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MINISTRY OF EDUCATION, YOUTH AND SPORTS

1st INTER MICRO project meeting

TELČ 2024

PROGRAM

&

ABSTRACT BOOK

MBU Institute of Microbiology Academy of Sciences of the Czech Republic

VYSOKÁ ŠKOLA CHEMICKO-TECHNOLOGICKÁ V PRAZE

CHARLES UNIVERSITY

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Obsah

PROJECT ORGANIZATION

WP 1 - JANA KAMANOVÁ *Words and sentences in microbial communication*			
	Name	**Activity leader**	**Institution**
RO1-1	Signal molecules and cascades in bacterial cell-to-cell signalling and inter-kingdom communication	Jana Kamanová	IMIC, UCT
RO1-2	Protein substrates of secretion systems in bacterial interactions	Ladislav Bumba	IMIC
RO1-3	Metallophores and nutrient acquisition in microbial communities	Andrea Palyzová	IMIC
RO1-4	Carbohydrates in the interaction with human and soil microbiome - triggers of the human and plant immune response	Pavla Bojarová	IMIC, UCT
RO1-5	Identification of novel bioactive compounds to modulate the microbiome	Kateřina Valentová	IMIC, UCT
WP 2 - FRANTIŠEK ŠTĚPÁNEK *Talking to microbes – innovative signal delivery systems*			
RO2-1	Microchemical signal emitters	František Štěpánek	UCT, IMIC, CUNI
RO2-2	Microchemical signal scavengers	Viola Tokárová	UCT, IMIC
RO2-3	Encapsulated microorganisms	Jitka Čejková	UCT, IMIC
RO2-4	Programmable multi-signal synthesiser	Jitka Viktorová	UCT, IMIC, CUNI
WP 3 - MARTIN SCHWARZER *Microbial conversation for the host health*			
RO3-1	Microbial murmurs: Microbial small talk over dinner	Martin Schwarzer	IMIC; UCT
RO3-2	Barrier for bugs: Epithelial barrier as the means for communication	Jan Dobeš	CUNI; IMIC
RO3-3	Immunity to invaders: Dialogue with microbes for host's resilience	Miloslav Kverka	IMIC; CUNI
RO3-4	Belows from bowel: Gut microbiota calls to distant organs	Radka Roubalová	IMIC; CUNI
RO3-5	Songs from surroundings: Microbes as tools for communication between host and its environment	Jakub Kreisinger	CUNI; IMIC
WP 4 – PETR KOHOUT *Microbial conversation for the host health*			
RO4-1	Assembly and coalescence of microbial communities within and modulation of the plant microbiome	Ondřej Uhlík	UCT, IMIC
RO4-2	Fluxes of carbon and mineral nutrients between plant and soil – role of the microbiome	Jan Jansa	IMIC, UCT
RO4-3	Plant microbiomes under changing environment	Petr Kohout	IMIC

WP = work package; RO = research objective; IMIC = Institute of Microbiology; UCT = Institute of chemical technology; CUNI = Charles University

PROGRAM AT GLANCE

Monday 14 October

9:30-10:00	*Arrival, morning coffee, poster installation*
10:00-10:20	**Welcome and introduction**
10:20 – 12:15	**WP1 presentations**
12:15 - 13:45	*Lunch*
13:45 – 14:30	**WP2 presentations**
14:30 – 16:00	**WP3 presentations**
16:00-16:45	*Coffee break + posters (everyone´s own choice)*
16:45 – 17:45	**WP4 presentations**
18:00	*Dinner in the dining hall*
19:30 – 21:30	**Posters with drinks**

Tuesday 15 October

8:30 – 9:30	**Wrap up of workpackages 1 through 4**
9:30 – 10:15	**Wrap up of the project activities**
10:15 – 12:00	**Posters** *with coffee,*
	Time for ISAB to discuss conclusions
12:00 – 13:15	*Lunch*
13:15 – 14:30	**Feedback from the ISAB**,
14:30 - 15:00	**Conclusion** of the meeting, final announcements, departure

PROGRAM

Monday 14 October

9:30-10:00 Arrival, morning coffee, poster installation

10:00-10:20 Welcome and introduction

- **Jan Jansa** - Introduction of the ISAB members, meeting structure and objectives, INTER-MICRO project structure, objectives, deliverables, milestones, organizational announcements

10:20 – 12:15 WP1 presentations (12 + 2 min each). Chair: Lucie Najmanová

- **Ladislav Bumba** - A multifaceted role of filamentous hemagglutinin (FHA) in the virulence of *Bordetella* species
- **Michal Grulich** - Rational engineering of the penicillin G acylase: a promising tool for modulation biofilm development
- **Vladimír Křen** - Exploring Diverse Glycone Preferences of *Aspergillus niger* Rutinosidase
- **Kristýna Slámová** - Enzymatic remodeling of chitin into bioactive chitooligomers
- **Tomáš Vomastek** - GpsB coordinates StkP signaling as a PASTA kinase adaptor in *Streptococcus pneumoniae* cell division
- **Jana Kamanová** - Identification of type III secretion system effector protein families in *Aeromonas schubertii*
- **Zdeněk Kameník** - Deciphering Microbial Interactions Using Mass Spectrometry Metabolomics
- **Petr Man** - Hydrogen/deuterium exchange mass spectrometry to study large heterogeneous proteins

12:15 - 13:45 Lunch

13:45 – 14:30 WP2 presentations (12+3 min each) Chair: Peter Šebo

- **František Štěpánek**- Microcapsule communicators for bioactive payload delivery and recovery
- **Jitka Čejková** - Manipulation and Transport of Cell-Encapsulating Alginate Particles Using Artificial Chemotaxis
- **Viola Tokárová**- Microparticle development - future robots with the ability to emit/absorb signaling molecules

14:30 – 16:00 WP3 presentations (12+3 min each), chair: Petr Novák

- **Martin Schwarzer** - Germ-free piglet model of juvenile undernutrition
- **Jakub Kreisinger**- The effect of genetic admixture on wild house mouse gut microbiota is weak when accounting for spatial autocorrelation
- **Dagmar Šrůtková** - Immunomodulatory potential of bifidobacterial cell-wall molecules on airway immune response in murine experimental studies
- **Radka Roubalová**- How microbes shape eating behavior: an experimental animal model of anorexia nervosa
- **Anna Jelínková**- LC-MS Analysis of Derivatized Short Chain Fatty Acids
- **Valeriia Belova** - Synthetic ecology of mycorrhizal hyphosphere

16:00-16:45 coffee break + posters (everyone´s own choice)

16:45 – 17:45 WP4 presentations (12+3 min each), chair: Vladimír Křen

- **Ondřej Uhlík** - Uncovering novel microbial populations associated with soil and subsurface carbon processing or fixation
- **Caroline Krug Vieira** - Reconstituting mycorrhizal ecosystem function through microbiome manipulation
- **Jan Jansa** - Talking, living, and working with prokaryotic microbes in the INTER-MICRO project
- **Petr Kohout** - What can we learn about fungal ecology and plant associated lifestyle of fungi from global databases of fungal species distribution?

