays, metabolic profiling, and structural elucidation, we demonstrated how bacterial competitors inactivate Pseudomonas CLPs by linearizing the lipocyclopeptide structure. Furthermore, we showed that the size of the peptide macrocycle determined the sensitivity of CLPs towards hydrolysis and that phylogenetically different bacterial competitors exhibited distinct levels of hydrolysis activity. To shed light on the enzymes involved in CLP hydrolysis, we conducted proteomic analyses and identified candidate enzymes through mass spectrometry and heterologous expression. Overall, this study expands our understanding of the biotransformation of CLPs within specific niches, particularly upon interaction with neighboring rhizobacteria. Furthermore, since microbiome-driven, ecological processes are affected by secondary metabolites, our study highlights the importance of elucidating the fate of these molecules within microbiomes.

11.20-11.40

Discovery of novel metabolites and biotransformations in cellulose-based fungi–bacteria co-cultures

Ana M. Palacio-Barrera1,2**, Jethro L. Hemmann**1*, Ivan Schlembach1,2, Maurice Finger3, Jochen Büchs3, Gerald Lackner1,2, Miriam A. Rosenbaum1,2.
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Co-cultures of bacteria and fungi play an important role for the discovery of natural products involved in ecological interactions within microbiomes as well as for biotechnological applications. In particular, cellulolytic fungi enable access to carbon sources that would otherwise be unavailable to

partner organisms. However, co-cultivations present several challenges, such as controlling and monitoring the growth of each organism, prevention of the dominance of a single strain, and determining optimal conditions for secondary metabolite production. Here, we utilized a previously established cellulose-based co-cultivation system involving the fungus *Trichoderma reesei* and the natural product-producer *Streptomyces coelicolor*. In this system, *T. reesei* degrades cellulose, thereby providing soluble sugars to support the growth of *S. coelicolor*. By combining fluorescence-based biomass monitoring with high-resolution LC-MS/MS-based metabolomics, we explored how population dynamics impacts metabolite production at a wide range of inoculation ratios. By analyzing known secondary metabolites from *S. coelicolor*, distinct production profiles where observed. Some metabolites were produced predominantly in *S. coelicolor*-dominated cultures (e.g. actinorhodin), while others were maximal in balanced co-cultures (e.g. undecylprodigiosin). Interestingly, methylenomycin A levels were significantly induced in co-cultivations compared to axenic controls. To identify additional metabolites predominantly produced in co-cultures, we performed untargeted metabolomic analysis. Our analysis revealed that the siderophore dimerumic acid, produced by *T. reesei*, was converted by *S. coelicolor* into the dehydroxylated derivatives eleutherazine B and talarazine A, thus inactivating the siderophore. Further, we identified a set of novel metabolites highly induced in co-cultures that seemed to be structurally related to steroidal lactones of the withanolide class. Withanolides have not been described to be produced by any of the two organisms, thus highlighting the potential of the co-cultivation system for the discovery of natural products. In the future, the approach will be further expanded to explore the interaction of *T. reesei* with other Streptomyces strains.

11.40-12.00

Identification of specialized root exudates associated with microbiome assembly of wild and domesticated tomato

Jie Hu 1, Nejc Stopnisek1, Stalin Sarango-Flores1, Muhammad Rizaludin1, Somayah S. Elsayed2, Paolina Garbeva1,3, Jos M. Raaijmakers1, 2
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Domestication of plant species has significantly impacted on the rhizosphere microbiome composition, but the underlying chemistry of microbiome assembly in wild and domesticated plant species remains largely elusive. Here, we collected and identified water-soluble root exudates and root volatile compounds (VOCs) from 4 wild and 4 domesticated tomato species and analyzed their chemical composition via untargeted LC-MS/MS and GC-MS analyses. Secondly, we repeatedly inoculated the extracted root exudates into soils from the center of origin and from the center of tomato production to determine specific shifts in the soil microbiome. Thirdly, we deployed a transparent soil system to investigate root colonization and root metabolite degradation of three bacterial species in wild and modern tomato rhizosphere. *Streptomyces arenae* was found to be more associated with modern tomato rhizosphere, while *Cellvibrio* and *Sphingobium* spp. prefer

colonizing the wild tomato rhizosphere. First, we showed that the different tomato genotypes have distinct rhizosphere microbiome composition when grown in greenhouse production soil and native soil. Moreover, root exudate composition was distinctly different between wild and modern tomato genotypes, with specific mass features significantly more abundant in the root exudates of the wild tomato species. Preliminary results showed that *Streptomyces* colonized better the rhizosphere of domesticated tomato, while *Cellvibrio* was more abundant in the wild tomato rhizosphere. LC-MS/MS analysis further revealed that *Cellvibrio* can better cope with specific root exudate metabolites produced by wild tomato species. Collectively these results suggest that tomato plant specialized metabolites consititutes an important driver for recruiting specific microbial taxa. By integrating 'metabolomics' and 'microbiomics', we are now investigating and validating which specialized metabolites are key compounds in tomato domestication and the concomitant change in microbiome assembly.

Session 4: Secondary metabolite mediated interactions in microbiomes and microbiome-host-systems

11 June 2024

13.00-13.30

Back to the Roots

Jos Raaijmarkers Head of the department of Microbial Ecology, Netherlands Institute of Ecology (NIOO), Professor, Leiden University, The Netherlands

Microorganisms living on and inside plant tissues are key to plant growth and health, and modulate diverse phenotypic responses by their host, below- as well as aboveground. Plants divert a substantial part of their photosynthetically fixed carbon into the exudation of primary and secondary metabolites, thereby influencing the composition and beneficial activities of their microbiome. Microorganisms competing to establish and sustain lucrative associations with their host plants also invest in producing metabolites that may directly modify the environment, act as signals, antimicrobials or nutrient-scavenging agents. To date, the genomic diversity of plant microbiomes, their functional traits and the mechanisms underlying the chemical cross talk within the microbiome and between the microbiome and their hosts remain largely elusive. In my presentation, I will provide insight into the rich but largely untapped functional diversity of the plant microbiome, and the molecular and chemical dialogues within the microbiome and between the microbiome and their host. Fundamental knowledge on networking and small talk in plant microbiomes can be instrumental for new microbiome-assisted approaches to maximize the life-support functions of the microbiome for crop resilience.

13.30-14.00

Specialized metabolites play functional and defensive roles in stingless bees' microbiomes

Mônica T. Pupo, Professor, School of Pharmaceutical Sciences of Ribeirão Preto, University of São Paulo, Brazil

Stingless bees (Tribe Meliponini) are a monophyletic group of eusocial insects belonging to a larger group known as the corbiculate bees (Hymenoptera: Apidae), which also includes honeybees, bumble bees, and orchid bees. Stingless bees occur in all tropical and subtropical regions of the world, with approximately 550 species and 61 genera. Brazil harbors around 300 species of native stingless bees, which play important roles in pollination and meliponiculture. Similar to that observed for other social insects such fungus growing ants and termites, microbial symbiosis in stingless bees ranges from nutritional to defensive interactions. Our research group has pursued efforts in uncovering functions of microbial specialized metabolites in such symbiotic interactions. We have demonstrated that osmophilic yeasts in the genus Zygosaccharomyces grow inside the brood cells and supply ergosterol as a precursor for ecdysteroid biosynthesis by the larvae. We have

also isolated actinobacteria from bees, which seem to be involved in defensive symbiosis by producing different families of specialized metabolites, including new compounds, active against entomopathogens. In situ detection of microbial metabolites in bee colonies reinforces their ecological roles. Our results indicate that stingless bees establish different microbial interactions mediated by small molecules. In addition to defensive roles, microbial specialized metabolites have also demonstrated antimicrobial activities against human pathogens, including bacteria, fungi and protozoan parasites. Therefore, microbial symbionts of stingless bees represent a promising source for chemical ecology research and natural products discovery.

14.30-14.50

Co-culture of algal microbiome members *Sulfitobacter* sp. and *Halomonas* sp. inhibits fish pathogen *Vibrio anguillarum*

Dóra Smahajcsik*, Matilde Emídio de Almeida, Sheng-Da Zhang, Lone Gram
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Section for Microbial and Chemical Ecology, Department of Biotechnology and Biomedicine, Technical University of Denmark, 2800 Kgs Lyngby, Denmark

Microbial secondary metabolites play a crucial role in mediating interactions within microbial communities and often exhibit diverse biological activities, such as pathogen inhibition. Whole-genome sequencing of many bacteria has revealed a much larger secondary metabolite potential inherent within microbial genomes than what is observed under laboratory cultivation. In natural environments microbial interactions are influenced by complex abiotic and biotic factors, which cannot be reproduced under standard laboratory conditions. Conventional single-strain culturing often results in rediscovery of known metabolites; however, inducing silent genes could lead to discovering novel chemical diversity. Co-cultivation is a promising approach to elicit dormant biosynthetic gene clusters (BGCs). In our study, culturable bacterial strains have been isolated from the microbiome of the microalgae *Isochrysis galbana.* This algal microbiome can inhibit the fish pathogen *Vibrio anguillarum*. Inhibition of *V. anguillarum* by monocultures and co-cultures of five chosen isolates (*Alteromonas*, *Halomonas*, *Phaeobacter, Roseovarius* and *Sulfitobacter* sp.) was investigated in an agar-based assay. Pure cultures of *Phaeobacter* were inhibitory, but also the co-culture of a *Sulfitobacter* sp. and a *Halomonas* sp. inhibited *V. anguillarum*, that was not observed in the respective monocultures. Current efforts aim to unravel the mechanism underlying the inhibition. This entails whole genome sequencing of both strains using NanoPore technology and predicting their metabolic potential using antiSMASH. Furthermore, we characterize metabolites extracted from the inhibition zone to identify active compounds in the co-culture. Additionally, we aim to verify whether the observed inhibition extends to a liquid culture, confirming its relevance in a more dynamic system and a potential practical application. With this study, we strive to shed light on the factors behind the observed inhibition, offering valuable insights for aquaculture management strategies and potential novel bioactive metabolites.

14.50-15.10,

gutMAGIC: gutMicobriome analysis for metaGenomic identification of bioactive microbial compoundsk

Jacob Agerbo Rasmussen * & Morten T. Limborg
* Presenting Author, Postdoc, University of Copenhagen, Copenhagen, Denmark

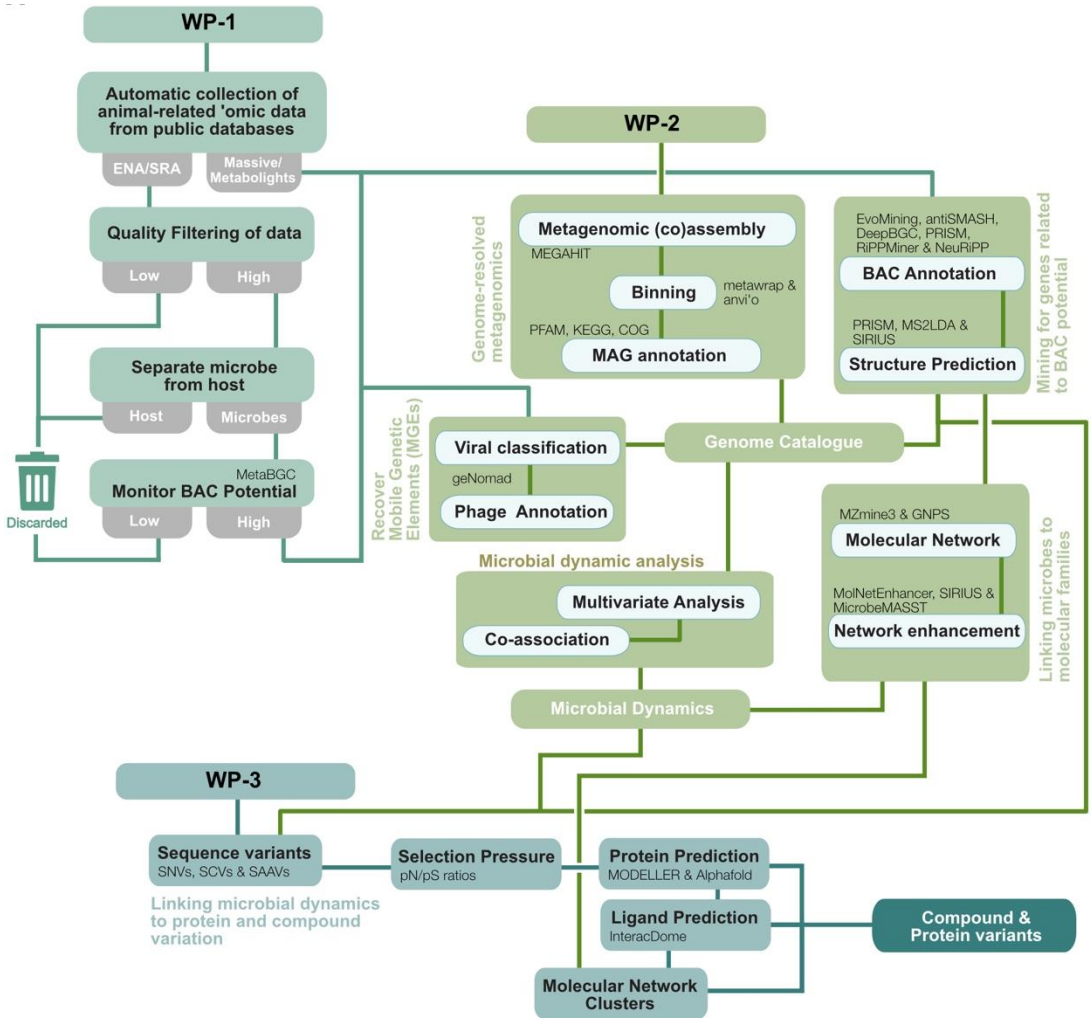
Is it possible to utilise microbial ecology to discover bioactive compounds and to increase the prediction of unknown compounds analogues in intestinal microbiomes?

The gut microbiota is a community of microorganisms that dwell in a mutualistic relationship with hosts in the gastrointestinal tract. Although the importance of gut microbiota has been recognised for a long time, it is only in recent decades that our understanding of gut microbiota began to surge because of the progress in advanced omic datasets, like metagenomics, metatranscriptomics, and metabolomics. Subsequently, the omics methods have advanced the search for microbial secondary metabolites, also known as specialised metabolites, which are organic compounds produced by microorganisms. These molecules are distinct from primary metabolites essential for the growth and development of the microbe.

Understanding the ecology and interplay of intestinal bacteria and phages is crucial for an increased understanding of the host-microbe-secondary-metabolite axis. Despite increased interest, less effort has been made to systematically survey secondary metabolites and phages in intestinal ecosystems, with most research focused on a single host. Hence, more research should be done to comprehensively investigate across multiple species to discover secondary metabolites and patterns related to synthesis and variation of secondary metabolism by the gut microbiome and how this relates to host-microbe interactions.

My proposed research proposes a new bioinformatic pipeline and aims to link microbe ecology to secondary metabolites in intestinal environments across animals and subsequently increase the understanding of the compound variation of secondary metabolites, using only public databases. I have accessed public nucleotide archives resulting in 5,452 animal-related metagenomes holding 24.26 Tbases of data, suggesting sufficient data to investigate host-microbe interactions and potential BGCs.

I propose a comprehensive perspective and the development of a new framework for mining intestinal for bioactive compounds in the lens of host-microbe interactions, using public databases.



15.10-15.30

Deciphering the antifungal activity of two ant-associated bacterial isolates: *Pseudomonas* sp. I3 and *Bacillus velenzensis* 1Ic.

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Danish Wood ants (Formica polyctena) may offer a sustainable strategy for controlling plant pathogenic fungi that avoids synthetic pesticides. This is partially due to the fact that the ants harbor bacteria with antifungal activity on their bodies and legs. These antifungal bacteria can be deposited onto the surroundings where the ant roam, and potentially form a protective shield against plant pathogens. We have isolated several ant-associated, antifungal strains of Bacillus, Pseudomonas, and Rouxiella. Members of these genera are well-known for their role in biological control, which makes ants and their associated microbiomes promising candidates for application in biological control.