18:00 Dinner in the dining hall

19:30 – 21:30 Posters with drinks

(posters arranged according to WP – see p. 24 and on, presenters should be onsite)

Tuesday 15 Oct

8:30 – 9:30 wrap up of workpackages 1 through 4 (10-15 min each), chair: Jan Jansa

- **WP1 – Jana Kamanová**
- **WP2 - František Štěpánek**
- **WP3 – Martin Schwarzer**
- **WP4 – Petr Kohout**

9:30 – 10:15 wrap up of the project activities, main challenges ahead, general discussion, proposing conclusion of the meeting

- **Jan Jansa**

10:15 – 12:00 posters with coffee, informal discussions,

time for ISAB to discuss conclusions, recommendations etc in a separate room

12:00 – 13:15 lunch

13:15 – 14:30 feedback from the ISAB, formulation of the report for the Ministry of Education,

14:30 - 15:00 conclusion of the meeting, final announcements, removing posters, departure

LIST OF POSTER PRESENTATIONS

WP1

1. **Gabriela Balíková Novotná**: Antibiotics in communication – interplay of ABCF proteins in decoding the antibiotic signal
2. **Pavel Branny**: Adaptor function of GpsB in regulation of StkP signaling pathway in *S. pneumoniae*
3. **Monika Čížková**: Deciphering the role of BscX and BscY subunits in the *Bordetella* Type 3 Secretion System
4. **Michaela Grobarčíková**: Identification of residues involved in post-translational modification of *Bordetella pertussis* adenylate cyclase toxin
5. **Jiří Mašín**: Acylated segment play a key role in the folding and cell penetration capacity of *Bordetella pertussis* adenylate cyclase toxin
6. **Andrea Palyzová**: Alternative enzymatic strategy for disruption of quorum sensing signaling molecules: promise for reducing bacterial biofilms
7. **Jitka Viktorová**: Plant secondary metabolites enhance the effectiveness of antibiotics
8. **Milada Kambová**: Bimodal Expression of Type 3 Secretion System 2 Enables Cooperative Virulence among Intracellular *Salmonella* Typhimurium
9. **Barbora Petránková:** Aryl sulfotransferases as a tool for polyphenol sulfonation
10. **Tommaso Stefani:** Enhancing Polar Metabolite Coverage in LC-MS Basted Metabolomics

WP2

11. **Adéla Brejchová**: Encapsulation in Glucan Particles for Improves Antimicrobial Activity and Wound Healing
12. **Eva Králová**: Enhancing Antioxidant and Anti-inflammatory Activity of Propolis through Glucan Particle Encapsulation
13. **Suada Dukaj**: Development of intestinal biosimilar mucus to study drug transport through the mucus barrier
14. **Martin Krov**: *title not authorized for public viewing*

WP3

15. **Tereza Novotná:** Impact of selected probiotic bacteria on juvenile host growth upon malnutrition in gnotobiotic mouse model
16. **Janet Ježková:** Anti-neuronal antibodies and the gut microbiota in patients with central hypersomnolence disorders
17. **Zdeněk Kukačka:** Fast FluoroAlkylation of Proteins as a Novel Strategy for Characterization of Membrane Protein in Bacteria
18. **Evgeniya Biryukova:** Development of Software for LC-MS Data Processing of Oligonucleotides
19. **Michal Kraus:** Gut microbiota protects mice from acute colitis by improving intestinal barrier function
20. **Janaina L.S. Donadio:** Dietary fiber pectin from papaya fruit (Carica papaya) modulates gut microbiota in a mouse model of colon carcinogenesis
21. **Veronika Motúzová:** High-protein diet accelerates the initial stage of colorectal cancer
22. **Miloslav Kverka**: Oral metronidazole reduces delayed-type hypersensitivity by modulating mucosal immunity

23. **Dagmar Čížková**: Convergence of gut phageomes but not bacteriomes after experimental transplantation of wild mouse bacteriophages into captive house mice
24. **Halil Mert Solak:** The gut microbiome and diet composition of Papua New Guinea Rodents among different altitudes

WP4

25. **Petr Baldrian:** Selective cutting preserves the health of temperate forest soil microbiome
26. **Jakub Skřivánek**: A comparison of the realized and fundamental niches of selected arbuscular mycorrhizal fungal species along a nitrogen gradient
27. **Tomáš Větrovský**: GlobalFungi and GlobalAMFungi Databases: Powerful tools to explore fungal mycobiomes
28. **Felix Wesener**: Soil mycobiome diversity peaks in the mountains
29. **Barbora Křístková**: Nitrile synthesis catalysed by aldoxime dehydratase immobilized on metal affinity resins
30. **Ludmila Martínková**: Nitriles and cyanides in microbe-plant communication
31. **Jáchym Šuman**: The role of soil prokaryotic communities in the biodegradation of lignin
32. **Petra Bukovská**: Stable isotope probing to disentangle carbon for nitrogen trading in mycorrhizal hyphosphere
33. **Michal Strejček**: Exploring unique microbial pathways on novel prokaryotic phyla in extreme subsurface environments

ABSTRACTS

Red-highlighted names indicates project personnel, presenter´s name is underlined

ORAL PRESENTATIONS – Work package 1 (Words and sentences in microbial communication)

A multifaceted role of filamentous hemagglutinin (FHA) in the virulence of *Bordetella species*

David Jurnečka[1], Katerina Vocadlova[1], Abdul Samad[1], Petra Kasparova[1], Josef Chmelik[1], Jana Holubova[1], Ondrej Stanek[1], Ludmila Brazdilova[1], Elodie Lesne[2], Andrew Gorringe[2], Peter Sebo[1] and Ladislav Bumba

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Filamentous hemagglutinin (FHA), a major virulence factor of classical *Bordetella*e, is a rod-shaped molecule that plays an important role in the adherence of bacteria to ciliated epithelial cells of the upper respiratory tract and suppresses the host innate and adaptive immune response. FHA is translated as a 360-kDa FhaB precursor that is exported across the outer bacterial membrane by a two-partner secretion mechanism involving the outer membrane protein FhaC and shed into external environment as an N-terminal 'mature' 220-kDa FHA protein after processing by surface-exposed SphB1 protease. The remaining C-terminal 130-kDa FhaB prodomain is thought to regulate maturation process and rapidly degraded in the periplasm. We show here that both the extreme C terminus (ECT) of the FhaB prodomain and the mature FHA play the pivotal roles in the virulence of *B. pertussis*. The NMR-based structural analysis of ECT, a highly-conserved the C-terminal 100 residues of the FhaB precursor, revealed that the ECT polypeptide adopts a rigid structure with a 'pilin-like' protein fold. Deletion of the sequence encoding ECT (ΔECT) resulted in a significant decrease in bacterial colonization within the nasal cavity of infected mice, comparable to *B. pertussis* strain lacking the FhaB precursor (ΔFhaB). Intriguingly, the ΔECT strain exhibited a complete loss of its ability to bind cilia on human nasal epithelial cells grown at the air-liquid interface, emphasizing the indispensable role of ECT in the adherence of Bordetella cells to ciliated epithelial cells. Furthermore, we demonstrate the mature FHA confers resistance of *B. pertussis* to complement-mediated killing, highlighting its involvement in protection of bacterial cells against the host's innate immune response. Collectively, these results provide novel insights into FHA biology, unraveling its multifaceted role in the virulence of pathogenic *Bordetellae*.

Rational engineering of the penicillin G acylase: a promising tool for modulation biofilm development

Michal Grulich[1], Bartlomiej Surpeta[2,3], Andrea Palyzová[1], Helena Marešová[1], Jan Brezovský[2,3]

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The rapid rise of antibiotic-resistant bacteria necessitates searching for alternative, unconventional solutions, such as targeted modulation of bacterial communication systems. Signaling disruption can be achieved by enzymatic degradation of autoinducers, reducing the expression of genes responsible for virulence, biofilm formation, and drug resistance. Quorum quenching (QQ) processes disrupt bacterial communication via the QS system, which bacteria use as an active way to sense the environment and deliver information to its own community, as well as to other species. Although many enzymes exhibit QQ activity against various bacterial autoinducers, their mechanisms of action are poorly understood, limiting their potential optimization as QQ agents. Using molecular dynamics simulations of the free enzymes and their complexes with two autoinducers of different lengths, followed by quantum mechanics/molecular mechanics MD simulations of two catalytic steps, we elucidate the molecular processes underlying QQ activity. A newly constructed penicillin G acylase (PGAEc) variant leads to the specific degradation of short to medium N-acyl homoserine lactones (AHLs), which serve as autoinducers for Gram-negative bacteria. PGAEc variants designed in silico carry mutations in the acyl-binding pocket of PGAEc to deepen it, narrow and stabilize its initial part, or elongate the pocket. The modelling pipeline, docking, and molecular dynamics simulations are combined with the iterative cycle process. Critical biochemical parameters of the resulting PGAEc variants are optimized to effectively inhibit biofilm development, specifically in *Pseudomonas aeruginosa*. In general, the strategy should provide a truly functional solution in the fight against AHL-producing G-bacteria and may help limit antibiotic use in essential cases.

Exploring Diverse Glycone Preferences of *Aspergillus niger* Rutinosidase

Vladimír Křen,[1] Katerina Brodsky,[1,2] Lucie Petrásková,[1] Martina Hurtová,[1] Jitka Brouzdová,[1] and Pavla Bojarová[1]

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Introduction

Rutinosidases (6-α-L-rhamnosyl-β-D-glucosidases) represent a class of retaining diglycosidases pivotal in cleaving the glycosidic bond between saccharide moieties and their respective aglycones in glucosides and rutinosides. Among these, the most extensively studied is the rutinosidase derived from *Aspergillus niger* (*An*Rut) [1]. Insights from its crystal structure and molecular modeling [2] reveal a robust, specific affinity towards aromatic aglycones (flavonoids) and remarkable adaptability at the glycone binding site. Notably, this enzyme demonstrates a broad acceptance of both β-glucopyranosides and rutinosides, showcasing significant flexibility in binding to the glycone moiety [3].

Objectives

We aimed to investigate the variability of various mono- and diglycosides, mostly as *p*-nitrophenyl glycosides. Our objective was to explore the potential utilization of alternative carbohydrates, focusing on their applicability in transglycosylation reactions with alcoholic and phenolic acceptors.

Results

We have shown that *An*Rut can cleave β-glucopyranosides glycosylated at C-6 of the glucose moiety – natural substrates rutinosides but also isomaltosides (6-α-d-glucopyranosyl-β-d-glucosides). 1-4-linked diglycosides (e.g., maltoside and lactoside) are not hydrolyzed, showing a certain preference for the C-6 substitution of Glc. *An*Rut cleaves unsubstituted β-glucopyranosides well and we have now shown that other β-glycosides, such as β- d-galactopyranoside, β-d-xylopyranoside and α-l-arabinopyranoside (which is a structural analog of β-d-Gal), are also well accepted by the enzyme. Transglycosylation with various acceptors, including phenolic acceptors was also successfully tested.

Conclusion

This study demonstrates the great substrate flexibility of rutinosidase at the glycone site, so that the substrate specificity of *An*Rut can be extended to the C-6-modified glucosides [4] and some other monoglycosides. The high transglycosylation potential of this enzyme, which also enables the glycosylation of phenolic acceptors, extends the application potential of this versatile enzyme.

References

[1] Šimčíková D, Kotik M, Weignerová L, Halada P, Pelantová H, Adamcová K, Křen V. *Adv. Synth. Catal*. 2015, 357,107–117.

[2] Brodsky K, Kutý M, Pelantová H, Cvačka J, Rebroš M, Kotik M, Kutá Smatanová I, Křen V, Bojarová P. *Int. J. Mol. Sci*. 2020, 21, 5671.

[3] Křen V, Bojarová P, *Biotech. Adv*. 2023, 108217.

[4] Brodsky K, Petrásková L, Kutý M, Bojarová P, Pelantová H, Křen V. *ChemCatChem*. 2024, 16, e202400028.

Enzymatic remodeling of chitin into bioactive chitooligomers

Kristýna Slámová[1], Zuzana Mészáros[1], Lucie Petrásková[1], Natalia Kulik[1], Vladimír Křen[1]

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Chitooligomers (COS, β-1-4-linked oligomers of *N*-acetylglucosamine (GlcNAc) and glucosamine (GlcN)) are bioactive compounds with diverse beneficial effects. Importantly, COS are key signaling molecules in plant-pathogen interactions as they activate plant defense mechanisms and protect plants from diseases indirectly as elicitors of resistance against bacterial, fungal, and insect pathogens [1]. The biological activity of COS depends on factors like the degree of polymerization (DP) and the degree of acetylation (DA). Longer COS of DP 6-8 exhibit higher antifungal activity and stronger affinity to the respective receptors than shorter COS of DP 4-5, inducing thus chitin-responsive genes more effectively. Chitin, the source of COS, is abundant in crustacean shells, creating a considerable amount of biowaste in the seafood industry. Enzymatic synthesis faces challenges in converting chitin to well-defined chitooligomers and developing a robust and scalable method for the synthesis of COS with DP ≥ 6 is of utmost importance.

Here we describe a pioneering enzymatic approach for the efficient transformation of chitin, the waste material of the food industry, into valuable chitooligomers with a degree of polymerization ranging from 6 to 11. In the first step, chitin was hydrolyzed using engineered variants of the novel fungal chitinase from *Talaromyces flavus* with increased hydrolytic activity to generate low-DP chitooligomers, followed by an extension to the desired DP using the high-yielding Y445N variant of β-*N*-acetylhexosaminidase from *Aspergillus oryzae* [2], achieving yields of insoluble COS (DP ≥ 6) up to 57%. The innovative enzymatic route demonstrates sustainability and feasibility for the transformation of waste chitin into bioactive chitooligomers, which can be further enzymatically deacetylated to increase their biological effects.

[1] Liaqat F and Eltem R (2018) Chitooligosaccharides and their biological activities: A comprehensive review. *Carbohydr. Polym.,* 184: 243-259

[2] Mészáros Z, Petrásková L, Kulik N, Pelantová H, Bojarová P, Křen V, and Slámová K (2022) Hypertransglycosylating variants of the GH20 β-*N*-acetylhexosaminidase for the synthesis of chitooligomers. *Adv. Synth. Catal.*, 364: 2009-2022.

GpsB coordinates StkP signaling as a PASTA kinase adaptor in *Streptococcus pneumoniae* cell division

Václava Stauberová[1], Bohumil Kubeša[1], Merrin Joseph[2] , Mattia Benedet[3], Berenice Furlan[3], Karolína Buriánková[1], Aleš Ulrych[1], Rudolf Kupčík[4], Tomáš Vomastek[1], Orietta Massidda[3], Ho-Ching T. Tsui[2], Malcolm E. Winkler[2], Pavel Branny[1] and Linda Doubravová[1]

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StkP, the Ser/Thr protein kinase of the human pathogen *Streptococcus pneumoniae*, monitors cell wall signals and regulates growth and division in response. *In vivo,* StkP interacts with GpsB, a cell division protein required for septal ring formation and closure that affects StkP-dependent phosphorylation. Recent phosphoproteomic analyses revealed phosphorylation of GpsB at multiple Ser/Thr residues. Here, we confirmed that StkP directly phosphorylates GpsB *in vitro* and *in vivo*, with T79 and T83 being the major phosphorylation sites. StkP has intrinsic kinase activity, but GpsB directly promotes the autophosphorylation of StkP and phosphorylation of StkP substrates. *In vitro*, GpsB phosphoablative substitutions had a reduced potential to stimulate StkP activity, whereas phosphomimetic substitutions were functional regarding StkP activation. Bacterial two-hybrid assay and co-immunoprecipitation of GpsB from cells with differentially active StkP indicated that increased phosphorylation of GpsB resulted in a more efficient interaction of GpsB with StkP. Our data suggest that GpsB acts as an adaptor that directly promotes StkP activity by mediating interactions within the StkP signaling hub, ensuring StkP recruitment into the complex and substrate specificity. Consequently, the interaction with GpsB and its phosphorylation and dephosphorylation dynamically modulate StkP activity during exponential growth and under the cell wall stress of *S. pneumoniae*, ensuring proper functioning of the StkP signaling pathway. In summary, we have demonstrated that GpsB plays a crucial role in the StkP-dependent signaling complex, facilitating the transfer of environmental signals into cellular responses.