The primary objective of this study is to develop and implement a pipeline streamlined to identify the antifungal compounds from the ant-associated bacteria. The pipeline integrates the combination of different methods, including chemical extraction and isolation of bioactive compounds, with a focus on genome mining for biosynthetic gene clusters (BGCs) and on the application of the highly manipulative CRISPR-associated transposon (CAST) system to generate sgRNA-guided knockouts of selected BGCs.

The initial findings, based on phenotypic assays and on crude extracts from lipopeptide-specific chemical extractions, suggest that both isolates produce lipopeptides with antagonism against plant pathogenic fungi. Furthermore, genome mining of BGCs also revealed the presence of lipopetide-encoding non-ribosomal peptide synthetase (NRPS) clusters in both isolates. Ongoing Bioassay-guided fractionation of crude extracts and CAST-mediated genome editing experiments will further elucidate the true nature of these antifungal compounds.

1

Bacterial Interactions in Soil regulate Pyoluteorin production in *Pseudomonas protegens* DTU9.1

Adelė Kaltenytė[1], Michael S. Cowled[2], Scott A. Jarmusch[2], Mikael L. Strube[2] & Lars Jelsbak[2]

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Bacterial communities engage in intricate interactions that play a vital role in the plant rhizosphere. Some members of the soil microbiome benefit plants by producing antifungal and antimicrobial agents, which can eliminate certain rhizosphere-colonizing parasites. Our model strain, Pseudomonas protegens DTU9.1 (DTU9.1), has the potential to serve as a biocontrol agent due to its ability to produce a wide range of secondary metabolites (SMs). One of the prominent SMs in DTU9.1 is an aromatic PKS-NRPS hybrid antibiotic known as Pyoluteorin (Plt). While Plt is a well-studied and documented molecule, there have been limited studies exploring its social role within bacterial communities.

In this study, we aim to place Plt in an ecological context and elucidate its role in bacterial interactions. Our findings reveal a significant increase in pyoluteorin production when DTU9.1 interacts with various bacterial strains, adding another layer of regulatory complexity to the Plt production pathway. Employing microbiological, molecular, and chemical techniques, we describe and clarify the interactions that lead to Plt production in DTU9.1 as well as look into the bacteria that causes Plt production.

Overall, our study seeks to uncover previously unexplored microbial mechanisms that govern the regulation of Pyoluteorin biosynthetic gene cluster. Bacterial interactions illustrate another angle to look into the regulation and production of Plt and give us a more comprehensive picture of what happens in the soil. This research will enhance our knowledge of SM induction, production, and their broader ecological roles within the soil community.

2 Linking fungal secondary metabolites and their biosynthetic gene clusters.

Adriana Romero-Otero, Steen S. Brewer, Jakob B. Hoof, Kai Blin, Tilmann Weber, Thomas O. Larsen.

Fungi are well-recognized for their ability to produce a variety of secondary metabolites, which bring opportunities to produce food additives, colorants, cosmetics, enzymes, and biopesticides. However, some fungal metabolites can generate serious health hazards and enormous economic losses, the mycotoxins.

An early assessment of the strains' metabolite production potential is a trending concern in industrial and academic biotechnology to avoid safety hazards and save time and money during product development. This is especially important for start-up companies developing novel fungal fermented foods since even strains generally recognized as safe (GRAS), such as *Aspergillus niger* and *A. oryzae*, out of the well-established conditions of its intended use, could potentially produce mycotoxins.

Dereplication and applying metabolomic high-throughput techniques, including mass networking tools, allow fast identification of compounds in complex samples. However, metabolomic approaches only reflect chemical production under the specific tested conditions.

Genome mining approaches, on the other hand, enable the identification of biosynthetic gene clusters (BGCs) responsible for forming a particular molecule on an organism, giving an estimation of its production capabilities. However, there is a mismatch between the number of predicted metabolites, known molecules, and molecules linking to their respective BGC, even for the most well-studied fungal species.

Therefore, to contribute to the easy assessment of fungal strains, new BGCs still need to be identified. This work uses a synergistic approach combining metabolomics, genome mining, and molecular biology tools to accomplish this task. Using genome mining approaches and retrobiosynthesis analysis, high-quality sequences of Aspergillus strains from the section Nigri allowed us to establish potential BGCs responsible for the biosynthesis of the detected compounds by untargeted analysis. Additionally, targeted detection methods were developed by liquid chromatography coupled to high-resolution mass spectrometry (MS), which will enable the fast dereplication of the target compounds during the forthcoming knock-out experiments.

3

Unveiling the surface-metabolome of Antarctic macroalgae towards temperature adaptations for Climate Change

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Climate Change has become a reality, and we are currently observing its consequences worldwide. Most negative scenarios suggest a rise in sea water temperature of up to +6° C, where polar environments are most vulnerable to these fluctuations. Antarctic macroalgae are key ecological players of polar intertidal niches, being their diversity and distribution a suitable model for biomonitoring Climate Change alterations. Previously, it has been suggested that some Antarctic macroalgae, such as *Adenocystis utricularis*, may be able to withstand the estimated increased temperatures. However, the role of the seaweed microbiome in conferring temperature fluctuations tolerance remains unknown. Therefore, in this project our main goal is to understand how *A. utricularis*, as a holobiont, is able to tolerate such thermal shifts, with a focus on its microbiome and its associated surface-metabolome. To accomplish this, we collected *A. utricularis* intertidal samples from King George Island, Antarctica, and submitted them to a thermal shift, from 2 °C as control, to 8 °C as climate change's most negative scenario. Additionally, macroalgae samples were treated with an anti-microorganisms cocktail to decrease surface microbiome loads, and also submitted to the same temperature shifts. After 5 days, all samples were collected for surface-metabolite extraction and subsequent untargeted metabolomics analyses. Chemoinformatic pipelines, involving mzMine, GNPS, SIRIUS with additional PCA statistics were conducted. Comparative metabolic profiling analyses show that there is a significant distance between the chemical space observed in depleted-microbiome samples, being specifically affected at the treatment of temperature shift. These results suggest the presence of metabolically-active microbial communities, producing compounds that are key in the macroalgae response to thermal stress. Future efforts are directed into understanding these global metabolic changes, with dereplication and characterization of the chemical nature of these compounds.

Funding: INACH Regular project RT_30-21

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4 Revealing the immuno-modulatory mediators of *Lactobacillus paracasei*-derived postbiotics using bioactivity-guided chromatographic fractionation

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Postbiotics are the metabolic products resulting from the fermentative metabolism of the probiotic strains inhabiting gut microbiota and sharing symbiotic relationship with the host. Despite the relevant evidence decribing the pivotal role of these microrganisms in several local and systemic functions of human metabolism, the precise chemical signature of the corresponding metabolites and their functionally-relevant molecules are yet to be defined. In this context, the current project aims to fully characterize a *Lactobacillus paracasei CNCM-I-5220*-derived postbiotic formulation produced following the proprietary PBtech® fermentative process. The major goal is understanding the molecules involved in the postbiotic mixture and responsible for mediating its functional effects. This is done through the multimodal, multistep, bioactivity-guided chromatographic separation of the postbiotic mixture, whose fractions are then tested by using *in vitro* functional assays and mass spectrometry analyses. Experimental evidence show that the anti-inflammatory and immuno-modulatory effect of the postbiotic formulation is mediated by its aqueous, polar components: these latter mainly consist of small molecules some of which were manually annotated as immunologically relevant based on what is reported in the literature. Size Exclusion and Ion Exchange Chromatography separation of the postbiotic mixture result in several fractions varying greatly in abundance and bioactive properties: fractions not displaying any functional effects were reported, as well as fractions sharing greater or even opposite effect to the full postbiotic. These results suggest that the functional properties displayed by the whole complex mixture result from the combination of several synergistic or antagonistic effects mediated by different molecular entities. Coherently, the chromatographic fractionation paradigm can be used as both a successful approach for the in-depth characterization of the whole postbiotic mixture and an effective tool for the production of a purified formulation with optimized functional performance.

5

Enhancing Antimicrobial Discovery: Microscale Fungal Cultures and Metabolomics for Rapid Evaluation and Prioritization of extracts.

Alexandre Bory[a,b], Marine Cosseron[a,b], Alexandre Luscher[c], Sylvain Schnee[d], Thilo Köhler[c], Katia Gindro[d] and Jean-Luc Wolfender[a,b]

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Subject headline: Synthesis and production of microbial secondary metabolites in microbiomes

Abstract: The challenge posed by antimicrobial resistance presents a growing concern for global health, emphasizing the urgent need for novel antimicrobials possibly featuring new modes of action. Microbial-derived natural products remain strong candidates, not only due to their established efficacy (Dai *et al.*, 2020), but also because their producers coexist with bacteria in shared ecological habitats, thus, constituting a promising avenue for the discovery of antimicrobial compounds. However, the time-intensive nature of their cultivation and extraction, coupled with the recurrent rediscovery of known active compounds, impedes the efficient screening of natural extracts.

To address this issue, we have recently devised a drug discovery platform leveraging the OSMAC approach (Bode *et al.*, 2002). This platform entails the cultivation of fungal strains, extraction processes, bioassays, and metabolomic analyses conducted in a standardized 96-well plate configuration, facilitating high-throughput capabilities and compatibility across screening and analytical platforms.

In our research endeavor, sixty fungal strains were cultured in triplicates across eight distinct liquid media, yielding approximately 1,500 fungal extracts. These extracts underwent enrichment via solid-phase extraction and were systematically subjected to antimicrobial and antivirulence bioassays alongside metabolomic profiling.

Among these extracts, I exhibited activity in several culture media. High-resolution mass spectrometry (HRMS) data revealed the presence of a metabolite with a chemical formula of $C_{22}H_{34}O_5$, strongly indicating the presence of the known antibiotic "pleuromutilin." As a proof-of-concept, *Clitopilus sp.* strain was upscaled, leading to the isolation of the active metabolite pleuromutilin along with a glycosylated analog.

This successful outcome underscores the efficacy of our workflow in swiftly identifying antibiotic compounds. This presentation will highlight the effectiveness of integrating rapid generation of highly reproducible extracts with systematic metabolomic profiling and antimicrobial screening to explore chemical diversity and prioritize extracts before targeted isolation of active candidates.

References:

Bode, H.B., Bethe, B., Höfs, R., Zeeck, A., 2002. Big Effects from Small Changes: Possible Ways to Explore Nature's Chemical Diversity. ChemBioChem 3 (7): 619–27.

Dai, J., Hand, R., Xud, Y., Lie, N., Wanga, J., Danc, W., 2020. Recent Progress of Antibacterial Natural Products: Future Antibiotics Candidates. Bioorganic Chemistry 101 (August): 103922.

6

A workflow for discovery and production of natural products useful for sustainable agriculture

Ana Calheiros de Carvalho, Agustina Undbarrena-Canusso, Carolina Cano-Prieto, Naiara Hurtado-Lopez, Miriam von Bargen, Luis Caleb Damas Ramos, Daniela Rago, Pablo Cruz-Morales and Jay D Keasling.

The continuous growth of the global population, coupled with the severe consequences of pests on crop yields intensified by climate change, persistently challenges the security of the world's food sources. Entomopathogenic fungi, capable of infecting and killing insects or altering their behavior, present potential for finding sustainable approaches to managing insect pests. In this study, our goal was to unveil new biosynthetic pathways and identify compounds and biosynthetic gene clusters (BGCs) with potential applications as biopesticides.

Through dereplication strategies involving chemical and phylogenomic analyses, we systematically annotated and classified molecules and their corresponding BGCs. This process included assessing the taxonomic distribution of these compounds and discovering new biosynthetic pathways. We established a strain collection of 92 insect-associated fungal strains and sequenced their genomes. Cultivating them under different conditions, we extracted their natural products for analysis using LC-MS/MS. Subsequently, we annotated and classified their BGCs, integrating chemical and genetic data to establish connections between metabolites and the corresponding gene clusters.

Our investigation resulted in a database containing hundreds of annotated compounds and their respective BGCs. We believe that this biosynthetic catalog of Hypocreales fungi can serve as a foundation for designing cell factories to discover and produce sustainable and more effective pesticides, as well as to engineer biocontrol strains. We believe that our findings will contribute to the advancement of sustainable agriculture and the reduction of dependence on synthetic pesticides.

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7

Gene-editing Approach to unveil Novel Bioactive Molecule in Sponge-associated *Streptomcyes*

Andrea Del Rio[a], Lina M. Bayona[a], Stephen A. Jackson[b], Alan D.W. Dobson[b], Gilles P. van Wezel[a]

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The application of antibiotics is indispensable for maintaining human health. However, the depletion of microbial drug discovery pipelines and the rapid spread of antibiotic resistance mean that bacterial infections may become untreatable. Marine microorganisms represent a vast, yet largely unexplored, reservoir of Natural Products (NPs). The MARBLES project (https://marblesproject.eu/) seeks to unlock the potential of untapped marine microbial biodiversity for the production of previously unknown or cryptic NPs.

Silent and cryptic Biosynthetic Gene Clusters (BGCs) can be activated through various methods, including the use of elicitors mimicking environmental signals, ribosome engineering, over-expression of regulatory elements, and DNA-editing techniques [1]. In this study, we investigate the biosynthetic potential of sponge-associated Streptomycetes. Initial metabolic screening of these strains revealed the production of antimycins by different Streptomycetes irrespective of their ecological niche or phylogenetic origin. Antimycins are a class of macrolides known for their broad and potent bioactivity [2], However, the constitutive and strong production of antimycins may mask other bioactivities, thereby impeding drug discovery efforts [3].

To address this challenge, we employ a genome-editing approach to either inactivate the antimycin BGC or silence it transcriptionally via modification of its activator binding site. This strategy aims to redirect metabolic flux towards alternative biosynthetic pathways and to liberate the Transcription Factor (TF) for activation of different BGCs characterized by lower-affinity TF binding sites. Our bioinformatic analysis identified several high-scoring target sequences within each analyzed genome that potentially regulate the expression of unidentified BGCs. This approach is designed to induce the expression of silent BGCs and facilitate the downstream identification of bioactive compounds with minor abundance or novel structural features.

This revealed major changes in the metabolome due to the activation of other biosynthetic pathways.

Our results will be discussed, focusing on the exploration of chemical diversity and bioactivity within marine sponge-associated Streptomyces.

References

1. Zhu, H., Sandiford, S.K. & van Wezel, G.P. Triggers and cues that activate antibiotic production by actinomycetes. J Ind Microbiol Biotechnol 41, 371-386 (2014).
2. Seipke, R.F. & Hutchings, M.I. The regulation and biosynthesis of antimycins. Beilstein J Org Chem 9, 2556-2563 (2013)
3. Culp, E.J. et al. Hidden antibiotics in actinomycetes can be identified by inactivation of gene clusters for common antibiotics. Nat Biotechnol 37, 1149-1154 (2019).

8

Trichlorination by a single cyanobacterial nonheme iron- and 2-oxoglutarate-dependent halogenase

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Halogenases are pivotal enzymes in natural products research by catalyzing the incorporation of halogen atoms into activated or unactivated organic molecules. Halogenation enhances not only the structural diversity, but also the bioactivity of these molecules making halogenating biocatalysts a main target for the synthesis of novel compounds with potential pharmaceutical application.

Among bacteria, cyanobacteria are a rich source of halogenases, mainly of nonheme iron- and 2-oxoglutarate-dependent and flavin-dependent halogenases. To date, there is a handful of cyanobacterial halogenases characterized such as AmbO5, WelO5, BarB1 and BarB2, JamE, CurA, and HctB to name a few. Those enzymes catalyze the incorporation of one to two chlorines per enzyme into the molecule.