Identification of type III secretion system effector protein families in *Aeromonas schubertii*

Hana Michova, Jan Pliva, Anezka Jirsova, David Jurnecka and Jana Kamanova

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Aeromonas, a genus of Gram-negative bacteria, is commonly found in aquatic environments around the world and is a regular component of fish intestinal microbiota. However, environmental stress or fish injury can cause mesophilic *Aeromonas* species to induce motile aeromonad septicemia (MAS), leading to high fish mortality and severe economic losses in aquaculture. Furthermore, these γ-proteobacteria are increasingly recognized as opportunistic human pathogens, responsible for gastroenteritis, wound infections, and septicemia. Despite their impact, little is known about their fitness factors, including the type III secretion system (T3SS) and its role in both commensal and pathogenic lifestyles. Interestingly, besides the widely distributed type III injectisome API-1 belonging to the *Yersinia* Ysc family, some *Aeromonas* strains also encode a second injectisome, API-2, from the *Salmonella* Inv-Spa family. Here, we aimed to identify the effectors translocated into host cells by *A. schubertii* API-1 and API-2 and their contribution to host interaction. We show that effectors translocated by API-1 cause cytotoxicity in HeLa cells, while API-2 effectors are essential for intracellular survival within predatory amoebas. Mass spectrometry analysis of secretomes of wild-type, ΔAPI1, and ΔAPI2 mutants indicates presence of two known effectors, AopH and AopO, and five putative effectors, PteR, PteI, PteJ, PteL a PteN for API-1, and two putative effectors PteC and PteD for API-2. We further employed a split-luciferase system to verify the translocation of putative API-1 effectors into HeLa cells. The next steps will address the individual roles of these effectors, their molecular mechanisms of action, and the analysis of signaling cascades responsible for their transcription and translation and the biogenesis of *Aeromonas* API-1 and API-2 injectisomes.

Deciphering Microbial Interactions Using Mass Spectrometry Metabolomics

Zdeněk Kameník, Tommaso Stefani.

Institute of Microbiology, Czech Academy of Sciences, Videnska 1083, 142 20 Prague 4, Czech

To study interactions between microbes and between microbes and their hosts, we have acquired the TimsTOF HT high-resolution mass spectrometer, coupled with ion mobility, as a major investment of the InterMicro project. In this lecture, we will introduce this advanced instrumentation and outline its potential applications in metabolomics studies relevant to our project. We will highlight key hardware features and then focus on data processing options and potential outcomes, illustrated through several case studies we have conducted so far.

Hydrogen/deuterium exchange mass spectrometry to study large heterogeneous proteins

Zuzana Kalaninova, Sarka Knoblochova, Peter Sebo, Jiri Masin, Petr Man

[1]Institute of Microbiology of the Czech Academy of Sciences, Czech Republic

CyaA is a bifunctional toxin that exhibits both cell-invasive adenylate cyclase (cytotoxic) and hemolytic (pore forming) activities on a variety of cells. It plays an important role in the early stages of respiratory tract colonization by the *Bordetella pertussis* pathogen. CyaA is a large multidomain protein containing 1706 amino acid residues. According to its function, CyaA is divided into the following domains: an N-terminal adenylate cyclase (AC) domain that binds calmodulin and converts host cell cytosolic ATP to cAMP; a linker segment that regulate the balance between the AC domain translocating and pore-forming capacities of CyaA; a hydrophobic region capable of penetrating host cell membranes to form pores; an acylation region containing two acylation sites critical for CyaA function; and a C-terminal calcium-binding RTX domain. Due to its size, structural studies focused on just smaller parts of the protein. Hydrogen/deuterium mass spectrometry has virtually no size limitation, works under various experimental conditions, and thus represents an excellent tool for probing the structural aspects of CyaA. Intact CyaA can be prepared and is stable in a denatured state devoid of calcium ions. Lowering the denaturing agent and adding Ca^{2+} induces folding. Here, we developed a workflow that allowed rapid screening of the folding process and monitoring of structural effects of various functionally relevant mutations. It provided spatially resolved information about the effect of different mutations and the thermodynamic stability of CyaA. Work with this heterogeneous protein system also underlined the importance of sufficient biological replications to capture the true structural effects and differentiate them from batch-to-batch variability.

ORAL PRESENTATIONS – Work package 2 (Talking to microbes – innovative signal delivery systems)

Microcapsule communicators for bioactive payload delivery and recovery

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Communication between the microbial and the macroscopic worlds by means chemical signals has been traditionally achieved by “bulk” methods, e.g. by pipetting a solution of the investigated substance to a Petri dish or a culture flask and observing the response. While this approach may be appropriate for “global” signalling scenarios, it has limitations when local concentration gradients or temporal variations of the local signalling substance concentration are of importance. This talk will provide an up-to-date overview of our research on engineered microcapsules that can either release or absorb bioactive substances “on demand” and therefore act as chemical communicators able to modify local spatial and temporal concentration profiles of various substances that can range from metal ions trough small organic molecules to peptides and RNA. The talk will cover the design, manufacturing, characterisation and application of several classes of microcapsule carriers, based on mesoporous silica particles, whole yeast glucan particles, and magneto-liposomes. Special focus will he places on the release kinetics of the bioactive payload and methods for its active control, e.g. to achieve on-off release scenario at predefined time steps. Examples of in vitro and in vivo drug delivery to model organisms will be provided. Potential for further prospects of such microcapsules within the INTER-MICRO project will be discussed.

Manipulation and Transport of Cell-Encapsulating Alginate Particles Using Artificial Chemotaxis

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This work aims to explore the use of cell-encapsulating alginate capsules in various environments to study intercellular communication. To enable movement within the environment, these capsules can be manipulated in several ways: they can be carried by fluid flow, controlled with magnets if embedded with magnetic particles, or directed using chemical gradients. Here we investigated whether alginate capsules, which provide a suitable environment for cells due to their hydrogel nature, could be transported by decanol droplets – a method previously shown to transport various objects via artificial chemotaxis. By introducing a surfactant during alginate cross-linking, we successfully integrated these hydrophilic capsules into hydrophobic decanol droplets, creating a novel transport system. Our research builds on previous work with self-moving and transforming droplet systems, including chemotaxis, shape changes, maze-solving, and swarming. These systems offer a simplified model for understanding key processes of living systems and hold potential for innovative applications in bioengineering and microbiology.

Microparticle development - future robots with the ability to emit/absorb signaling molecules

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Encapsulation involves enclosing different active substances (such as antimicrobials, aromas, and oils) or cells in polymeric carriers. This method is widely used in various industrial and scientific sectors, including pharmaceuticals, food, and cosmetics. It can serve to protect the encapsulated material from the surrounding environment and control the transport of molecules in and out of the system. This work will focus on the encapsulation process and the optimisation of process parameters to determine particles' final size, shape, and structure. The presentation will include a representative system of core-shell particles containing a model lipophilic drug with lipid-based cores and polymeric alginate shells and encapsulation strategies for bacterial cells using the Büchi Encapsulator B-395 Pro.

Germ-free piglet model of juvenile undernutrition

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We have previously shown that the specific strain *Lactoplantibacillus plantarum* WJL (LpWJL) is able to promote the growth of malnourished *Drosophila* larvae and juvenile gnotobiotic and conventional mice. Due to its similarity to humans in anatomy, physiology, and genetics, the pig is a frequently used translational animal model in biomedical research. We established a piglet model of juvenile malnutrition and tested the ability of LpWJL to promote the growth of gnotobiotic piglets.

Germ-free (GF) minipigs (Animal Research Institute, Kostelec nad Orlici, Czech Republic) were obtained by hysterectomy on the 112th day of gestation. Piglets were reared in fiberglass isolators with a heated floor and bottle-fed with autoclaved cow's milk-based diet (Mlekarna Hlinsko, Hlinsko, Czech Republic) 6–7 times per day, and the amount of food intake was recorded. On day of life (DOL) 4, a group of piglets was colonized by LpWJL and their weight and length were followed daily until sacrifice at DOL 15. In adition, groups of piglets were at DOL 4 transferred to irradiated 5% (undernutrition) and 25% (full nutrition) ROMELKO MILK (RM) comercial powder formula (De Heus, Ede, Netherlands).

Our preliminary data show that LpWJL was able to stably colonize the milk-fed piglets. Compared to the GF milk-fed piglets, LpWJL-colonized grew better in terms of both weight and length and showed better feed conversion ratio. 5% RM feeding of GF piglets induced severe undernutrition with edema and macroscopic growth similar to that of GF milk-fed piglets.

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The effect of genetic admixture on wild house mouse gut microbiota is weak when accounting for spatial autocorrelation

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The question of how interactions between the gut microbiome and vertebrate hosts contribute to host adaptation and speciation is one of the major problems in current evolutionary research. Using bacteriome and mycobiome metabarcoding, we examined how these two components of the gut microbiota vary with the degree of host admixture in secondary contact between two house mouse subspecies (Mus musculus musculus and M. m. domesticus). We used a large data set collected at two replicates of the hybrid zone and model-based statistical analyses to ensure the robustness of our results. Assuming that the microbiota of wild hosts suffers from spatial autocorrelation, we directly compared the results of statistical models that were spatially naive with those that accounted for spatial autocorrelation. We showed that neglecting spatial autocorrelation can strongly affect the results and lead to misleading conclusions. The spatial analyses showed little difference between subspecies, both in microbiome composition and in individual bacterial lineages. Similarly, the degree of admixture had minimal effects on the gut bacteriome and mycobiome and was caused by changes in a few microbial lineages that correspond to the common symbionts of free-living house mice. In contrast to previous studies, these data do not support the hypothesis that the microbiota plays an important role in host reproductive isolation in this particular model system.

Immunomodulatory potential of bifidobacterial cell-wall molecules on airway immune response in murine experimental studies

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Live probiotic *Bifidobacterium* strains are considered to be beneficial for its consumers. However, recent focus has been shifted towards bacteria-derived defined molecules, so called postbiotics. Postbiotics are defined as "a preparation of inanimate microorganisms and/or their components that confers a health benefit on the host". The excellent source of these effector macromolecules is the bacterial cell wall.

1) Using germ-free (GF) BALB/c mice we analysed the immunomodulatory potential of three components isolated from cell wall of probiotic *Bifidobacterium longum* ssp. *longum* CCM7952 strain. Crude exopolysaccharide (EPS), protein (PROT) or peptidoglycan (PG) were isolated from surface of this bifidobacterial strain and applied intranasally to GF mice in isoflurane anesthesia three times in one-week interval. One week after the third administration, bronchoalveolar lavage and lung tissue for histological analysis, qPCR and cell digest homogenates were collected and analyzed. 2) Further, these surface components were tested for their potential to alleviate the airway allergic inflammation in mouse model. BALB/c mice were intraperitoneally sensitized with the ovalbumin (OVA) emulsified with Alum adjuvant. Before each sensitization and intranasal OVA-challenge, selected cell-wall components were applied intranasally to the lungs of mice.

1) In bronchoalveolar lavage of GF mice, we detected only slight lymphocyte and neutrophil infiltration into lung tissue in mice with PG treatment. By qPCR analysis we detected up-regulation of transcription factors (RORgt, FoxP3, TBX21) and pattern-recognition receptors (NOD1, TLR2) in lungs of mice treated by PG or PROT components. In supernatants of lung cell homogenate cultures we found out the distinct pattern of cytokine and chemokine stimulation.

2) Among the analysed surface molecules, only peptidoglycan was able to reduce levels of Th2-related OVA-specific IgE, cytokine response in splenocyte cultures, number of eosinophils in bronchoalveolar lavage and reduce the infiltration of leucocytes into lung tissue, and thus alleviate the airway allergic inflammation in murine model.

We proved that cell-wall components of *Bifidobacterium longum* ssp. *longum* CCM7952 are able to modulate immune response in lung tissue and especially peptidoglycan is promising candidate for alleviation of immune-aberrant reactions in lungs of the host. This work relates to the INTER-MICRO project objectives RO3-1-3 and RO3-3-5 with the aim to test and define the effect of bacteria-derived components in modulation of immune response and in the prevention of airway allergy in mouse models.

How microbes shape eating behavior: an experimental animal model of anorexia nervosa

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Keywords: anorexia nervosa, neuropeptides, activity, gut microbiota

The communication on the microbiota-gut-brain axis is currently being thoroughly investigated in the context of various neuropsychiatric disorders. The pathophysiology of the eating disorder anorexia nervosa is not entirely clear. It is hypothesized that the participation of the gut microbiota and related immune responses may contribute to the development of the disease.

To study this phenomenon, we introduced a mouse model of anorexia nervosa (activity-based model, ABA) in conventional, antibiotic-treated, and germ-free BALB/c mice. In these mice with substantially different gut microbiota backgrounds, we observed differences in their running-wheel activity already at the beginning of the experiment. During food restriction, the differences in activity between the three groups of mice became even more pronounced.

We also observed significant differences in appetite regulation in mice with different gut microbiome. Our results suggest that induction of the anorexia nervosa phenomenon in mice is associated with a considerable dysregulation of appetite-regulating neuropeptides and that this dysregulation differs depending on the microbial background of mice.

LC-MS Analysis of Derivatized Short Chain Fatty Acids

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ABSTRACT

Gut microbiota-associated metabolites have been extensively studied for their roles in physiology and disease. These include communication between gut microbiota and the host, providing nutrients for host cells, and regulating its metabolism and immunological functions. Therefore, analysis of gut microbiota-associated metabolites is crucial to explore the host–gut microbiota interactions.

Some gut microbiota-associated metabolites have been identified, such as short-chain fatty acids (SCFAs). SCFAs are organic acids with a length of 1-6 carbon atoms formed as fermentation products of intestinal microbiota. These products of the metabolism of anaerobic bacteria significantly influence the physiological state of the host organism and participate in setting the overall homeostasis. They serve as the main source of energy for colonocytes, they regulate the setting of metabolic pathways in cells and the function of cells of the immune system. Thanks to their low molecular weight and polarity, these substances penetrate the intestinal epithelium and spread throughout the body through the blood. Also, they affect the function of organs, including the brain, and serve as signaling molecules in the brain-gut pathway. The determination of these substances in complex matrices is currently required both in research and clinical medicine. However, it remains problematic, given by their high polarity and non-compatibility with reverse-phase chromatography.

To understand the phenomena of microbial metabolites in general, we can utilize models of gnotobiotic animals. This pilot study aimed to analyze SCFAs in stool samples of three cohorts of gnotobiotic mice with different gut microbial populations (germ-free, defined microbiota, conventional mice) by derivatization of SCFAs and subsequent analysis by liquid chromatography with mass detection (LC-MS). Samples were extracted and derivatized with 3-nitrophenyl hydrazine (3NPH). Gradient elution and a chromatographic column with a C18 ligand were used for separation.

Based on the successful application of the method, an extension will be carried out to include the overall quantitative analysis of not only basic SCFAs, but also organic acids produced in the citrate cycle, and subsequently interlaboratory validation of this method. Further, different derivatization agents targeting more functional groups will be used for a deeper understanding of the role of microbial metabolites.

KEYWORDS: Microbiome, germ-free, metabolomics, derivatization

Synthetic ecology of mycorrhizal hyphosphere

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Arbuscular mycorrhizal (AM) fungi establish symbiosis with most plant species currently on Earth. This ancient association dates back to the origin of vascular plants hundreds of millions years ago and the fungi are particularly efficient in gathering inorganic nutrients such as orthophosphate and ammonium in soil and exchanging them for reduced carbon compounds (sugars and lipids) with their host plants. On the other hand, the fungi lost the capacity to degrade complex organic molecules such as cellulose, chitin or phytate, which means that they rely on other soil microbes to release the mineral nutrients contained in such sources before these could be utilized by the AM fungus. Whereas several case studies confirmed the above concept, most experiments to date were carried out with laboratory strains of only a few prokaryotic taxa added to the microcosms separately, a situation which is far away from typically hyperdiverse soil ecosystems. Therefore, here we will prepare synthetic microbial communities with variable composition and diversity, and observe possible synergistic or antagonistic effects of such communities or their members on nutrient uptake by AM fungi from organic sources. Movement of bacteria along the hyphal networks and feedbacks of the prokaryotes on AM fungal growth and development (incl. spore formation) will be addressed with the aim of identification the specific chemical signals at play.

Uncovering novel microbial populations associated with soil and subsurface carbon processing or fixation

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The enormous diversity of microorganisms is not only astonishing, but also a key to understanding how organisms evolve and adapt to different environmental conditions. The metabolic activity of microorganisms is essential for maintaining the functions of all ecosystems in the biosphere. However, in different environments, different microbial populations may be associated with biogeochemical processes and use different metabolic pathways. In this presentation, the activities of the Laboratory of Microbial Ecology of the University of Chemistry and Technology, Prague, within the OP JAK project *Talking Microbes - understanding microbial interactions within One Health* will be presented.