In this work, we discovered a trichlorinated new natural product from Nostoc sp. through fatty acid supplementation experiments. We were able to connect the detected metabolite to its matching BGC and confirmed the halogenase substrate specificity in vitro through usage of fatty-acyl SNAC substrate surrogates. Rather than generating a trichloromethyl group, this halogenase demonstrates the ability to install three chlorine atoms onto different unactivated carbons of a fatty-acyl chain, namely in terminal and non-terminal positions.

The newly discovered cyanobacterial halogenase conducting an unprecedented trichlorination in multiple alkyl chain positions opens the door to engineer new halogenating catalysts and to discover additional halogenated natural products.

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9 Unusual cationic lasso peptides

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Lasso peptides are ribosomally synthesized and post-translationally modified peptides (RiPPs) produced by microorganisms. We have studied triculamin, an antibiotic against mycobacteria originally isolated in 1967. Through bioactivity-guided isolation and genome mining, we have discovered that it is, in fact, a very unusual lasso peptide. Unlike any known lasso peptides, their precursor peptides appear to have a follower instead of a leader peptide, and many of the processing enzymes remain cryptic. Our current genome mining attempts indicate that this unusual group of lasso peptides is represented in both canonical and cryptic triculamin-like biosynthetic gene clusters (BGCs). The canonical lasso BGCs are mainly found in Bacilli, Betaproteobacteria, and Gammaproteobacteria, whereas the non-canonical BGC is found in Actinobacteria, yet both encode similar lasso peptides. Currently, we are cultivating bacteria with either canonical or non-canonical BGCs to confirm the production of triculamin-like lasso peptides and performing heterologous expression. I will present some of our ongoing work attempting to understand the different biosynthesis of these similar yet unusual lasso peptides.

12

Building Biotechnological Platforms of Non-Ribosomal Peptides in *Saccharomyces cerevisiae*

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Non-Ribosomal Peptides (NRPs) are one of the main fountains of specialized metabolites and exhibit diverse natural roles such as interaction among microorganisms in a community (e.g. lipopeptides). Well-known NRPs are Penicillin, Daptomycin, Pyoverdines, Ferricrocin and the most potent known immunosuppressant, Cyclosporin. Access to the production of many of these compounds is a challenge, especially if the microbial strain is embedded in a microbial community. Heterologous expression of non-ribosomal peptide synthetases (NRPSs) let us discover and characterize novel compounds and their natural role. However, this approach is challenging due to the natural features of NRPSs: their structural complexity, their internal repetitive sequences, their capacity to incorporate non-proteinogenic amino acids that are synthesized by the host.

In our group, we aim to develop a biosustainable platform for the heterologous expression of large and complex NRPSs in the cell factory *Saccharomyces cerevisiae* to ensure the access of all types of NRPs, independently of their microbial origin. To achieve our goal, we have begun to build the house from the roof by reconstructing synthetically the NRPS Cyclosporin synthetase. Cyclosporin synthetase is a 1.6 MDa canonical NRPS encoded by 46.5 Kb codifying DNA. This mega-synthetase possesses four modules (M2, M3, M8 and M10) highly similar and responsible for the incorporation of Methyl-Leucine. Besides Cyclosporin synthetase activates and incorporates the non-proteinogenic amino acid 2-Butenyl-4-Methyl-L-Threonine (BmT) which is biosynthesized by a polyketide synthase.

To carry out our aim, we have synthesized twenty-one gBlocks and assembled them into eleven BioBricks to proceed to assemble and clone the entire synthetase. Here we are presenting our first achievements in the heterologous expression of Cyclosporin synthetase using an entire Synthetic Biology pipeline in *Saccharomyces cerevisiae*

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Towards NMR Spectroscopy-based Metabolomics

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Nuclear Magnetic Resonance, NMR spectroscopy is a powerful analytical tool applicable in a wide range of (bio)chemical fields of research. NMR spectroscopy has become the "gold standard" for structure elucidation, verification, and quantification due to its unique advantages such as remarkable reproducibility, automation and minimal sample preparation.[1] This poster seeks to present an overview of the historical, current and future developments within NMR spectroscopy relevant to the investigation of complex mixtures and molecular structures of biological origin.

The practice of metabolomics involves the broad range exploration and identification of measurable compounds in a biological metabolome. By focusing on complex mixtures, metabolomics seeks to link spectral patterns, or chemical fingerprints, to specific phenotypes such as disease states, ecological factors and genetic alterations.[2, 3]

The field of metabolomics is typically divided into two main categories. The untargeted approach entails conducting comprehensive chemometric analysis of all measurable metabolites to identify metabolite changes, interactions and possibly new entities. In contrast, a targeted approach seeks to track specific metabolite classes, their bio- synthetic potential, -activity and -transformation in biological systems.[4]

Highly variable metabolite concentrations pose a significant challenge for NMR-based metabolomics. The dynamic range of measured signals can easily hide important low concentration metabolites in a sea of highly upregulated chemistries. Stable isotope labelling is one approach to overcoming this challenge. The introduction of ^{13}C and ^{15}N can enhance both general and specific aspects of the metabolome aiding both targeted and untargeted metabolomics endeavours.[5]

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References:

[1] D. S. Wishart, L. L. Cheng, V. Copié, A. S. Edison, H. R. Eghbalnia, J. C. Hoch, G. J. Gouveia, W. Pathmasiri, R. Powers, T. B. Schock, et al, "NMR and Metabolomics—A Roadmap for the future," *Metabolites* 2022, 12, 678-698

[2] O. Fiehn, "Metabolomics – the link between genotypes and phenotypes", *Plant Mol. Biol.*, 2002, 48, 155-171

[3] Nicholson, J. and Lindon, J., Metabonomics, *Nature*, 2008, 455, 7216, 1054–1056

[4] L. D. Roberts, A. L. Souza, R. E. Gerszten, and C. B. Clish, "Targeted Metabolomics", *Curr. Prot. Mol. Biol.* 2012, 30.2, 1-24

[5] McKay, R. T., "Metabolomics and NMR," *Metabolomics and Its Impact on Health and Diseases. Handbook of Experimental Pharmacology*, 2022, 277, 74-108

14

High Throughput Culturomics Yields Potential Metabolic Tools in the Fight Against A Deadly Plant Pathogen

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The plant microbiome is a promising avenue for the next generation of crop improvement. However, most of our understanding of the plant microbiome is limited to culture-independent studies that are difficult to recapitulate and deploy. Looking to move beyond DNA based inferences, “culturomics” seeks to improve our ability to isolate microbiome members from complex environments and understand their contributions to their host. One area of study in which plant microbiomes have a distinct implication is within the presentation and potential treatment of the fatal disease of citrus plants, Huanglongbing. This insect-vectored bacterial disease is considered the biggest threat to continued cultivation of citrus worldwide. Previous studies have indicated that the citrus microbiome may have a distinct role in Huanglongbing development, and that certain microbiome members possess the capability to alleviate disease severity. Additionally, our group previously examined how Huanglongbing severity varied between clonal trees, and we found that this range of severity may be explained by the different microbial composition of trees with severe symptoms and those with less severe symptoms. To better understand the chemistries that may allow the citrus microbiome to suppress this disease, we established a pipeline to isolate and screen citrus associated bacteria from field grown trees against *Liberibacter crescens*, the culturable surrogate for the associated causal agent of Huanglongbing. Utilizing an automated high throughput microbial cultivation array technology, made possible by the Prospector® from Isolation Bio™, we established a pipeline that increased the diversity of a previously established citrus plant-associated microbial culture collection. Furthermore, our screening pipeline identified several bacterial isolates, new to our culture collection, that produce metabolites inhibitory to the pathogen surrogate. Through genomic characterization and bioassay guided fractionation, we are characterizing these chemistries, defining their activity as potential disease management tools, and unraveling their role in the citrus microbiome.

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Genome-mining for the discovery of biosynthetic gene clusters of known and novel antibiotics

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The advent of affordable long-read genome sequencing technologies coupled to our increasing understanding of the molecular basis directing secondary metabolite biosynthesis has revolutionized the field of natural products with the implementation of bottom-up discovery approaches.

Here we show that *in silico* analysis of the genome from the globomycin-producing strain *Streptomyces* sp. CA-278952 led to the identification of its hitherto unknown biosynthetic gene cluster (BGC). CRISPR-cBEST was employed to generate a null mutant that was unable to produce globomycin in any of the fermentation conditions tested. To further confirm the identity of the cluster, we successfully cloned and heterologously expressed the BGC in *S. coelicolor* M1146 and *S. albus* J1074, thus proving that the cluster is indeed responsible of globomycin biosynthesis and paving the way for the generation of novel *unnatural* globomycin derivatives.[1]

On the other hand, *in silico* analysis of the remaining BGCs revealed a cluster encoding a putative lipopeptide with an amino acid sequence containing an Asp-Gly-Glu-Ala motif. We envisioned that this motif could mimic the canonical Asp-X-Asp-Gly sequence found in calcium-dependent lipopeptide antibiotics. Chemical investigation of the fermentation broths led to the discovery of three new lipodepsipeptides, dilarmycins A-C. The calcium-dependent antibacterial activity of the compounds was established against methicillin-resistant *Staphylococcus aureus* and vancomycin-resistant *Enterococcus*, strongly suggesting a potential role of the Asp-Gly-Glu-Ala motif as a new calcium-binding motif that will need to be further explored.[2]

1. D. Oves-Costales, T. Gren, E. Baggesgaard Sterndorff, J. Martín, F. J. Ortiz-López, T. S. Jørgensen, X. Jiang, F. Román-Hurtado, F. Reyes, O. Genilloud and T. Weber, Synth. Syst. Biotechnol. 2023, 8, 206-212

2. I. Fernández-Pastor, F. J. Ortiz-López, D. Oves-Costales, J. Martín, P. Sánchez, A. Melguizo, F. Reyes, T. Weber and O. Genilloud, Org. Lett. 2024, 26, 1343-1347

16

The plant Aster tataricus harbours more than one fungal endophyte producing cytotoxic specialized metabolites

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Plants have been an important source of pharmaceutically relevant specialized metabolites in the past. They often harbour bacterial or fungal endophytes.[1,2] Novel fungal species, and in turn new producers of specialized metabolites with potential pharmaceutical use, are frequently isolated from plants. One famous example is the endophytic fungus *Cyanodermella asteris*, which has been found to be the actual producer of the cytotoxic astins,[3,4] initially isolated from the plant *Aster tataricus* that is used in traditional Chinese medicine.[5] During our work on *C. asteris* and *A. tartaricus*, we isolated several additional endophytes from the plant. Chemical screening of these endophytes and a tandem mass spectrometry based molecular networking study using GNPS[6] indicated the presence of several compounds that likely were not reported in the literature, yet. The producing fungus is a new species we named *Tengochaeta bulbillosa,* belonging to a genus from the Chaetomiaceae family which previously had only one other representative. Assays using the nematode *C. elegans* and cytotoxicity assays indicated biological activity of the strain's extract. Subsequent isolation and structure elucidation of the active compounds revealed new members of the azaphilone family, compounds known for their cytotoxic activity. One of the isolated compounds (Figure 1) was profiled in a screening against 60 cancer cell lines, revealing an interesting bioactivity profile.

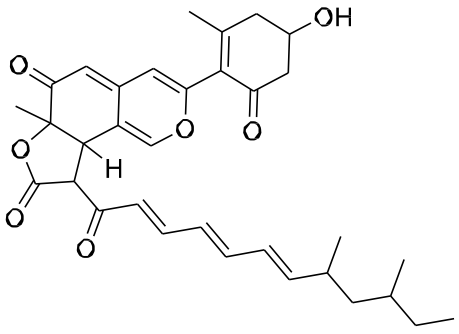

Figure 1 An azaphilone isolated from T. bulbillosa

References

Hardoim, P. R.; van Overbeek, L. S.; Berg, G.; Pirttilä, A. M.; Compant, S.; Campisano, A.; Döring, M.; Sessitsch, A. *Microbiol Mol Biol Rev* **2015**, *79*, 293–320, DOI: 10.1128/mmbr.00050-14.

Li, Z.; Xiong, K.; Wen, W.; Li, L.; Xu, D. *Int. J. Mol. Sci.* **2023**, *24,* DOI: 10.3390/ijms24021153.

Jahn, L.; Schafhauser, T.; Pan, S.; Weber, T.; Wohlleben, W.; Fewer, D.; Sivonen, K.; Flor, L.; van Pée, K.-H.; Caradec, T. *et al. mycotaxon* **2017**, *132*, 107–123, DOI: 10.5248/132.107.

Schafhauser, T.; Jahn, L.; Kirchner, N.; Kulik, A.; Flor, L.; Lang, A.; Caradec, T.; Fewer, D. P.; Sivonen, K.; van Berkel, W. J. H. *et al. Proceedings of the National Academy of Sciences of the United States of America* **2019**, *116*, 26909–26917, DOI: 10.1073/pnas.1910527116.

Wang, R.; Xiao, S.; Niu, Z. *AJTCAM* **2017**, *14*, 142–147, DOI:

10.21010/ajtcam.v14i2.15.

Nothias, L.-F.; Petras, D.; Schmid, R.; Dührkop, K.; Rainer, J.; Sarvepalli, A.; Protsyuk, I.; Ernst, M.; Tsugawa, H.; Fleischauer, M. *et al. Nat. Methods* **2020**, *17*, 905– 908, DOI: 10.1038/s41592-020-0933-6.

17

Uncovering the biosynthetic potential of uncultured cyanobacteria

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Cyanobacteria are prolific producers of structurally diverse secondary metabolites. However, their chemical exploitation based on cultured strains has been limited by low production titers and overall slow growth rates. The decreasing discovery rates of new compounds produced by cultured microbes together with the enormous unexplored biosynthetic potential of uncultured microorganisms boosted the development of culture-independent methodologies to uncover new natural products. Namely, metagenomics has enabled the capture and heterologous expression of biosynthetic gene clusters (BGCs) directly from environmental DNA to uncover the encoded metabolite. In this work, 20 environmental samples collected during CIIMAR sampling campaigns were screened for cyanobacterial diversity. Metagenomics allowed the recovery of 92 medium- and high-quality cyanobacterial MAGs from 9 distinct phylogenetic orders. AntiSMASH analysis of the recovered MAGs revealed 1612 BGCs from different biosynthetic classes. A microviridin BGC (*mvd*BGC) from a *Planktothrix* MAG was heterologously expressed in *E. coli* using Direct Pathway Cloning coupled with Sequence- and Ligation-Independent Cloning (DiPaCSLIC).
Two versions of the BGC were tested: one containing genes orf1-*mvd*ABCDEF, and other containing genes orf1-*mvd*ABCDEF-orf2. LC-HRESIMS analysis revealed differences between the chromatograms obtained from extracts of *E. coli* carrying the orf1-*mvd*ABCDEF BGC and those obtained for bacteria transformed with the orf1-*mvd*ABCDEF-orf2 BGC. Nonetheless, several ions linked to the *mvd*BGC were encountered in extracts from both transformants. None of the detected ions could be linked to the expected final peptide. The differences point towards a possible role of orf2 in microviridin biosynthesis. However, additional experiments are required to validate this hypothesis. Furthermore, an indole BGC from a *Calothrix* MAG and a microginin BGC from a *Planktothricoides* MAG are currently being assembled into *E. coli* for heterologous expression and compound identification. To the best of our knowledge, this is the first report of the use of DiPaC-SLIC to capture and heterologously express cyanobacterial MAG-derived BGCs.

18

Investigating interactions between *Zymoseptoria tritici* and *Pseudomonas* bacteria through multi-omic approaches

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Zymoseptoria tritici, the causal agent of Septoria tritici blotch disease (STB), is the most economically important wheat pathogen in Western Europe, resulting in up to 50% yield losses. Resistance to conventional fungicides is becoming an increasing problem, with very few effective control strategies available to farmers. New chemistry and fungicide targets are therefore urgently required. *Pseudomonas* are ubiquitous, gram-negative bacteria that can act as biocontrol agents of many plant pathogens and produce antimicrobial compounds. *Pseudomonas* bacteria possess a largely untapped diverse secondary metabolite repertoire, which has potential for the discovery of new fungicides or *Z. tritici* antagonistic compounds. We present the use of an *in vitro Z. tritici* antagonism assay, that can be used both qualitatively and quantitatively, to identify *Pseudomonas* isolates with *Z. tritici* antagonistic potential, through the production of secreted secondary metabolites. Combining genome-mining and gene disruption, we aim to identify and characterize BGCs mediating interactions between antagonistic *Pseudomonas* and *Z. tritici*. Using this approach, we have characterized *Pseudomonas* bacteria producing the known antifungal compound 2,4-diacetylphloroglucinol using site-directed mutagenesis, to link the BGC to antagonism phenotype. We also identified *Pseudomonas* isolates where the mechanisms of *in vitro* fungal antagonism are currently unknown. We found statistically significant differences in the responses of genetically distinct *Z. tritici* isolates to antagonism by pseudomonads in vitro. These findings suggest that combining in silico BGC prediction with *in vitro Z. tritici* antagonism assessment could reveal potentially novel antagonism mechanisms, providing insights for further novel BGC characterization through analytical chemistry approaches. In a wider context, these results highlight the potential need for multiple genotypes of fungal pathogen to be assessed in biocontrol studies, particularly for genetically diverse species such as *Z. tritici*, as resistance to bacterial antagonism may exist as a quantitative trait within natural populations.

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Bacillus stimulates the production of antimicrobial secondary metabolites upon perception of Pseudomonas secondary siderophores.

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Molecular interactions between microbes in competitive environments such as the rhizosphere are key for understanding microbiome functioning. It is particularly interesting for bacterial species known as strong producers of multifunctional Bioactive Secondary Metabolites (BSMs), such as *Bacillus velezensis*. Although BSM production is considered as an ecological adaptative trait, it is still poorly known how this arsenal is produced under natural conditions. In that context, interactions between these rhizobacteria and other members of the plant microbiome represent a key component that may help us to elucidate the modulation of the production of their BSMs. Our research work focuse on the outcomes of interspecies interactions on the expression of the secondary metabolome of the plant-associated and beneficial bacterium *B. velezensis*. We observed that, by interacting with a *Pseudomonas* competitor, *B. velezensis* specifically stimulates the production of polyketides and cyclic lipopeptides leading to an enhanced antimicrobial potential. We identified the *Pseudomonas* secondary siderophore enantiopyochelin (ePCH), as main trigger of this *Bacillus* response and are investigating the mechanistics underpinning ePCH perception. Our results further indicate that this chelator sensing phenomenon is not specific to *B. velezensis* and ePCH but extends to other soildwelling *Bacillus* species that can also perceive other bacterial secondary siderophores. We therefore anticipate that this concept of chelator sensing may represent a new facet of siderophore-mediated bacterial interactions beyond the concept of competition for iron and siderophore piracy.

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Wood ants and their associated microorganisms inhibit plant pathogens

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Danish Wood ants (*Formica polyctena*) may offer a sustainable strategy for controlling plant pathogenic fungi that avoids synthetic pesticides. This is partially due to the fact that the ants harbor bacteria with antifungal activity on their bodies and legs. These antifungal bacteria can be deposited onto the surroundings where the ant roam, and potentially form a protective shield against plant pathogens. We have isolated several ant-associated, antifungal strains of *Bacillus, Pseudomonas, and Rouxiella*. Members of these genera are well-known for their role in biological control, which makes ants and their associated microbiomes promising candidates for application in biological control.

The primary objective of this study is to develop and implement a pipeline streamlined to identify the antifungal compounds from the ant-associated bacteria. The pipeline integrates the combination of different methods, including chemical extraction and isolation of bioactive compounds, with a focus on genome mining for biosynthetic gene clusters (BGCs) and on the application of the highly manipulative CRISPR-associated transposon (CAST) system to generate sgRNA-guided knockouts of selected BGCs.

The initial findings, based on phenotypic assays and on crude extracts from lipopeptide-specific chemical extractions, suggest that both isolates produce lipopeptides with antagonism against plant pathogenic fungi. Furthermore, genome mining of BGCs also revealed the presence of lipopetide-encoding non-ribosomal peptide synthetase (NRPS) clusters in both isolates. Ongoing Bioassay-guided fractionation of crude extracts and CAST-mediated genome editing experiments will further elucidate the true nature of these antifungal compounds.

22

Discovery of antibiotic producing marine microorganisms via highly parallelized microfluidic droplet cultivation

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The continued advance of antimicrobial resistance (AMR) poses a critical challenge to public health, underlining the imperative need for the discovery of novel antibiotics. As traditional antibiotic sources become increasingly depleted, the rich microbial diversity and biosynthetic potential of marine environments emerge as a promising yet underexploited reservoir for novel antimicrobial agents. In this study, we harness the power of droplet microfluidics technology to cultivate novel marine microorganisms and screen for their antimicrobial activity against a fish pathogen, *Vibrio anguillarum*, in a high-throughput fashion. Employing a comprehensive methodological framework that includes single-cell droplet encapsulation, pico-injection with a fluorescently tagged pathogen, and fluorescence-activated droplet sorting (FADS), we screened 750,000 droplets from which a subset of 752 were sorted out based on a reduction in the fluorescent signal from the pathogen. This led to the successful cultivation of five bacterial isolates for which antimicrobial activity against *V. anguillarum* could be confirmed through well-plate assays. These were assigned to the species *Vibrio splendidus, Vibrio rumoiensis, Vibrio lentus, Sulfitobacter marinus*, and the genus *Enterovibrio*. In addition to the *Enterovibrio* sp. isolate, which is likely a novel species, other isolates, for which inhibition was not observed in the well-assay, had 16S rRNA gene sequence similarities around the species cutoff of 97%, suggesting that we have isolated novel species of the genera *Psychromonas* and *Mesorhizobium* as well. Future work will include genome sequencing of these isolates to identify potential biosynthetic gene clusters and secondary metabolites responsible for their bioactivity. Collectively, our findings demonstrate the efficacy of droplet microfluidics in uncovering previously uncultured microorganisms from the environment, and that the highly parallelized nature of the approach successfully allows for screening of hundreds of thousands of micro cultures at once.

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Autoinducer 3: A one-step construction of the DPO ring system and the formation of both DPO isomers

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Salmonella typhimurium utilizes quorum sensing to coordinate collective behavior in a density dependent manner. In recent years autoinducer-3 has been detected in first, Vibrio Cholerae as 3,5 DPO and since E. coli as 3,6 DPO. Both isomers were proposed to play a significant role in regulation of biofilm formation and virulence which makes it an interesting target for anti-infective treatment. Since then both isomers have been detected in Salmonella typhimurium though their role in virulence regulation is yet to be revealed. Previously it was established that 3,5 DPO is the predominant isomer of the two proposed structures but to further investigate the biological effects the development of a selective synthesis of 3,5 DPO and its analogous structure was required. We will present research on a one-step construction of the DPO ring system and the formation of both DPO isomers.

24

Detection of Flavin-dependent Halogenases in antiSMASH: Covering a wide range of canonical and recently explored enzymes

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Small bioactive compounds produced by microorganisms are important to develop new drugs, agrochemicals, and more. These specialized metabolites were traditionally discovered by chemical isolation, purification, and bioactivity testing of compounds from environmental isolates. With the explosion of data available from genome sequencing, this approach is supplemented with genome mining of producer organisms to identify interesting biosynthetic gene clusters (BGCs).

antiSMASH[1] currently is the most popular tool for specialized metabolite genome mining. Besides providing information about the genes present in BGCs, it also gives a rough core structure prediction of the possibly produced natural product (NP).

The more we can predict about the chemical structure of a NP, the better researchers can tailor their lab-based approach to explore its production and the more informed idea they can have about its bioactivity. Therefore, this project aims to improve the structure prediction features in antiSMASH by considering the important effect of halogenases in the BGC. The first step was to create a computational workflow to detect and categorize flavin-dependent halogenases based on their substrate and the position of the substitution. This workflow is suitable for detecting canonical enzymes that possess all the conserved motifs in the family, and bifunctional or uncanonical members that lack these patterns.

Here, I will present the methods applied in the computational process and the biological information used as the basis of categorization. This work will improve the utility of sequence-based mining and move towards accurate structural prediction of halogenated compounds.

1: antiSMASH 7.0: new and improved predictions for detection, regulation, chemical structures, and visualisation Kai Blin, Simon Shaw, Hannah E Augustijn, Zachary L Reitz, Friederike Biermann, Mohammad Alanjary, Artem Fetter, Barbara R Terlouw, William W. Metcalf, Eric JN Helfrich, Gilles P van Wezel, Marnix. H Medema & Tilmann Weber Nucleic Acids Research (2023) doi: 10.1093/nar/gkad344.

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Unprecedented Antifungals Inspired by Actinobacteria-Fungal Interactions

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Fungal phytopathogen and fungi-like oomycetes contribute to substantial global yield losses in agricultural production annually, with several fungi known to produce toxins that have severe impacts on animal and human health. While synthetic pesticides have been widely used to chemically control microbial infestation in agricultural fields, their extensive use has raised serious concerns for human health and the environment. With a vision to create more resilient and sustainable food systems to feed a growing world population, the European Commission has recently committed to reducing the overall use and risk of chemical pesticides by 50% by 2030.

Biological controls, involving the introduction of naturally occurring agents such as beneficial microorganisms or crude extracts from natural products directly into a natural ecosystem, have emerged as one of the most promising approaches to mitigate crop diseases. As Actinobacteria are the most important antibiotic producer and co-exist with fungi in nature, we aim to investigate the biotechnological potential of Actinobacteria and their secondary metabolites for use in the biocontrol of phytopathogens through advanced chemical ecology and natural product chemistry.

This presentation will cover the isolation of Actinobacteria from Danish soil and unveil the roles and interactions of the isolated Actinobacteria and their derived secondary metabolites against the fungal phytopathogens, *Alternaria solani* and *Phytophthora infestans*. Additionally, LCMS-based metabolomics analysis of the prioritized strains will be described, and the on-going workflow involves large-scale fermentation and bioassay-guided isolation for the identification of antifungal compounds.

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Deciphering the chemical dialogue between Bacillus and pathogenic fungi

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In nature, bacteria frequently form bacterial communities known as biofilms, where cells are embedded within an extracellular matrix (ECM) that provides protection against external aggressions or facilitates the efficient uptake and utilization of available resources. Interactions with other microbes can notably alter the community structure and, consequently, the nature of the relationship with the environment1. Previous studies of our laboratory have demonstrated the significance of biofilm formation in the antagonistic interaction between Bacillus and the phytopathogenic fungi Botrytis in the melon phyllosphere2. Our hypothesis is that the ECM plays a complementary role to the structural aspects of this antagonistic interaction.

In this study, we dissect how the different components of Bacillus ECM mediate the adhesion of bacterial cells to Botrytis hyphae, which could enhance the efficient release of antifungal metabolites. We also describe how several purified components of the ECM and specific secondary metabolites of Bacillus participate in the chemical communication between Bacillus and Botrytis, thereby altering the physiology and metabolism of Botrytis. Our findings unveil that during this antagonistic interaction, Botrytis secrets different oxylipins, defence molecules capable of killing Bacillus. In response, Bacillus increases the production of several secondary metabolites, which appears to have antifungal effects.

Our results underscore the urgency of further investigation of these interactions with the aim of identifying and describing adaptation processes that either lead to the exclusion or coexistence of two initially antagonistic microorganisms.

[1] Dragoš A, Kovács ÁT. The Peculiar Functions of the Bacterial Extracellular Matrix. Trends Microbiol. 2017 Apr;25(4):257-266. doi: 10.1016/j.tim.2016.12.010. Epub 2017 Jan 11. PMID: 28089324.

[2] Berlanga-Clavero, M.V., Molina-Santiago, C., Caraballo-Rodríguez, A.M. et al. Bacillus subtilis biofilm matrix components target seed oil bodies to promote growth and anti-fungal resistance in melon. Nat Microbiol 7, 1001–1015 (2022). https://doi.org/10.1038/s41564-022-01134-8

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Whole-cell biosensors for detection of bacterial and plant signals present in the soil microbiome

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The soil microbiome inhabiting the plant rhizosphere and the interactions between microbes and plant roots play essential roles in plant health and resilience. Most inter- and cross-species interactions between microbes and plants can are mediated by key secondary metabolites (SecMet). However, it remains challenging to capture these chemical interaction events *in situ*. Therefore, our understanding of how plant-beneficial rhizobiomes are formed and how they function is limited. The aim of the project is to design and implement bacterial whole-cell biosensors to not only detect various SecMets, but also record the detection event in genetic memory devices such that we retain the spatial and temporal dynamics of the SecMet production. As opposed to typical sense-and-report biosensors utilizing e.g., fluorescent reporters, we store the detected signal as irreversibly "flipped" DNA sequences on the sensor plasmid for subsequent analysis. The flipped part can either be a fluorescent reporter protein (e.g. GFP) or a 50 bp DNA barcode. In the former system, the proportion of "flipped" cells within a population can be determined with flow cytometry methods whereas in the ladder, quantitative PCR reactions with specified primers are used. Currently, we have developed sensors capable of detecting five different SecMets from both bacterial and plant species, namely: tetracycline, 2,4-Diacetylphloroglucinol, pyoluteorin, salicylate and naringenin. The sensor regulatory circuit are to be chromosomally integrated in root-colonizing bacteria and the resulting whole-cell biosensors can then in various combinations (sensor cocktails) be inoculated in the rhizosphere.

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High-efficiency genome editing of marine bacterial isolates enables monitoring of TDA-mediated interactions between microbial community members

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The secondary metabolism of marine bacteria is a research field met with increasing interest, in part because many secondary metabolites are known to possess antimicrobial activities. For example, several species of the obligate marine genus *Phaeobacter* are promising biocontrol agents, capable of eliminating pathogenic *Vibrio* spp. from aquacultures by virtue of the antimicrobial secondary metabolite tropodithietic acid (TDA). However, secondary metabolites such as TDA carry out a broader spectrum of ecological functions than being mere molecules of competition. Likewise, recent environmental studies demonstrate that the members of a marine microbial community, including a *Muricauda* sp. and a *Mameliella* sp., are tolerant and able to co-exist with a TDA-producing *Phaeobacter* sp. These observations raise the question of whether a dedicated molecular mechanism exists in these microbial community members that allows them to sense and deflect TDA, thereby explaining their tolerance. In order to identify the pathways and associated biomolecules of the surrounding microbiome which interact directly with TDA released from *Phaeobacter* spp., tools for efficient engineering of marine microbiomes are needed. For this purpose, the recently developed DNA-editing all-in-one RNA-guided CRISPR-associated transposase (DART) system will be used to perform precise genome editing in *Phaeobacter piscinae* strains, with and without the ability to produce TDA, as well as in the two non-model marine bacterial isolates *Muricauda* sp. and *Mameliella* sp. for which there are currently no genetic toolbox available. We aim to show how the DART system can be delivered efficiently into these bacteria via conjugation, and followingly demonstrate how different fluorescent reporter genes can be integrated at specific genomic locations with no or few off-targets. Tagging each bacterium with a unique fluorophore using the DART technology will allow us to discriminate them in co-culture setups, in turn enabling us to monitor TDA-mediated interactions in the microbiome.