In soils, plants are the major suppliers of organic carbon, with some compounds being quite recalcitrant. An example of such a compound is lignin, a complex plant biopolymer of considerable ecological and industrial importance. Although the synthesis of lignin in plants is well understood, its degradation by soil microorganisms is still not fully understood. While fungi are considered to be the main lignin degraders, recent studies and our results suggest that some bacteria produce enzymes capable of efficient lignin degradation. Therefore, our research aims to elucidate the role of bacterial populations in the degradation of lignin and its structural motifs (phenolic compounds) and to better understand the link between this degradation and the degradation of organic pollutants. In oligotrophic subsurface environments, on the other hand, carbon fixation is our primary interest. Our recent results show that we have detected microbial populations with an unusual arrangement of the Wood-Ljungdahl pathway, indicating the importance of horizontal gene transfer in isolated subsurface ecosystems. Overall, these results contribute to our ever-growing knowledge of the fascinating phylogenetic and metabolic diversity of environmental microorganisms.

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Reconstituting mycorrhizal ecosystem function through microbiome manipulation

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Arbuscular mycorrhizal (AM) fungi are dependent on association with saprotrophic microbes (chiefly the bacteria) in the soil to efficiently recycle organic sources of nitrogen (N) such as chitin. This has been firmly established using in-vitro experiments where different bacteria have been added or not to AM fungal hyphosphere and release and fungal acquisition of N from biopolymers was measured by isotopic tracing. However, questions remain whether the rates of organic nutrient recycling are affected by microbial diversity or rather presence of highly efficient degraders, whether specific microbes could migrate along the AM fungal hyphae to reach the nutrient sources, and how resilient are soil microbial communities to experimental manipulation. Linking microbial community composition in the AM fungal hyphosphere with the rates of organic N recycling will thus be carried out upon experimental manipulation of complex microbial communities using biocides (antibiotics, fungicides), suppressing specific microbial guilds, and the consequences of this manipulation on ecosystem processes will be quantified.

Talking, living, and working with prokaryotic microbes in the INTER-MICRO project

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Prokaryotes, be it bacteria or archaea, colonize nearly all environments on the planet, interacting (talking) with each other mainly via chemical cues. Further, the prokaryotes also interact with the other (eukaryotic) organisms, forming interaction networks and important part (besides protists) of the associative microbiomes of fungi, plants and animals including humans. Prokaryotic members of the microbiomes often complement or add important functions to their eukaryotic hosts, and this is increasingly acknowledged by using the term „holobiont“ for a eukaryotic host with its associated microbiomes. Importantly, microbes could also exploit its host, causing disease, reduction of competitiveness and eventually of fitness – which is important both from the health perspective of the host and the ecosystem stability including,.e.g., agricultural pathogen control. The INTER-MICRO project offers a rare opportunity to join forces and mutually enrich different scientific disciplines ranging from detailed study of various microbial models, functions and interaction processes through gnotobiotic systems with reconstructed microbiomes of fungi, plants and animals, up to complex ecosystems including the global change perspective. A central focus on the microbial manipulation through targeted/sequential delivery or scavenging of established and novel molecular signals allows for major cross fertilization of various research disciplines which would only rarely meet otherwise.

What can we learn about fungal ecology and plant associated lifestyle of fungi from global databases of fungal species distribution?

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GlobalFungi and GlobalAMFungi databases are open sources and open access initiatives that compile the most comprehensive atlases of fungal taxa distribution, so far. Altogether these databases provide almost 100,000 samples of fungal communities from all continents and environments and source materials, which were analysed using next-generation sequencing technologies. Besides the applicability of such dataset to study biogeography and global diversity patterns of fungi, it also offers unique opportunity to better understand ecology of individual fungal species. In this talk we would like to present recent advances in understanding global diversity patterns of various fungal ecological groups.

We will show different importance of host plant distribution of global diversity patterns of arbuscular mycorrhizal, ectomycorrhizal and ericoid mycorrhizal fungi. Besides that, we will demonstrate the applicability of GlobalFungi and GlobalAMFungi databases to identify various fungal traits and their applicability to better understand assembly rules structuring fungal communities. Specifically, we calculated the dependence of fungal species on plant-associated life history based on their preferential occurrence. Hundreds of fungal taxa were identified to preferentially occur in plant tissue (shoot and root) rather than in soil. Interestingly, this plant-associated trait was then the best predictor of endophytic fungal species responses to host plant identity in an independent case study. Similarly, we identified preferential investment of arbuscular mycorrhizal fungal taxa into intraradical vs. extraradical biomass. In subsequent application, we found higher occurrence of arbuscular mycorrhizal fungal taxa with preferential investment to soil biomass on sites with low levels of nutrient concentrations, while arbuscular mycorrhizal fungal taxa with preferential investment to root biomass dominated in highly fertile soils.

Altogether, GlobalFungi and GlobalAMFungi databases proved to be unprecedented source of data with potential to significantly improve our understanding of fungal ecology and biology.

ABSTRACTS - POSTER PRESENTATIONS

Poster No. 1

Antibiotics in communication – interplay of ABCF proteins in decoding the antibiotic signal

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Antibiotics play an important role in shaping bacterial communities not only by inhibiting growth but also by acting as signaling molecules that trigger various cellular responses depending on their target and concentration. Bacteria have developed a number of mechanisms to defend themselves and respond to antibiotic signals. As an important antibiotic target is the bacterial ribosome, these mechanisms include the ubiquitous family of ABCF proteins, which catalyze the displacement of antibiotics from the 50S ribosomal subunit. Our research has shown that the action of displacement not only confers resistance but also fine-tunes gene expression to ensure bacterial survival under stress conditions[1]. Bacteria typically encode 4 ABCFs, but the number of ABCF paralogs in the genome correlates with the complexity of the bacterial lifestyle and can be as high as 10 in microorganisms with rich secondary metabolism such as the soil-dwelling Actinomycetota.

The presented study focuses on two closely related ABCF proteins from the ARE5 subfamily encoded in Streptomyces coelicolor. Functional analysis revealed their different roles in decoding antibiotic signals at the ribosome.

Reporter gene fusions expression, validated by toeprinting assays, demonstrates that both proteins are regulated by a ribosome-mediated attenuation mechanism in response to antibiotics from the lincosamide, streptogramin A, and pleuromutilin groups. These antibiotics are known to inhibit the early stages of bacterial translation. Supported by proteomic analysis and reporter gene fusions, we also demonstrate that each protein responds at different times to a distinct range of antibiotic concentrations, ensuring that their expression is mutually exclusive. Moreover, only one of the proteins, TiaA, confers high resistance to basidiomycete-produced pleuromutilins, thus representing an instrument of interkingdom warfare. Overall, our study revealed a delicate interplay between two ABCF proteins, reflecting the hormetic effect of antibiotics which enables response at low antibiotic concentrations and survival at high, inhibitory concentrations.

1. Koberska et al, Mbio12(5), e01731-21 (2021).

Poster No. 2.

Adaptor function of GpsB in regulation of StkP signaling pathway in *S. pneumoniae*

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Streptococcus pneumoniae is a Gram-positive bacterium and an opportunistic pathogen with a commensal relationship with its obligate human host. Pneumococci colonize the human upper respiratory tract and are among the most common bacterial causes of infections, including otitis media, sinusitis, pneumonia, sepsis, and meningitis. The Ser/Thr protein kinase StkP monitors cell wall signals and, in response, regulates the growth and cell division of pneumococci. StkP interacts with the cell division protein GpsB, required for StkP-dependent phosphorylation in vivo. Phosphoproteomic analyses revealed that GpsB is phosphorylated at several Ser and Thr residues in a StkP-dependent manner. Here, we confirmed that StkP phosphorylates GpsB in vitro. StkP has intrinsic kinase activity, but GpsB directly stimulates autophosphorylation of StkP and phosphorylation of StkP substrates. To study the effects of phosphorylation on GpsB function, we performed mutagenesis of the GpsB phosphoacceptor residues. We analyzed the phenotypes of the mutants concerning cell division and StkP-dependent phosphorylation. To investigate the effects of phosphorylation on GpsB interactions, we performed a bacterial two-hybrid assay and immunoprecipitation of GpsB from cells with differentially active StkP. Our data suggest that GpsB is necessary for the proper functioning of the StkP signaling pathway and likely acts as an adaptor that directly promotes StkP activity by mediating interactions within the StkP signaling hub, which ensures StkP recruitment into the complex and substrate specificity.