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FunToxins: A comprehensive database that integrates experimental and biosynthetic data on fungal toxins

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Mycotoxins pose a serious threat to human and animal health. These fungal-derived natural products can cause both acute toxicity and long term effects such as immune deficiency and cancer. Mycotoxins are commonly produced by food spoiling fungi and also by strains used for industrial food and enzyme production, especially *Aspergillus, Penicillium* and *Fusarium* species. In order to evaluate the mycotoxin producing potential of these strains, it is essential to identify which genes are involved in mycotoxin biosynthesis. At the moment, there is no comprehensive database available that contains biosynthetic information about fungal toxins. We present FunToxins; a new open source database that collects manually curated biosynthetic and experimental data of over 250 mycotoxins, including information on the type of toxicity and the fungal species that produce them. Where possible, compounds are linked to biosynthetic gene clusters represented in the Minimum Information about a Biosynthetic Gene cluster (MIBiG) database. Having this information openly available will aid in the identification of mycotoxins in food and prevent them from causing harm.

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Quorum sensing autoinducer-3 in *Salmonella* Typhimurium: from its biosynthesis to its impact on cell physiology

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Quorum sensing (QS) is a bacterial intercellular communication system using small, secreted molecules (auto-inducers, AIs) to coordinate population wide-behaviors in a cell-density dependent manner. The multispecies AI-3 has been reported to play roles in virulence and biofilm formation, and recently, in *Escherichia coli* and *Vibrio cholerae*, the structure of AI-3 was proposed to be either 3,5- or 3,6-dimethylpyrazin-2-ol (DPO) (Figure 1). However, AI-3 synthesis and its role in the major human pathogen *Salmonella* Typhimurium (STM) remains largely unknown. In this study, we first developed a method for measuring the production of DPO in complex biological samples using UHPLC-MS/MS. Our method also, for the first time, enables distinction between the two isomers 3,5- and 3,6 DPO. Using this method, we were able to monitor the biosynthesis of 3,5/3,6 DPO in *V. cholerae, E. coli* and STM and explore the genetics of its biosynthesis. In STM, we find that biosynthesis of both isomers is dependent on the presence of L-threonine and the enzyme L-threonine dehydrogenase encoded by the *tdh* gene. With that knowledge, we explored the STM transcriptional response to DPO biosynthesis, and showed that virulence behavior in a mice infection model was promoted by biosynthesis of this proposed AI-3. Through future experiments, we hope to reveal the potential receptor for DPO in STM and unfold the molecular response to this newly found interspecies signaling molecule AI-3.

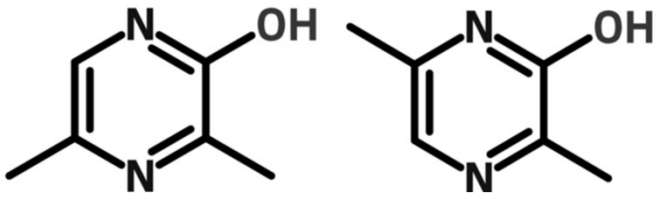

Figure 1 : DPO molecule. On the left, 3,5-dimethylpirazin-2-ol and on the right, 3,6-dimethylpyrazin-2-ol

Keywords: *Salmonella* Typhimurium, Quorum sensing, autoinducer, biofilm, virulence

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Secondary metabolites are strong predictors of inhibition in biofilms.

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Listeria monocytogenes is a frequent contaminant of industrial surfaces and in food products. One hypothesis is that *L. monocytogenes* benefits from living in multispecies communities, and thereby persist despite of cleaning, because neighbor bacterial species produce a protective matrix. We set out to investigate the basic principles underpinning this hypothesis and tested whether some species enhance the number of *L. monocytogenes* adhered to steel surfaces after washing. To test this, we cultivated *L. monocytogenes* Scott A harboring plasmid pNF8 (encoding GFP) as biofilms on steel beads with 52 different isolates, respectively. Due to the green fluorescence encoded by the plasmid, it was possible to quantify the number of *L. monocytogenes* by flow cytometry. Interestingly, none of the strains increased the number of *L. monocytogenes* significantly, while several of the strains reduced the number of this focal species. By quantifying phenotypic and genotypic characteristics of the cohabitants, we found that genome size was the strongest predictor, and cell-free supernatants also were able to reduce growth of *L. monocytogenes*. A Pearson correlation matrix revealed that genome size correlated strongly with number of genes encoding for secondary metabolites. Thus, we proposed a new hypothesis; The number of secondary metabolites encoding genes in the genome is a predictor of inhibition in dual-species biofilms. To test, we assessed the abundance of two different focal species, namely *L. monocytogenes* and *Vibrio anguillarum*, when mixed with 64 new isolates, respectively. Here, the number of secondary metabolite gene clusters showed a strong negative correlation with the abundance of the focal species (R^2=0.61). Closer inspection revealed that some secondary metabolite encoding genes were strongly associated with reduction, while others were not. All in all, this study suggests that intelligent design of bacterial communities that aim to reduce contamination can benefit from taking secondary metabolites into consideration.

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Exploring the Taxonomic and Chemical Diversity of Wheat Root-Associated Fungi

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Plants host a variety of microbes that confer important benefits, including nutrient acquisition, pathogen suppression, phosphorus solubilization, and abiotic stress tolerance. Understanding the taxonomic composition and chemical potential of the rhizosphere microbiome, and how it differs from that of the bulk soil microbiome, is crucial for manipulating it to improve plant performance. Here, we explored community composition of wheat root-associated fungi and its chemical diversity through the integration of culture-dependent methods and untargeted metabolomics. We isolated and identified over 350 fungal strains from the rhizoplane, rhizosphere, and bulk soil of wheat plants, collected under controlled greenhouse conditions and from fields in Denmark. Our analysis revealed a predominance of fungi belonging to the Penicillium genus, particularly within the sections *Lanata-Divaricata, Canescentia*, and *Fasciculata*, along with other isolates from genera *Trichoderma, Fusarium, Mucorales*, and *Talaromyces*. Distinct fungal communities were identified across rhizocompartments, with rhizoplane hosting a small number of abundant fungi, while the bulk soil exhibited high diversity, including *Fusarium sp., Rhizopus sp.*, and *Mucor sp*. found predominantly in this compartment. The fungal community composition of field-grown plants demonstrated similarities with those under controlled greenhouse conditions. Chemical profiling of fungal strains was conducted using LC-MS/MS and molecular networking analysis. The dereplication of fungal extracts revealed the presence of several known bioactive metabolites, such as mycophenolic acid, gliotoxin, fumagillin, pseurotin A, roquefortine C, griseofulvin, and penicillin acid. The exploration of the molecular network indicated the presence of previously unknown analogs. This study identifies core fungal species and their chemistry, providing a starting point for the development of synthetic microbial communities.

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Featuring cyclic lipopeptides as key players in *Bacillus* chemical ecology in the rhizosphere

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Microbial natural products are widely explored for their therapeutic potential, yet understanding the underlying evolutionary and adaptive forces driving their production remains a fundamental question in biology. Amphiphilic cyclic lipopeptides, a prominent category of bacterial specialized metabolites, show strong antimicrobial activity, particularly against phytopathogens. It is thus assumed that these compounds are deployed by soil- or rhizosphere-dwelling *Bacillus* spp. as microbial weapons in competitive natural environments. Here we challenge this reductionist perspective and present evidence that *Bacillus* CLPs are prominent chemical mediators of ecological interactions deployed to communicate, compete, defend against predators, or cooperate and establish mutualistic relationships with other (micro)organisms. This broader perspective underscores the need for further investigation into the role of CLPs in shaping the adaptive strategies of key rhizobacterial species. It includes resolving the mechanistics underpinning functional specificity of lipopeptides taking into account their outstanding chemical diversity. This requires combined expertises in molecular biology, structural chemistry and biophysics to comprehensively understand their complex interactions with biological membranes. Further insights into the natural functions of key metabolites such as CLPs are needed first for better understanding the chemical ecology of keystone bacteria but the ultimate objective is to harness these insights for practical purposes, such as developing environmentally friendly pest management strategies or discovering new pharmaceutical compounds.

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Investigations of the NRP/PK hybrid, BE-43547, with hypoxia-dependent bioactivity and production from the marine isolate *Micromonospora* sp. RV43

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It is widely understood that the Actinobacterial genomes often encode 20-40 biosynthetic gene clusters (BGCs), but the full potential remains under-explored. There is simply a huge discrepancy between the potential and actual production. Intrigued by rare hypoxia-selective cytotoxicity and antimicrobial activity, we have taken interest in an NRP/PK hybrid natural product family containing a 4-amido-2,4-pentadienoate (APD) moiety[1]. While applying the OSMAC strategy (One Strain Many Compounds)[2] in an attempt to circumvent the problem of having tightly regulated specialized metabolites in an unselective approach, we hypothesized that bioactivity could be used to predict regulation. We, therefore, investigated the possibility of a connection between the hypoxia-selective bioactivity and production conditions of the BE-43547 APD-containing compounds. Facilitated by bioreactors capable of controlling aeration during cultivation of the marine isolate *Micromonospora* sp. RV43[3], we found a significant increase in production titers during hypoxia. Combining this approach with genome mining, we hypothesize that bioinformatically predicted novel natural products of this family can be produced, leading to the identification of yet unknown compounds

References

1. Villadsen, Nikolaj L., Kristian M. Jacobsen, Ulrik B. Keiding, Esben T. Weibel, Bjørn Christiansen, Thomas Vosegaard, Morten Bjerring, et al. “Synthesis of Ent-BE-43547A1 Reveals a Potent Hypoxia-Selective Anticancer Agent and Uncovers the Biosynthetic Origin of the APD-CLD Natural Products.” Nature Chemistry 9, no. 3 (March 2017): 264–72. https://doi.org/10.1038/nchem.2657
2. Bode, Helge Björn, Barbara Bethe, Regina Höfs, and Axel Zeeck. “Big Effects from Small Changes: Possible Ways to Explore Nature’s Chemical Diversity.” ChemBioChem 3, no. 7 (2002): 619–27. https://doi.org/10.1002/1439-7633(20020703)3:7<619::AID-CBIC619>3.0.CO;2-9
3. Abdelmohsen, Usama Ramadan, Kristina Bayer, and Ute Hentschel. “Diversity, Abundance and Natural Products of Marine Sponge-Associated Actinomycetes.” Natural Product Reports 31, no. 3 (February 11, 2014): 381–99. https://doi.org/10.1039/C3NP70111E

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Learning chemical capabilities of biosynthetic gene clusters with CHAMOIS

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The rapid development of genome mining methods in the last decade has enabled the prediction of large numbers of biosynthetic gene clusters (BGCs) from genomic and metagenomic data. For most of these predictions, however, the produced compounds are unknown, and can only be elucidated through experimental validation. Existing methods for characterizing *de novo* BGC predictions mostly rely on genomic similarity to known, experimentally-validated BGC, thus working well for close homologs, but failing to characterize predicted BGCs with novel architecture.

To address this gap, we developed CHAMOIS, a method that uses machine learning to identify chemical features of compounds produced by BGCs. Taking the protein domains of a BGC as input, CHAMOIS predicts the placement of the (unknown) produced compounds into the ChemOnt ontology. Because the method only uses the genomic content of the BGCs as features, it is suitable for use with BGCs obtained *in silico* from genome mining.

The current version of CHAMOIS, trained on the MIBiG database version 3.1, can predict a fingerprint of more than 400 independent chemical features for any BGC. It accurately identifies some broad classes of compounds (e.g benzenoids, organonitrogen compounds), as well as more specific chemical substructures (e.g quinoxalines, benzodiazepines, hydroxamic acids) with low representation in MIBiG. The fingerprints predicted by CHAMOIS can map BGCs to chemical space. The can be used to screen BGC predictions to identify the producer of a known compound, or to search the compound for an uncharacterized BGC in a catalog such as the Natural Product Atlas.

The model uses logistic regression to learn links between protein domains in BGCs and substructures in the produced compound, without prior knowledge. The weights resulting from training are sparse and can be inspected to extract the list of domains contributing to the prediction of a particular chemical class. These lists can be used for validating the model by verifying high associations against the literature (for instance between YcaO domains and thiazole compounds), but they can also be used to discover the putative function of novel biosynthetic domains.

The method developed here represents a novel, data-driven approach for exploring the chemical space of BGCs, independently of their type or origin.

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The inhibitory potential of marine microalgae-associated microbiomes against the fish pathogen *Vibrio anguillarum*

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The spread of Antimicrobial Resistance (AMR) has become a global challenge and alternatives to antibiotics must be found. These include probiotics, defined as living microorganisms capable of providing a health benefit to the host. The purpose of this project was to determine the inhibitory potential of microalgae-associated microbiomes against a highly virulent bacterial fish pathogen, *Vibrio anguillarum* strain 90-11-286. A constitutively expressed GFP gene was integrated in the chromosome of 90-11-286. Consequently, the growth kinetics of 90-11-286 in co- and complex cultures could be monitored by measuring the fluorescence intensity of GFP. The growth and inhibition assays were conducted in a sea salt medium supplemented with casamino acids and glucose. Growth inhibition as measured by decrease in fluorescence intensity was first verified by co-culturing the GFP-tagged 90-11-286 with a known antagonistic probiotic bacterium, *Phaeobacter piscinae* S26. The GFP-tagged pathogen was subsequently introduced in enrichments with complex microbiomes from non-axenic marine microalgae, *Isochrysis galbana*. The microbial community of *I. galbana* was inhibitory against *V. anguillarum* strain 90-11-286. Culturable bacteria have been isolated from the inhibitory microbiomes, and will be identified using 16S rRNA gene sequencing and tested for mono-culture inhibition of the pathogen. Current work also involves sequence analyses of 16S rRNA gene amplicons to determine the microbial composition of the inhibitory microbial algal community. Metabolomic analysis of the inhibitory microbiomes will be performed to identify metabolites potentially involved in inhibition.

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Maramycin, a cytotoxic isoquinolinequinone terpenoid produced through heterologous expression of a bifunctional indole prenyltransferase /tryptophan indole-lyase in *S. albidoflavus*

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In our recent work on azodyrecin biosynthesis[1] we discovered a novel unrelated molecule produced in a heterologous host. Structure analysis of the novel compound revealed that the compound belongs to the isoquinolinequinone family, representing an important family of natural alkaloids with profound biological activities. We were able to confirm that the production of the novel molecule is linked to an introduction of a rare bifunctional indole prenyltransferase /tryptophan indole-lyase enzyme from *Streptomyces mirabilis* P8-A2 in *S. albidoflavus* J1074. The structure of the molecule, that we named maramycin, was determined by analysis of spectroscopic (1D/2D NMR) and MS spectrometric data. The prevalence of this bifunctional biosynthetic enzyme was explored and found to be a recent evolutionary event with only a few representatives in Nature. Maramycin exhibited moderate cytotoxicity against human prostate cancer cell lines, LNCaP and C4-2B. The discovery of maramycin enriched the chemical diversity of natural isoquinolinequinones and also provided new insights into crosstalk between the host biosynthetic genes and the heterologous biosynthetic genes in generating new chemical scaffolds.

1. Maleckis, M.; Wibowo, M.; Gren, T.; Jarmusch, S. A.; Sterndorff, E. B.; Booth, T.; Henriksen, N. N. S. E.; Whitford, C. M.; Jiang, X.; Jørgensen, T. S.; Ding, L.; Weber, T. Biosynthesis of the Azoxy Compound Azodyrecin from Streptomyces Mirabilis P8-A2. *ACS Chem Biol* **2024**. https://doi.org/10.1021/ACSCHEMBIO.3C00632

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Biosynthetic potential of Archaea in costal Danish waters

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Archaea harbor intriguing physiologies and are of considerable ecological importance. However, the biosynthetic potential of archaea is often overshadowed by bacteria and eukaryotes, despite the diverse metabolisms and biochemistry employed by archaea, representing a vast and untapped potential. In addition, evidence is emerging that archaea produce secondary metabolites, which could function as signaling molecules or antimicrobials, mediating microbial interactions. Previous research has shown antiarchaeal activities of two secondary metabolites from halophilic archaea. However, knowledge about the potential of secondary metabolites in temperate marine environments is limited. To address this, 33 metagenome assembled genomes (MAGs) of archaea from Danish coastal waters were screened for putative bioactive compounds with antiSMASH. The analysis indicated that 75% of the archaeal genomes had biosynthetic gene clusters, with terpenes being the most commonly occurring type, in line with previous findings. Initial analysis suggests different putative secondary metabolites between Marine Group I (*Nitrosopumilaceae*), Marine Group IIa (*Poseidoniaceae*) and Marine Group IIb (*Thalassarchaeaceae*). Two MAGs showed multiple putative ribosomally synthesized and post-translationally modified peptide (RiPP) recognition elements (RREs), raising the question of novel RiPPs within proximity to theses RREs, which will be further manually investigated. Overall, our analysis provides a first glimpse into the biosynthetic potential of archaea in Danish coastal waters and can guide future in-depth investigations on the function of specific compounds and their role within microbial communities.

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Studies of a cryptic biosynthetic C-H oxidation and its applicability in chemoenzymatic synthesis

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Polyether ionophores are complex natural products with potent antimicrobial activities, which have not been clinically applied in humans due to cellular toxicity. Synthetically derived structural analogues of the natural compounds have shown enhanced antibacterial selectivity, and methods to introduce late-stage modifications to obtain a greater variety of analogues are therefore of keen interest. A structural investigation of the compound class has shown that several contain a common hemi ketal hydroxylation in a hydrobifuran motif, which cannot be derived from the canonical biosynthesis. It was hypothesised that a cytochrome P450 enzyme could be responsible for the introduction of this hydroxylation as a tailoring enzyme. A model ionophore from the lysocellin family was chosen for further investigation. The biosynthetic gene clusters of the ionophore-producing *Streptomyces* strain were analysed using antiSMASH, which revealed a P450 enzyme in close proximity to the biosynthetic gene cluster. The enzyme was knocked-out by applying the base editing system, CRISPR-BEST. The knockout strain produced an ionophore analogue lacking the hydroxyl group of interest, confirming the hypothesis. The bioactivities of the knockout compound varied drastically from those of the natural product, highlighting the importance of tailoring of the compound to introduce the hydroxyl group. The enzyme was heterologously expressed in *E. coli* and explored for its applicability in chemoenzymatic synthesis. The synthetic strategy aims to utilise the enzyme to afford new polyether ionophore analogues, which will facilitate further investigation of the antibacterial selectivity of these novel compounds.

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Deciphering the antifungal activity of two ant-associated bacterial isolates: Pseudomonas sp. I3 and Bacillus velenzensis 1Ic.

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Danish Wood ants (Formica polyctena) may offer a sustainable strategy for controlling plant pathogenic fungi that avoids synthetic pesticides. This is partially due to the fact that the ants harbor bacteria with antifungal activity on their bodies and legs. These antifungal bacteria can be deposited onto the surroundings where the ant roam, and potentially form a protective shield against plant pathogens. We have isolated several ant-associated, antifungal strains of Bacillus, Pseudomonas, and Rouxiella. Members of these genera are well-known for their role in biological control, which makes ants and their associated microbiomes promising candidates for application in biological control.

The primary objective of this study is to develop and implement a pipeline streamlined to identify the antifungal compounds from the ant-associated bacteria. The pipeline integrates the combination of different methods, including chemical extraction and isolation of bioactive compounds, with a focus on genome mining for biosynthetic gene clusters (BGCs) and on the application of the highly manipulative CRISPR-associated transposon (CAST) system to generate sgRNA-guided knockouts of selected BGCs.

The initial findings, based on phenotypic assays and on crude extracts from lipopeptide-specific chemical extractions, suggest that both isolates produce lipopeptides with antagonism against plant pathogenic fungi. Furthermore, genome mining of BGCs also revealed the presence of lipopetide-encoding non-ribosomal peptide synthetase (NRPS) clusters in both isolates. Ongoing Bioassay-guided fractionation of crude extracts and CAST-mediated genome editing experiments will further elucidate the true nature of these antifungal compounds.

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Elucidating the transcriptional effect of tropodithietic acid (TDA)-producing *Phaeobacter* spp. on the microbiome of the microalga *Tetraselmis suecica*.

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Over the last few decades, fish production by capture has stagnated, and aquaculture has become the major supplier of fish and shellfish. Bacterial infections, especially at the larval stage, are a severe constraint and often caused by *Vibrio* spp. Antibiotics used in aquaculture to control such infections can lead to antibiotic resistance, potentially spreading to human pathogenic bacteria, and hence other disease control alternatives are needed.

Phaeobacter spp., that produce the antimicrobial compound TDA, can inhibit *Vibrio* spp. and have been proposed as probiotics for aquaculture. The inhibition is pronounced in algal cultures (used as live feed), however, despite repeated attempts, TDA has not been chemically detected in such microalgal cultures. The purpose of this project was to determine if the TDA biosynthesis pathway is expressed in microalgal cultures, and how the presence of a TDA-producing bacterium affects the microalgal microbiome. The study used gene expression as the major analysis relying on RT-qPCR and metatranscriptomics for measuring *tdaC* gene expression and the global transcriptome, respectively. Non-axenic *Tetraselmis suecica* were grown with either *P. piscinae* S26 or *P. inhibens* DSM17395 (both probiotic candidates) or with no added bacteria for four days. RT-qPCR revealed that the expression of *tdaC*, a core gene in the biosynthetic gene cluster of TDA, was detectable on day 1, 2, and 4 with day one having the highest expression per CFU, for both strains of *Phaeobacter* spp. Preliminary analyses of the metatranscriptomics data showed that ~15% of the expressed genes were of bacterial origin and current work is analysing the functionality of these. Thus, algal microbiome metatranscriptomics can be used to determine effects of added probiotic bacteria on the native microbiome.

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Succession of microbial community composition and secondary metabolism during marine biofilm development

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In nature, secondary metabolites mediate interactions between microorganisms residing in complex microbial communities. However, the degree to which community dynamics can be linked to secondary metabolite potential remains largely unknown. In this study, we address the relationship between community succession and secondary metabolism variation. We used 16S and 18S rRNA gene and adenylation domain amplicon sequencing, genome-resolved metagenomics, and untargeted metabolomics to track the taxons, biosynthetic gene clusters, and metabolome dynamics in situ of microorganisms during marine biofilm succession over 113 days. Two phases were identified during the community succession, with a clear shift around Day 29, where the alkaloid secondary metabolites, pseudanes, were also detected. The microbial secondary metabolite potential changed between the phases, and only a few community members, including Myxococotta spp., were responsible for the majority of the biosynthetic gene cluster potential in the early succession phase. In the late phase, bryozoans and benthic copepods were detected, and the microbial nonribosomal peptide potential drastically decreased in association with a reduction in the relative abundance of the prolific secondary metabolite producers. Conclusively, this study provides evidence that the early succession of the marine biofilm community favors prokaryotes with high nonribosomal peptide synthetase potential. In contrast, the late succession is dominated by multicellular eukaryotes and a reduction in bacterial nonribosomal peptide synthetase potential.

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Unveiling the chemical diversity and the anti-infective potential of marine extremophilic bacteria from Antarctica

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Rapid increase in resistance development, the rise of new pathogens and the lack of effective treatments against the most problematic human and environmental pathogens pose an important threat to the global health, and lead to big losses of crops and fisheries. Climate change is expected to exacerbate all infectious diseases due to the induced changes in their geographical and temporal distribution, seasonality, and transmission intensity. These facts highlight the need for discovery of new antibiotics. In this context, marine extremophilic microorganisms represent excellent sources of new bioactive scaffolds due to their metabolic adaptation to extreme environments. Actinobacteria are prolific producers of complex bioactive secondary metabolites; about two-thirds of the antibiotics used in clinics originate from them. In this study, we investigated a number of Antarctic bacteria isolated from marine sediment. We identified several Actinobacteria showing antimicrobial activity against a panel of human, agriculture, and aquaculture associated-pathogens. Next, we employed an OSMAC (One-Strain-Many-Compounds) strategy coupled with a time course approach to identify the best culture conditions for such slow growing bacteria. The extracts were assessed for cytotoxic and antimicrobial activity and analyzed by an LC-MS/MS based untargeted metabolomics approach using Feature-Based Molecular Networking workflow. The cultivation time and culture medium significantly affected the metabolome composition. Marine Broth extracts displayed the highest antimicrobial activity; while extended cultivation time (days 10 and 20) extracts led to the highest chemical diversity, i.e., the highest number of specific features. Notably, only a few molecular families were annotated, indicating novel chemistry in those bacteria. Antarctic extremophilic bacteria represent a promising opportunity for discovery of novel antimicrobial compounds. Our future research will focus on metabolomics- and bioactivity- assisted isolation, and chemical characterization of new antibiotic metabolites, plus genome mining of the key components of the Biosynthetic Gene Clusters (BGCs).

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Synechococsins: lanthipeptides as a defensive signal?

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Natural products (NP) play a crucial role in shaping the ocean bacterial community dynamic, biochemical ecosystem cycles, and marine nutrient recycling. Within the diversity of NP, ribosomally produced and post-translationally modified peptides (RiPPs) are known to impact the biodiversity of microbial communities. Lanthipeptides, a subgroup of RiPPs, are predominantly antimicrobials (known as lantibiotics). However, the biological functions of non-antimicrobial lanthipeptides, yet bioactive molecules remain unknown. This is particularly intriguing in the case of prochlorosins, a distinctive lanthipeptide family produced by marine picocyanobacteria. A single strain of *Synechococcus* can produce multiple substrates (SyncAs) modified by a single modification enzyme (SyncM), resulting in a highly diverse repertoire. To investigate the function of these lanthipeptides, we challenge a marine *Synechococcus* RS9116 (SyncA-containing) strain with a synechococsin from *Synechococcus* MITS9509 and analyze the transcriptomic response. Intriguingly, we observed a specific response involving the downregulation of genes related to putative antimicrobial peptides: microcin-C-like, putative RiPPs, and proteins that could be involved in their further processing. These results suggest that some synechococsins might function as a defensive/alert signal when in the proximity of other Synechococci. This is by inhibiting bacteriocins synthesis that would compete with the SyncA6-Synechococus producer; thereby enhancing their competitive advantage.

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Interactions in Marine Microbial Synthetic Communities

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Microbial secondary metabolites play significant roles in shaping microbial communities and confer important physiological and ecological functions for the producing organisms. The most renowned secondary metabolites are famous for their antimicrobial activities, while their actual ecological roles in nature are often not understood. Furthermore, some secondary metabolites are only expressed as part of inter-species interactions in complex ecological communities and can therefore be challenging to study. In the marine environment, such complex communities can often be found in association with macro-organisms or on inert surfaces. However, in situ study of bacterial interactions in these communities remain challenging. A way to bridge the gap between in situ studies and studying bacteria as mono-cultures is to establish synthetic communities that can be controlled and manipulated in the laboratory. The purpose of the present study is to assemble a synthetic marine bacterial community to study cross-chemical interactions between community members facilitated by secondary metabolites. The community is assembled from bacteria isolated from marine biofilms rich in bryozoans (moss animals) (Jyllinge harbor, Denmark), since we have discovered that the bryozoans are a hotspot for the occurrence of potent secondary metabolite producing bacteria. The genomes of the bacteria have been mined for their potential as secondary metabolite producers. Mutants devoid of secondary metabolite production as well as reporter fusions to key biosynthetic gene clusters will be constructed genetically and employed to explore the impact of secondary metabolites on interactions within the synthetic community, and microbial community assembly and function as well. Furthermore, this project will use a broad range of omics analyses (including metabolomics and transcriptomics) to unravel the functional role(s) of bacterial natural products in bacterial physiology and the development of complex microbial systems.

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Generating a TDA-overproducing Phaeobacter piscinae strain to uncover the ecological roles of tropodithietic acid (TDA) in marine microbiome.

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Phaeobacter species play pivotal ecological roles in shaping the microbiome structures and combatting fish pathogens in aquaculture, owing mainly to the production of tropodithietic acid (TDA), a potent antibiotic compound. However, the molecular mechanism of interactions driven by TDA within the microbiome remains unclear, due to the challenges associated with the *in situ* detection of TDA in complex cultures. Despite the importance, *Phaeobacter* does not seem to be the most abundant member in communities, making the subsequent detection of TDA production barely feasible using current chemical detection approaches. This project aims employ metabolic engineering to generate a TDA-overproducing *Phaeobacter piscinae* S26 mutant that can be used to address these challenges. TDA is synthesized by enzymes encoded by *tda* genes utilizing an intermediate compound of the phenylacetic acid (PAA) catabolic pathway as the precursor. Genomic analysis of strain S26 found that the genes involved in PAA catabolic pathway displayed 95-98% similarity to those in *P. inhibens DSM16374* and 35-51% similarity to *Escherichia coli*. Additionally, both *Phaeobacter* species harbor a second copy of *paaZ* gene, named paaZ2, which may be co-transcribed with *tdaF* gene from the same promoter. The product of *paaZ* is a bifunctional enzyme. In *Phaeobacter,* the C-terminal enoyl-CoA hydrotase domain catalyzes the reaction to produce the TDA-precursor compound; however, the N-terminal aldehyde dehydrogenase (ALDH) of PaaZ catalyzes such intermediate into the PAA catabolic pathway. We hypothesized that the inactivation of ALDH domain of PaaZ and PaaZ2 will lead to the accumulation of TDA-precursor and subsequently increase TDA production in *Phaeobacter*. We are currently generating in-frame deletion and point mutation of the ALDH domain in PaaZ and PaaZ2 of S26. TDA production in these mutants will be quantified through UHPLC-MS/MS-based chemical detection. The TDA-overproducing mutant will be introduced into the microbial communities to elucidate the molecular-level interactions facilitated by TDA. Maybe add the certain % range of similary to here?

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Unveiling the Genetic Diversity of the Cyanobactin Family Through Metagenomic Exploration

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Microbial natural products, mainly from bacterial origin, exhibit an immense structural diversity and complexity. Among chemically-rich bacteria, the phyla Actinobacteria, Cyanobacteria and a few other groups are responsible for the production of a great number of bioactive molecules with potential applications in pharmaceuticals, agriculture, and industry. The study of DNA obtained directly from an environmental sample (metagenomics) accesses the collective genomes and biosynthetic potential of bacterial consortia. Metagenomics therefore provides a means of exploring novel metabolites from bacteria that are known to be present in a variety of environments, but which remain difficult to culturing. Moreover, metagenomics is particularly attractive for natural product discovery because the genetic information encoding the activities of interest are generally clustered on bacterial genomes, making it possible to clone an entire pathway on a heterologous host. In this study, we applied a function-driven screening strategy on microbiomes from different environments (freshwater, marine, subaerial and extreme habitats) in which Cyanobacteria are dominant. As query, we use the G protein from a cyanobactin BGC, encoding a kinase domain responsible for the phosphorylation of cyclic cyanobactins. This strategy led to the discovery of different cyanobactin biosynthetic pathways which can reflect new scaffolds of phosphorylated macrocyclic peptides. Also, these results expand the enzymatic diversity associated with cyanobactin family and provide additional biosynthetic logic to explore its genetic and chemical diversity.

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The hepatotoxin Microcystin shapes the microbiome of the cyanobacterium *Microcystis*

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Harmful cyanobacterial blooms formed by the photosynthetic species *Microcystis* pose an increasing threat to freshwater bodies worldwide. These mass developments of *Microcystis* blooms burden the entire ecosystem substantially. Additionally, some *Microcystis spp.* produce and release a variety of secondary metabolites – many of them being highly toxic to humans and animals alike. Especially, the compound class of Microcystins (MCs) has shown to be hazardous to mammalian life due to their hepatotoxic characteristics. However, the biosynthetic genes encoding for the biosynthesis of MCs have evolved much earlier than eukaryotic life suggesting that MCs may serve purposes going beyond a simple defense mechanism. Still, the biological function to the producing organism Microcystis is not fully understood.