Poster No. 3.

Deciphering the role of BscX and BscY subunits in the *Bordetella* Type 3 Secretion System

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The type 3 secretion system (T3SS), also known as the bacterial injectosome, is a multisubunit protein-export apparatus that enables the delivery of bacterial effector proteins directly from bacterial cytosol into the cytosol of the host cells. The structural organization of the injectosomes is highly homologous among the Gram-negative bacteria, but some additional regulatory and structural components are species-specific. In *Bordetella pertussis*, the causative agent of whooping cough, as well as in *Bordetella bronchiseptica*, which primarily causes respiratory infections of rodents, the T3SS comprises small protein subunits, BscX and BscY, of which the structure and function remain unknown. Here, we present the solution structure of the BscX-BscY heterodimer determined by nuclear magnetic resonance spectroscopy and demonstrate that both BscX and BscY subunits are critical for the proper function of the T3SS apparatus. The structure shows that BscY adopts a six helical fold, which is wrapped by BscX consisting of the long N-terminal unstructured region with three helices at the Cterminus of the protein. In vitro pull-down assays revealed that the BscX-BscY heterodimer directly interacts with the cytosolic domain of the BcrD protein, suggesting that the heterodimer might regulate the interaction of the inner membrane export apparatus with the cytoplasmic sorting platform. Even though both subunits appear to be tightly connected within the complex, the BscX subunit was also found to be secreted out of the bacterial cells, demonstrating a certain dynamics of the T3SS apparatus during the secretion of the effectors. Moreover, deletion of the individual *bscX* and *bscY* genes, or the removal of the N-terminal twelve residues of BscX rendered the bacterial mutant non-cytotoxic against the HeLa cells, indicating that the structural integrity of the BscX and BscY subunits is essential for the proper function of the *Bordetella* T3SS apparatus.

Poster No. 4

Identification of residues involved in post-translational modification of *Bordetella pertussis* adenylate cyclase toxin

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Bordetella pertussis adenylate cyclase toxin (CyaA) plays a crucial role in the virulence of *Bordetella pertussis*. CyaA translocates its N-terminal adenylyl cyclase enzyme (AC) domain into the cytosol of phagocytes and disrupts their bactericidal functions by unregulated conversion of ATP to cAMP. Additionally, CyaA permeabilizes the membranes of eukaryotic cells by forming cation-selective pores. The toxin preferentially binds to cells expressing the complement receptor 3 (CR3) but can also penetrate the membranes of cells not expressing CR3 on their surface.

CyaA is synthesized as a protoxin (pro-CyaA) and is activated to mature CyaA by covalent post-translational acylation that is catalyzed by the dedicated acyltransferase CyaC. The acyl groups are linked to e-amino groups of two lysine residues located within conserved acylation sites, namely Lys860 and Lys983 in CyaA. Using chimeric CyaA/HlyA molecules (HlyA, α-hemolysin, the RTX toxin of *Escherichia coli*), we identified the sequence essential for acyltransferase-mediated acylation of CyaA. Site-directed mutagenesis defined the residues involved in the recognition of the toxin acylation site by the acyltransferase. We demonstrate that substitution of Arg991 in CyaA causes a reduction in the extent of acylation of Lys983. Furthermore, this substitution decreases the cytotoxic and cytolytic capacity of CyaA towards model sheep erythrocytes and human macrophage THP-1 cells.

Our aim is to identify residues that are directly involved in the interaction of the acyltransferase CyaC with the pro-CyaA molecule. To achieve this, we are combining mutagenesis, functional assays and homologous modeling of CyaC structure. For structural modeling, we are using AlphaFold predictions and the known structure of ApxC (ApxC, the acyltransferase of *Actinobacillus pleuropneumoniae*).

Poster No.5

Acylated segment play a key role in the folding and cell penetration capacity of *Bordetella pertussis* adenylate cyclase toxin

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The adenylate cyclase toxin-hemolysin (CyaA) belongs to the Repeats in ToXin (RTX) family of leukotoxins and plays a key role in the virulence of the whooping cough agent *Bordetella pertussis*. CyaA translocates an adenylyl cyclase (AC) enzyme subunit into phagocytes expressing the complement receptor 3 (CR3, also known as the $\alpha_M\beta_2$ integrin, CD11b/CD18, or Mac-1) and subverts the bactericidal functions of phagocytes by unregulated conversion of cytosolic ATP to cAMP. In parallel, CyaA permeabilizes cellular membrane by forming small cation-selective pores. With a reduced efficacy, CyaA can also interact with a variety of epithelial and other host cell types that lack CR3, or even with naked lipid bilayer membranes. Both cytotoxic activities depend on the activation of the protoxin proCyaA to CyaA by covalent fatty acyl modification of two internal lysine residues, Lys860 and Lys983 by a dedicated acyltransferase CyaC. By combining site-directed mutagenesis, CD spectroscopy, mass spectrometry, hydrogen/deuterium exchange, and toxin activity assays, we have identified key residues of the acylated segment of CyaA that are involved in the overall folding and cell penetration capacity of the CyaA toxin. We therefore hypothesize that the acylated segment plays a key role in anchoring the toxin to the plasma membrane of target cells.

Poster No.6

Alternative enzymatic strategy for disruption of quorum sensing signaling molecules: promise for reducing bacterial biofilms

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Increasing antibiotic resistance may result in up to 10 million deaths per year globally in 2050. The targeted design of quorum-quenching (QQ) enzymes that negatively affect the communication process in Gram-negative bacteria, the so-called quorum sensing (QS) process leading up to biofilm formation, represents a smart strategy to combat this global threat. Tailor-made mutant variants of the biotechnologically well-established penicillin-G-acylase from *E. coli* (ecPGA), designed using advanced protein engineering approaches, show promising potential as an efficient tool for cleaving QS molecules, i.e., taxonomically specific N-acylhomoserin lactones (AHLs). In this study, novel *in-silico* QQ acylase (QQA) mutant variants have been designed that are capable of efficiently hydrolyzing a wider range of AHLs. The predicted QQA structures are based on highly stable ecPGA acylase from *Escherichia coli* and inspired of QQ enzymes with narrower AHL-substrate specificities (PvdQ acylase from *Pseudomonas aeruginosa* and KcPGA from *Klebsiella citrophila*). The structural genes encoding the proposed QQAs were then expressed in *Escherichia coli* BL21(DE3). The profile of AHL molecules synthesized by the bacterial population of *P. aeruginosa* growing in the form of a biofilm was identified in detail using mass spectrometry methods. Quantitative MS analysis of the cell-free culture supernatants revealed the presence of AHLs, including 3-o-C12-HSL, 3-o-C8-HSL, C4-HSL, C6-HSL, the molecules that play an essential role in intercellular communication. The inhibitory effect of the designed and constructed QQA mutant variants against individual types of these AHLs was monitored using a biosensor assay with *Chromobacterium violaceum*. The degradation efficiency of individual AHLs was verified using LC-MS. This study aims to increase the potential of QQ enzymes as an alternative to conventional antibiotic therapies and agents for medical and industrial use in the fight against the formation of multidrug-resistant biofilms.

Poster No. 7

Plant secondary metabolites enhance the effectiveness of antibiotics

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Antibiotic resistance is one of the most critical challenges facing modern medicine. Bacteria enhance their resistance by producing enzymes that chemically alter antibiotics into inactive forms or by modifying their target sites. This has led to a rapid increase in multi-resistant bacterial strains, resulting in infections that are untreatable with conventional antibiotics. This study introduces a method for high-throughput screening of substances capable of restoring the efficacy of common antibiotics against resistant strains, known as adjuvants.

To achieve this, we created a library of genetically modified bacteria expressing specific resistance genes. We employed the microdilution method for testing these substances, allowing us to investigate a broad spectrum of resistance mechanisms. The method was validated with four known inhibitors, after which we tested one hundred plant secondary metabolites for their ability to suppress antibiotic resistance in bacteria. Our results identified potential inhibitors targeting four clinically important enzymes responsible for resistance to aminoglycoside and polymyxin antibiotics.