In their natural habitat, *Microcystis spp*. usually appear in communities with heterotrophic bacteria, suggesting the existence of specialized phototroph-heterotroph interactions. Since the loss of MC has been shown to affect surface structures and primary metabolism of *M. aeruginosa PCC 7806*, our research is aimed to elucidate of the involvement of MCs in the mediation of directed phototroph-heterotroph interactions.

Using 16S-rDNA analysis and PCR-based discrimination of field-sampled single *Microcystis* colonies, we demonstrate that the presence of *mcyA* gene is indicative for lower alpha-diversity, hinting towards a more specialized microbiome in the MCproducing colonies than in in the non-producing. Furthermore, we identified taxa on the species-level, that were significantly differentially abundant based on the presence/absence of the *mcyA* gene.

In longitudinal experiments with synthetic communities consisting of a MC-producing *Microcystis* WT and a non-producing mutant together with 21 heterotrophic isolates, we identified two bacterial isolates that showed reciprocal relative abundance with respect to toxin production. Using these two candidates in tri-partite experiments, we aim to further understand the role of MC for the specialized recruitment of heterotrophic partners as well as the nature of the specialized interaction.

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Streptomyces iModulons: A knowledgebase of the S. albidoflavus regulatory network

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Streptomyces albidoflavus J1074 is a popular and genetically tractable platform strain used for drug discovery and production via the expression of heterologous biosynthetic gene clusters (BGCs). However, its transcriptional regulation network (TRN) and its impact on secondary metabolism are poorly understood. Here we characterized its TRN by applying an independent component analysis to a compendium of 142 high quality RNA-Seq transcriptomes, from both in-house and public sources spanning 58 unique growth conditions. We obtained 63 independently modulated sets of genes (iModulons), that quantitatively describe the TRN and its activity state across diverse conditions. Through analyses of condition-dependent TRN activity states, we (i) describe how the TRN adapts to different growth conditions, (ii) conduct a crossspecies iModulon comparison, uncovering shared features and unique characteristics of the TRN across lineages, (iii) identify putative regulators of several biosynthetic gene clusters, including Surugamide, Cyclofaulknamycin and Dudomycin, and (iv) infer potential functions of 40% of the uncharacterized genes in the *S. albidoflavus* J1074 genome. Our findings provide a comprehensive and quantitative understanding of the TRN of *S. albidoflavus* J1074, and suggest new strategies for rational strain design to optimize its production capabilities.

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Metabolomics tools to assess the impacts of chemical elicitors in the production of antibiofilm secondary metabolites in endolichenic fungi

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Endolichenci fungi are mutualists living within the lichen thallus. These group of fungi were different from lichen mycobionts and are less explored sources of natural products for drug discovery application. In this study, two Philippine endolichenic fungi:*Hypoxylon lateripigmentum* and *Daldinia eschscholtzii* were investigated. Both displayed excellent antibacterial and biofilm inhibition activity from preliminary screening works. Addition of two chemical elicitors (5-azacytidine (Aza) and suberoylbishydroxamic acid (SBHA)) in the potato dextrose broth media resulted in the enhancement of biological activities and chemical diversity. The antibacterial activity against methicillin-resistant *Staphylococcus aureus* of *H. lateripigmentum* treated with Aza were significantly improved by 7%. NMR-based metabolomics revealed that aromatic compounds were activated. In depth LC-HRMS metabolomic analysis unveiled the metabolites elicited such as quinone pigments, strepsilin, hypoxylone, etc. Meanwhile, the prebiofilm inhibition activity of *D. eschscholtzii* treated with SBHA increased significantly by 40%. This can be explained by the apparent enhancement in overall metabolite diversity based on NMR and LC-HRMS heatmaps. The elicited metabolites include a tripeptide, a dinaphthalene compound, and several unknown hits with high molecular weight. These putatively new compounds can then targeted for isolation works. Overall, the study highlights the significance of metabolomics to explain the secondary metabolite diversity of microorganisms.

Watch video pitch on youtube.com

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A ribosomally synthesised and post translationally modified peptide containing a β-enamino acid and a macrocyclic motif

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Ribosomally synthesized and post-translationally modified peptides (RiPPs) are structurally complex natural products with diverse bioactivities. Here we report discovery of a RiPP, kintamdin, for which the structure is determined through spectroscopy, spectrometry and genomic analysis to feature a bis-thioether macrocyclic ring and a β-enamino acid residue. Biosynthetic investigation demonstrated that its pathway relies on four dedicated proteins: phosphotransferase KinD, Lyase KinC, kinase homolog KinH and flavoprotein KinI, which share low homologues to enzymes known in other RiPP biosynthesis. During the posttranslational modifications, KinCD is responsible for the formation of the characteristic dehydroamino acid residues including the β-enamino acid residue, followed by oxidative decarboxylation on the C-terminal Cys and subsequent cyclization to provide the bis-thioether ring moiety mediated by coordinated action of KinH and KinI. Finally, conserved genomic investigation allows further identification of two kintamdin-like peptides among the kin-like BGCs, suggesting the occurrence of RiPPs from actinobacteria.

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Antioxidant extracellular vesicles from Baby Gut Microbiota: Insights into probiotic candidates and their metabolites

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Extracellular vesicles (EVs) are small bilipid layer-enclosed vesicles secreted by all microbial cells. They facilitate inter-kingdom communication between bacteria and mammalian (human) host cells by delivering proteins, lipids, metabolites, and nucleic acids. This study aimed to identify probiotic candidates with antioxidant potential and investigate their EVs as carriers for antioxidant compounds.

In the screening procedure, 151 isolates of Lactic acid bacteria (LAB) were obtained from baby stool, and their cell-free supernatants were assessed for antioxidant capacity using permanganate reducing antioxidant capacity (PRAC) and α, α-diphenyl-β-picrylhydrazyl (DPPH) assays. Five promising probiotic and antioxidant candidate strains were selected for whole genome sequencing. EVs were extracted from these strains using size exclusion chromatography, and their antioxidant activities were evaluated. Among the selected strains, EVs from *Lacticaseibacillus rhamnosus* E18 exhibited the highest antioxidant activity. Metabolomics and proteomic analyses were conducted on the cell-free supernatants and EVs. Gene functions were annotated using the Gene Ontology (GO) database, and pathways were annotated using the Kyoto Encyclopedia of Genes and Genomes (KEGG) database.

The analyses revealed a diverse range of biological processes, molecular functions, and cellular components, including metabolic processes, catalytic activities, binding, transporter activity, and antioxidant activity. L-ascorbic acid, a potent antioxidant, was detected in EVs from the strain with the highest antioxidant activity compared to the other selected strains. Proteomic analysis of EVs from LABs identified enzymes such as catalase, superoxide dismutase, and peroxidases, further supporting their antioxidant potential. These findings were corroborated by the whole genome sequence analysis, which revealed a significant number of oxidoreductase-related genes in the selected LABs. Overall, this study highlights the presence of metabolites and enzymes in the baby gut microbiota that contribute to antioxidant systems and suggests their potential for antioxidant treatments.

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Microbial volatile organic compounds as salinity stress busters and plant growth enhancers: Case study with mungbean

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Soil salinization is a major threat for agricultural sector because it impairs plant physiology and development. The conventional approach to mitigate salinity stress include application of chemicals which may conversely lead to more soil salinization. An eco-friendly approach is application of plant beneficial microbes as bioinoculants. But indigenous soil microbes outcompete the inoculated strains thereby leading to a reduction in the latter's survival and efficacy. One of the potent approaches is the application of microbial volatile organic compounds (mVOCs), which can enhance plant growth and resistance to salinity stress. This study aimed to investigate the potential of mVOCs in mitigating salinity stress in *Vigna radiata* (mungbean) plants. Top-down strategy of rhizosphere engineering was employed wherein the rhizosphere microbiome of *V. radiata* was subjected to salt stress acclimatization through repeated microbiome transplantation across successive plant growth cycles under salt stress.In our prior work, we reported isolation of the culturable fraction from the acclimatized microbiome and testing the strains for mVOC production using gas chromatographymass spectrometry (GC-MS) analysis of the volatiles collected from the strain headspace of a microcosm experiment set up based on closed-loop-stripping analysis (CLSA). mVOCs produced by plant growth promoting strains were identified, and shortlisted based on existing literature reports suggesting their potential role in promoting plant growth under salinity stress. In this study, plant growth experiments were conducted to assess the impact of these selected mVOCs on *V. radiata* under salinity stress conditions. The observed effects of the volatiles included both preventive and curative actions against salinity stress, leading to enhanced plant growth. Our study brings forth the potential of mVOCs from salt stress acclimatized microbes as a novel tool to improve crop productivity in a sustainable way.

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Structural Insights and Bioprospecting of the Deep-Sea Sponge Microbiome

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The deep ocean is the largest biome on Earth, accounting for >90% of the planet's marine environment. Sponges (phylum Porifera) harbour unique microbial communities which are a rich source of bioactive compounds for antimicrobial discovery. Much of the work to date has focused on sponges from warm and shallow coastal waters, while sponges from the deep ocean remain less well-studied. Our research initially focused on a metataxonomic assessment of the structure and diversity of the microbial community inhabiting these underexplored deep-sea sponges, revealing intricate, species-specific microbial communities. We uncovered communities dominated by ammonia-oxidizing archaea and reveal a strong influence of sponge phylogeny on microbial composition (1).

Secondly, we bioprospected the culturable members of the microbiome including the isolation of novel deep-sea species from genera including *Streptomyces*, *Micromonospora*, *Bacillus* and *Stappia*. Two of the novel species - *Micromonospora robiginosa* sp. nov. and *Streptomyces ortus* sp. nov. - demonstrated impressive bioactivity against MDR Gram-positive pathogens (2). We performed molecular networking analysis to identify two large clusters of anthracycline like compounds, NMR analysis identified two of the compounds as the known antibiotics kosinostatin and rudolphomycin. This highlights the deep-sea sponge microbiome as an untapped reservoir for novel microbial life with significant biosynthetic capabilities.

In conclusion, our study underscores the underexplored deep-sea sponge microbiome as a fertile source of antimicrobial agents and contributes to enriching our understanding of unexplored deep-sea microbial ecology.

1. Williams SE, Varliero G, Lurgi M, Stach J, Race PR, Curnow P. Diversity and structure of the deep-sea sponge microbiome in the equatorial Atlantic Ocean. bioRxiv [Internet]. 2024 Jan 1;2024.01.24.577104. Available from: http://biorxiv.org/content/early/2024/01/26/2024.01.24.577104.abstract
2. Williams SE, Back CR, Best E, Mantell J, Stach JEM, Williams TA, et al. Discovery and biosynthetic assessment of "Streptomyces ortus" sp. nov. isolated from a deep-sea sponge. Microb Genom. 2023 May 19(5):000996

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Screening marine bacteria for production of siderophores and identification of biosynthetic gene clusters

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Siderophores are small molecule chelating agents secreted by bacteria and fungi, with the ability to uptake iron under restricted iron conditions. Marine microbes typically require micromolar concentration of iron to flourish, yet the concentration of iron in the ocean surface water is only around 0.01–2 nM. To get around iron paucity, microorganisms produce siderophores that bind iron with high selectivity and affinity, and transport it into the cells. Siderophores find extensive use in iron chelation therapy, antibiotic carriers, biosensors, promotion of plant growth, biocontrol of plant and fish pathogens, and bioremediation. The aim of this study was to detect and quantify siderophore production in approx. 90 marine bacteria isolated from Baltic Sea macrophytes. The isolates were screened quantitatively and qualitatively for siderophore production using Chrome Azurol S assay. Utilizing both the conventional and modified microplate methods, the siderophore production was quantified in percent siderophore unit (psu). Qualitative estimation using well diffusion assay was consistent with the quantitative results, resulting in production of halo zones up to 14 mm. Currently, we are employing the antiSMASH bioinformatics tool to predict and annotate biosynthetic gene clusters in the best siderophore producers. The Rapid Annotation using Subsystem Technology (RAST) platform will also be utilized to annotate and analyze the genomic sequences for comparative analysis, and functional gene annotation.

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Cyclic-lipopeptides produced by *Pseudomonas* spp. are degraded by bacterial competitors

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The production of specialized secondary metabolites often governs bacterial interactions. In this regard, several species of bacteria secrete bioactive small molecules that can interfere with the growth and development of competing species. In response, competing bacteria may evolve mechanisms to cope with the effects of these antagonistic molecules. Here, we explored the interplay of these counteracting forces in nine *Pseudomonas* strains that produce structurally distinct cyclic-lipopeptides (CLPs) against five bacterial competitors. CLPs are a multifaceted family of natural products crucial in Pseudomonas ecology. They can act as antimicrobials, antagonizing other organisms but may also be required for motility, surface colonization, and signaling processes. By combining bioassays, metabolic profiling, and structural elucidation, we demonstrated how bacterial competitors inactivate *Pseudomonas* CLPs by linearizing the lipocyclopeptide structure. Furthermore, we showed that the size of the peptide macrocycle determined the sensitivity of CLPs towards hydrolysis and that phylogenetically different bacterial competitors exhibited distinct levels of hydrolysis activity. To shed light on the enzymes involved in CLP hydrolysis, we conducted proteomic analyses and identified candidate enzymes through mass spectrometry and heterologous expression. Overall, this study expands our understanding of the biotransformation of CLPs within specific niches, particularly upon interaction with neighboring rhizobacteria. Furthermore, since microbiome-driven, ecological processes are affected by secondary metabolites, our study highlights the importance of elucidating the fate of these molecules within microbiomes.

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Culturing the unculturable - Investigating cross-domain interactions in miniscule droplets

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Following the discovery and independent evolutionary status of Archaea, the study of their diversity and ecological relevance has expanded continuously. With high-throughput sequencing Archaea have been found in extreme environments including hot springs and hydrothermal vents, but also under mesophilic conditions like in soil, water, and in sediments. To date, the culturable Archaea represents only a fraction of the total diversity that exists. Emerging evidence from culture-independent approaches indicates Archaea may produce bioactive compounds such as secondary metabolites. Additionally, Archaea show potential to be used in bioremediation due to their ability to survive extreme conditions and utilize pollutants. Finally, Archaea may hold the key to eukaryogenesis and the evolution of multicellular life. However, cultures remain necessary for an understanding of the biochemistry, metabolism, and ecological and evolutionary dynamics of Archaea. Here, we aim to establish enrichment cultures of Archaea and their bacterial associates. To gain insight into which taxa engage in hydrogen-mediated syntrophic consortia and which metabolic functions are involved, we utilize a droplet-based microfluidic approach to generate a multitude of individual consortia while facilitating cross-feeding between H2 producing anaerobic Archaea and sulfate reducing bacteria. Based on the entangle-engulf-endogenize model, these droplet cultures may help shed light on the transformative events leading to eukaryogenesis, which plausibly occurred by Archaea engaged in a syntrophic relationship with different bacteria becoming increasingly interdependent and ultimately giving rise to the eukaryotic cell. By collecting sediment cores along the eastern coast of Zealand, we gain a spatial distribution along the salt and wastewater-discharge gradient between Kattegat and the Baltic Sea. This will enable a local scale mapping of Archaea and identification of environmental drivers and co-occurrence patterns with other prokaryotes. This can provide an understanding of how prokaryotes coordinate behavior across domains and allow us to tap into the under explored metabolism of Archaea.

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Unveiling Basidiomycete Biosynthetic Potential for Sustainable Chemical Production

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The heavy dependence on fossil chemicals as a finite and unsustainable resource calls for the exploration of alternative sources. The specialized metabolism of fungi holds promise for more sustainable chemical production. Cataloguing the fungal chemical and biosynthetic repertoire is crucial to leverage their full potential. Encompassing saprotrophs, mycorrhizal symbionts, and plant parasites, basidiomycetes are a major component of soil and plant microbiomes. However, this diverse fungal division remains surprisingly unexplored in terms of their specialized metabolism. A major challenge for genome-based efforts for specialized metabolite discovery is the lack of efficient bioinformatic tools for basidiomycete genomes. In this study, we present the genome-guided investigation of basidiomycete specialized metabolism. We developed a bioinformatic tool that facilitates gene-calling, improving prediction of biosynthetic genes encoded in basidiomycete genomes. This tool enabled a thorough investigation of biosynthetic potential across a wide range of basidiomycetes. This work paves the way for heterologous expression-based discovery of previously unknown chemical architectures and enzymatic functions, which we believe will help develop new processes for sustainable production of chemicals.

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An unusual but widespread lasso peptide

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Lasso peptides are ribosomally synthesized and post-translationally modified peptides (RiPPs) produced by microorganisms. We have studied triculamin, an antibiotic against mycobacteria originally isolated in 1967. Through bioactivity-guided isolation and genome mining, we have discovered that it is, in fact, a very unusual lasso peptide. Unlike any known lasso peptides their precursor peptides appear to have a follower - instead of a leader peptide and many of the processing enzymes remain cryptic. In addition, we have identified similar predicted lasso peptides across several bacterial phyla, suggesting an important ecological role. I will be presenting our ongoing work on understanding this intriguing secondary metabolite and its biosynthesis.

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Bridging the Gap: Understanding Triculamin Biosynthesis through In vitro Reconstitution and Genetic Analysis

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Triculamin is a lasso peptide with specific bioactivity against *Mycobacteria*, posing a unique biosynthetic challenge. Despite previous structural studies and identification of the putative biosynthetic gene cluster (BGC), the pathway for its synthesis remains elusive. Notably, the precursor is expressed as a core peptide with a follower sequence deviating from the conventional leader sequence. Furthermore, the BGC lacks both peptidase and RRE. We aim to unravel the details of triculamin biosynthesis by investigating the functional importance of individual genes within the putative BGC. Our approach involves *in vitro* reconstitution of triculamin. We've successfully expressed and purified the precursor and macrocyclase through heterologous expression in *E. coli*. However, preliminary in vitro results suggest that the macrocyclase alone may not be sufficient for lasso fold formation, indicating the involvement of additional factors. Interestingly, in silico identification of diverse bacterial strains has revealed genetic sequences similar to the triculamin core sequence, all situated within putative lasso peptide BGCs. The triculamin BGC and these newly found BGCs encode an acetyltransferase and a helix-turn-helix motif, in addition to the canonical lasso peptide genes. We incorporate these genes into our in vitro assays to shed light on their role in lasso peptide maturation. Our ongoing investigation seeks to discern the significance of triculamin-like lasso peptides across diverse bacterial species by identifying their molecular target. Hopefully, this will help us understand the apparent importance of expressing this peptide natural product.

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Biofilm formation and *tdaCDE* gene expression of *Phaeobacter piscinae* on polypropylene surfaces

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In the last decades, fish production in aquaculture has increased dramatically, paralleled by the emergence of antibiotic-resistant fish pathogens. Therefore, new sustainable ways to prevent bacterial infections must be found, and beneficial bacteria, probiotics, is one alternative strategy. Bacteria from the *Roseobacter* group, such as *Phaeobacter piscinae*, have been studied as potential probiotics in marine larval rearing. Their probiotic effect is linked to their ability to produce an antimicrobial secondary metabolite called tropodithietic acid (TDA). TDA production appears enhanced when the bacteria grow in a biofilm. The purpose of this study is to develop a setup for comparing biofilm formation and TDA production, measured by proxy using gene expression of a core TDA biosynthetic gene operon, *tdaCDE*.

P. piscinae was cultivated in Instant Ocean salts with casamino acids and glucose and formed biofilms on Polypropylene (PP) surfaces placed in microtiter plates. Surfaces were analyzed for biofilm formation of *P. piscinae* after rinsing and staining with Syto62 for 10 minutes, and fluorescent intensity of GFP expressed from the *tdaCDE* promoter was used to track the expression of core enzymes involved in TDA production. Z-stack images were acquired by confocal microscopy, and the images were used to quantify biovolume and gene expression of *tdaCDE* using the software BiofilmQ.

The biofilm volume of *P. piscinae* increased with significant change between 24 to 48 hours ($p=0.00791$) and 72 to 96 hours ($p<0.001$). The expression of *tdaC*DE also increased, albeit not statistically significant. GFP gene expression decreased at the 144-hour time point, indicating that there is an optimum time point for TDA production in the *Phaeobacter* biofilm. These results will contribute to identifying the optimal time points for comparing surface characteristics and identifying the best features to enhance *tdaCDE* expression.

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Insights into the nature of the microalgal toxins from the *Chrysochromulina leadbeateri* blooms in Northern Norwegian fjords

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This presentation will cover the discovery and analysis of two novel polyhydroxylated polyketides, leadbeaterin-1 (**1**) and leadbeaterin-2 (**2**), from *Chrysochromulina leadbeateri*, identified as potential fish-killing toxins. In May–June 2019, the microalga *C. leadbeateri* caused a massive fish-killing event in several fjords in Northern Norway, resulting in the largest direct impact ever on aquaculture in northern Europe due to toxic algae (Samdal and Edvardsen, 2020; John et al., 2022). Motivated by the fact that no algal toxins have previously been described from *C. leadbeateri*, we set out to investigate the chemical nature and toxicity of secondary metabolites in extracts of two strains (UIO 393, UIO 394) isolated from the 2019 bloom, as well as one older strain (UIO 035) isolated during a bloom in Northern Norway in 1991. Bioassay-guided fractionation using the RTgill-W1 cell line (Bols et al., 1994; Dorantes-Aranda et al., 2011) and metabolomics analysis pointed to a major compound affording $[M+H]^+$ ions at *m/z* 1399.8333 as a possible toxin. Moreover, our study unveiled a series of minor analogues exhibiting distinct patterns of chlorination and sulfation, together defining a new family of compounds, which we propose to name leadbeaterins. Leadbeaterin-1 (**1**) and leadbeaterin-2 (**2**) were isolated and purified from UIO 394 cultures. Their structures were determined by 1D and 2D NMR experiments and tandem mass spectrometry. Remarkably, these suspected toxins were detected *in situ* in samples collected during the 2019 bloom close to Tromsø, thereby substantiating their likely role in fish kills.

References:

Samdal, I., Edvardsen, B., 2020. Massive salmon mortalities during a Chrysochromulina leadbeateri bloom in Northern Norway. Harmful Algae News. 64, 4–6.

John, U., Šupraha, L., Gran-Stadniczeñko, S., Bunse, C., Cembella, A., Eikrem, W., Janouškovec, J., Klemm, K., Kühne, N., Naustvoll, L., Voss, D., Wohlrab, S., Edvardsen, B., 2022. Spatial and biological oceanographic insights into the massive fish-killing bloom of the haptophyte Chrysochromulina leadbeateri in northern Norway. Harmful Algae 118, 102287.

Bols, N.C., Barlian, A., Chirinp-Trejo, M., Caldwell, S.J., Goegan, P., Lee, L.E.J., 1994. Development of a cell line from primary cultures of rainbow trout, Oncorhynchus mykiss (Walbaum), gills. J. Fish Dis. 17, 601–611.

Dorantes-Aranda, J.J., Waite, T.D., Godrant, A., Rose, A.L., Tovar, C.D., Woods, G.M., Hallegraeff, G.M., 2011. Novel application of a fish gill cell line assay to assess ichthyotoxicity of harmful marine microalgae. Harmful Algae 10, 366–373.

Keywords: *Chrysochromulina leadbeateri*, algal bloom, ichthyotoxins, bioassay-guided fractionation, RTgill-W1

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Discovery and bioengineering of natural products from *Streptomyces* to improve plant stress resistance

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Severe challenges in agriculture have been aroused with the increasingly serious environment and climate change issues, such as drought and excessive salinity in soil. According to the literature, beneficial microorganisms and their secondary metabolites can help plants cope with different abiotic stresses. Our recent study showed that pteridic acids, which were produced by *Streptomyces* and can promote plant growth and improve stress resistance in planta at the concentration of 1 ng/mL. In this project, our main purpose is to find more bioactive natural analogues, confirm their modes of action, and obtain enough amount for application in agriculture to reduce water usage, and enhance plant stress resistance.

To achieve the goals, this project is ongoing, carried out through a series of research, combining the technology of microbiology, natural product chemistry, biotechnology with plant sciences. Firstly, we tried to discover new pteridic acids by genome mining, high throughout chemical determination, molecular networking and genetic editing. Secondly, we tested the effects of pteridic acids on different plants, such as *Arabidopsis*, wheat and mung beans. Meanwhile, we tried to elucidate the the structure-activity relationship and mode of action of pteridic acids by transcriptome analysis. Finally, we applied genetic editing and media optimization to improve the production of pteridic acids from *Streptomyces*.

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Perspective on in situ metabolomics and potential approaches for the future

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Chemical detection of secondary metabolites directly from the environment, in situ, allows for more unbiased studies of complex microbial systems, natural metabolite expression and interactions between microbial communities than what is afforded by traditional laboratory culturing of microbes. Historically *in situ* detection has been difficult, but with advances in both instruments, capture methods, and metabolomic approaches it is a promising avenue for answering basic research questions about the microbial world.

Terrestrial soil-based systems can be studied by utilizing high water saturation, such as in well-irrigated soil in crop fields, allowing for higher metabolite mobility, such as those seen in marine soil environments. Utilizing a resin kept within membrane filters and either gravimetric or vacuum assisted flow, the water can be pulled through the filters and depositing metabolites onto the HP-20 resin, thus achieving similar results to recent *in situ* marine metabolite capture studies[1] . Additional marine studies may be performed as well, placing resin capture devices in microbe-containing soil, around sea sponges, or coral reefs. These studies may allow for new metabolites to be discovered from organisms which have been shown to not culture in laboratory conditions.

MALDI-MSI allows observation of the spatial distribution of metabolites in situ and has seen prior use within CeMiSt[2] . These techniques can be further applied to *in situ* biological systems, such as microbial-plant root system interactions or biofilm-organism interfaces, for tracing the distribution, interactions, and possible biotransformations of symbiotic or pathogenic metabolites at the plant-microbe interface. There exist many metabolites which have been proven beneficial towards plant growth in drought or saline conditions, however their mechanisms of action remain unproven. With MALDI-MS imaging of metabolite-treated plant root cross sections, plant uptake and localization of the metabolites in the actual 3-dimensional space can help illuminate a metabolite's true mechanism of action. This can further be utilized with full microbial biofilms on plant root surfaces and observing the spatial distribution of the microbe's full metabolome through the plant system, as well as with fully wild plant systems.

[1] Bogdanov, A., Salib, M. N., Chase, A. B., Hammerlindl, H., Muskat, M. N., Luedtke, S., Barbosa Da Silva, E., O'donoghue, A. J., Wu, L. F., Altschuler, S. J., Molinski, T. F., Jensen, P. R., Da, S. L. E. B., Performed, S., & Analyzed, P. R. J. (2023). Small Molecule in situ Resin Capture – A Compound First Approach to Natural Product Discovery. BioRxiv, 2023.03.02.530684. https://doi.org/10.1101/2023.03.02.530684

[2] Lozano-Andrade, C. N., Nogueira, C. G., Henriksen, N. N. S. E., Wibowo, M., Jarmusch, S. A., & Kovács, Á. T. (2023). Establishment of a transparent soil system to study Bacillus subtilis chemical ecology. ISME Communications, 3(1). https://doi.org/10.1038/S43705-023-00318-5

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Breaking the witches' spell

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Striga is a genus of parasitic plants that pose major limitations on cereal production in Sub-Saharan Africa. This parasite, referred to as witch's weed, infects the root system of a broad range of crop species, including sorghum, millet, maize, and upland rice. The development of effective strategies to control *Striga* has been a major focus of research in the past decades. Among the approaches with most impact are those based on agricultural practices (e.g., push-pull), resistance breeding and chemistry. To date, however, none of these strategies is singularly effective on different crops and across a diverse range of agroecosystems. In the PROMISE (Promoting Microbes for Integrated *Striga* Eradication) program, we focused on characterizing microbial communities associated with *Striga* and the host plant sorghum to understand their role in the life cycle and infection process of this root parasitic weed. More specifically, we studied a diversity of mechanisms and the underlying chemistry by which soil and root-associated microorganisms could diminish the *Striga* seed bank or interfere with the early stages of root infection to enhance crop productivity. The experimental work ranged from studying the effects of edaphic factors on microbial community diversity and functioning across different Ethiopian agro-ecologies and sorghum cultivars to microbe-mediated changes of host root architecture, root exudation, and induction of *Striga* resistance. More specifically, we discovered the functional potential of soil and root-associated bacteria and fungi to: i) disrupt the early stages of the parasite's life cycle through the production of volatile organic compounds, ii) cause *Striga* seed decay or suicidal germination, iii) degrade host-derived germination signals and haustorium-inducing factors, and iv) induce structural barriers (aerenchyma, suberin) in the host plant roots. In collaboration with local research institutes, we developed strategies to augment *Striga*-suppressive activities of indigenous soil microbial communities to achieve Striga suppression in greenhouse and field settings.

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Fathoming the interaction between secondary metabolites of phylogenetically related *Bacillus* species and the plant metabolome

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Plant-beneficial microbes are known to provide multifaceted traits that contribute to plant health. *Bacillus* species, frequently found within the plant holobiont, are known to enhance seed germination, promote plant growth, and bolster defense mechanisms against phytopathogens[1], although the molecular shifts underlying these processes remain unstudied. Previous data has shed light on the topic, concluding that seed treatments lead to metabolic changes in adult plants. In this process the extracellular matrix component of *Bacillus subtilis*, TasA, and fengycin, a secondary metabolite, play crucial roles[2].

In this study, we hypothesize that this metabolic reprogramming at the seed level is conserved among closely related *Bacillus* species, while differences may arise from variations in secondary metabolite production. To evaluate this, we conducted an in-depth analysis of the metabolic profile of whole plants emerging from seeds treated with either *Bacillus subtilis* or *Bacillus velezensis*. We also analyzed the role of bacillomycin, a secondary metabolite mainly produced by the latter. We describe the different metabolomic patterns found in different parts of the plant, focusing specifically on leaves and stem sections, identifying tryptophan and alkaloids, in the case of *B. subtilis*, and a shift in the pool of lipids, for *B. velezensis*, as key metabolites in the specific response of each bacterium. Ultimately, we evaluated different abiotic and biotic stressors on plants emerging from treated seeds, finding an enhancement of the resistance of the adult plants in all cases. Moreover, plants emerging from seeds treated with *B. velezensis* exhibit a slowed-down program of development that does not compromise their favorable response to stressors.

These findings suggest that two different plant growth and resistance developmental programs, triggered by closely related *Bacillus* species, may converge toward a common goal.

[1] Bhattacharyya, P. N., & Jha, D. K. (2012). Plant growth-promoting rhizobacteria (PGPR): Emergence in agriculture. In World Journal of Microbiology and Biotechnology (Vol. 28, Issue 4, pp. 1327–1350). https://doi.org/10.1007/s11274-011-0979-9

[2] Berlanga-Clavero, M. V., Molina-Santiago, C., Caraballo-Rodríguez, A. M., Petras, D., Díaz- Martínez, L., Pérez-García, A., de Vicente, A., Carrión, V. J., Dorrestein, P. C., & Romero, D. (2022). Bacillus subtilis biofilm matrix components target seed oil bodies to promote growth and anti-fungal resistance in melon. Nature Microbiology, 7(7), 1001–1015. https://doi.org/10.1038/s41564-022-01134-8

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