The method presented in this work offers a simplified and efficient approach to researching antibiotic adjuvants, potentially accelerating the development of solutions to combat antibiotic resistance.

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Poster No. 8

Bimodal Expression of Type 3 Secretion System 2 Enables Cooperative Virulence among Intracellular *Salmonella* Typhimurium

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The Type 3 Secretion System encoded on the *Salmonella* pathogenicity island 2 (T3SS-2) enables bacteria to proliferate in *Salmonella*-containing vacuole inside host cells as well as systemic dissemination. However, not all intracellular *Salmonella* express this factor of virulence. Using infection of cell lines and subsequent analysis by flow cytometry and fluorescence microscopy we show that the expression of the T3SS-2 injectosome itself as well as of its effectors is bimodal. Furthermore, we demonstrate that all effectors of T3SS-2 necessary for the intracellular replication of *Salmonella* can be complemented *in trans*. Bacteria expressing and delivering the T3SS-2 effectors (T3SS-2^{on} bacteria) thus enable growth of the bacteria not expressing T3SS-2 (T3SS-2^{off} bacteria). T3SS-2^{off} bacteria had shorter division time *in vitro* and proliferated faster inside host cells than the T3SS-2^{on} bacteria. T3SS-2^{off} bacteria were more likely to escape from infected host cells and thus served as the bacterial pool for further dissemination of *Salmonella* into more distant host tissues, while the T3SS-2^{on} bacteria were more adapted for subsequent phagocytosis by macrophages. Taken together, our results suggest that bimodal expression of the T3SS-2 and its effectors enable division of labour among intracellular *Salmonella* and might be involved in bacterial dissemination through the host. The next goal is to investigate the factors influencing this bimodal expression of T3SS-2. By focusing on quorum sensing autoinducers and other metabolites, we will detect their impact on division of labour between intracellular *Salmonella*. To study the impact of interkingdom communication, we will use an untargeted bacterial scRNA-seq to investigate bacterial heterogeneity of T3SS-2 expression following interaction with the host.

RO1-1 Signal molecules and cascades in bacterial cell-to-cell signalling and inter-kingdom communication

RO1-1-4: Gut secondary metabolites that regulate heterogeneity and division of labour inbacterial communities

Poster No. 9

ARYL SULFOTRANSFERASES AS A TOOL FOR POLYPHENOLS SULFATION

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Polyphenols are the most common plant-derived natural substances. The largest group is represented by flavonoids, phenolic acids and catechols. Flavonoids have many benefits on the human body, but these benefits are limited by their poor absorption and a very rapid metabolism. Polyphenols are metabolized in the body during the biotransformation phase II, they can be sulfated, glucuronylated or methylated. The synthesis of sulfated metabolites could help to understand the biotransformation of polyphenols and their effects on the human body. Isolation of these metabolites from biological material is impractical, but they can be synthesized in vitro using chemical and chemo-enzymatic approaches[1,2].
The aim of this work was to produce and characterize new aryl sulfotransferases (ASTs) for the sulfation of phenolic compounds and production of sulfated (poly)phenolic substances.
So far, only a few ASTs have been described. The best known are the ASTs from *Desulfitobacterium hafniense*[3] and *E. coli* CFT073[4]. New ASTs were selected based on the sequences of the known enzymes. The new ASTs were produced in *E. coli* and purified to homogeneity. The enzymes were characterized and their substrate specificity was determined with various flavonoids (chrysin, apigenin, genistein, luteolin, hesperetin, fisetin, kaempferol, quercetin and myricetin) and phenolic acids (caffeic acid and ferulic acid).
Based on the screening of substrate specificity, all produced enzymes were capable to sulfate the selected substrates using *para*-nitrophenyl sulfate as a sulfate donor. The products of these reactions were confirmed by HPLC-MS analysis. Based on the tests of the individual ASTs, one of the new ASTs was selected for the preparative production of kaempferol sulfate. AST from *Desulfofalx alkaliphile* was used for the preparative production. The products of the synthesis were isolated and structurally characterized.
Some of the new ASTs, such as the ASTs from *Campylobacter fetus* and *Desulfofalx alkaliphila*, were more effective in sulfating phenols than previously known ASTs. These enzymes can also be used for the preparative sulfation of flavonoids, such as kaempferol, which was enzymatically produced for the first time in this study. The products of these reactions will be used as standards for the characterization of the metabolites of the biotransformation of polyphenolic substances.

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Poster No. 10

Enhancing Polar Metabolite Coverage in LC-MS Basted Metabolomics

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The interaction between microbiome and its host plays a crucial role in human health, largely through their diverse metabolic output. A comprehensive understanding of these interactions requires detailed analysis of the gut metabolome, particularly polar metabolites, which represent the most abundant class in biological samples. However, conventional separation techniques using Reverse Phase Chromatography (RP) often struggle with retaining these highly polar molecules, hindering their detection and quantification. Microbial metabolites, including Short Chain Fatty Acids (SCFAs), neurotransmitters, bile acids, and amino acids, are vital for gut microbial communication and host interactions. While their analysis is crucial, traditional RP chromatography suffers from limited retention of these polar molecules. Two main approaches have been employed to address this challenge: Hydrophilic Interaction Liquid Chromatography (HILIC) which offers broad retention of polar metabolites but can be challenging to set up and often achieves limited reproducibility for specific metabolites. Chemical Derivatization that allows the use of RP columns but may be suitable for only specific functional groups within the metabolites.
In this work we present our LC-MS workflow that combines the strengths of both HILIC and derivatization-based RP for enhanced coverage of polar gut microbial metabolites in fecal samples. Fecal samples from different cohorts of mice (conventional, oligoflora, and germ-free) were analyzed on Bruker's TimsTOF HT. Derivatized samples were analyzed by RP-LC, while underivatized samples were analyzed by HILIC-LC. A library of over 300 standards underwent the same protocol for spectral library creation.
The method can cover the major classes of polar compounds and polar functional groups within fecal samples. Moreover, it enhances the sensitivity of certain metabolites when compared with HILIC and allows for the detection of enantiomers without chiral chromatography. Additionally, the production of a derivatization library for our derivatized standards will aid in the metabolite annotation and data deconvolution. Finally, the easy and rapid protocols used in sample derivatization and analyses make them suitable for high throughput studies.

Poster No. 11

Encapsulation in Glucan Particles for Improves Antimicrobial Activity and Wound Healing

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To defend their hives from bacterial, fungal, and yeast pathogens, honeybees (*Apis mellifera L.*) produce propolis, a resinous material with potent antimicrobial properties. The diverse chemical composition of propolis, which includes flavonoids such as pinocembrin, galangin, and quercetin, makes it an effective natural antimicrobial agent. However, the therapeutic use of propolis is limited due to its poor water solubility and low bioavailability. In response, our study explores the encapsulation of propolis in glucan particles derived from *Saccharomyces cerevisiae* (baker's yeast) to improve its solubility, stability, and antimicrobial efficacy.

Encapsulation was achieved using spray drying and was confirmed using ATR-FTIR spectroscopy and SEM, while UV-VIS spectrophotometry was used to confirm encapsulation efficiency. Ultra-high performance liquid chromatography (UHPLC) was employed to analyze the chemical composition of each propolis sample, focusing on key bioactive compounds such as chrysin, apigenin, kaemferol, quercetin, pinocembrin, galangin, 10-hydroxyl-2-decenoic acid, and cinnamic acid. UHPLC results revealed that chrysin, galangin, and pinocembrin were predominant across all samples. The variations in composition were consistent with the geographical and temporal differences of the propolis sources. In biological assays, the encapsulated propolis exhibited antimicrobial activity against *Staphylococcus aureus*, *Escherichia coli*, and *Candida albicans*. Moreover, *in vitro* wound healing tests showed that encapsulated propolis accelerated wound closure significantly more than raw propolis extracts. These findings indicate that encapsulating propolis in glucan particles enhances its potential for treating infections and promotes wound healing.

Poster No. 12

Enhancing Antioxidant and Anti-inflammatory Activity of Propolis through Glucan Particle Encapsulation

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Propolis, a complex natural substance, is recognized for its antioxidant and anti-inflammatory properties, which are useful in treating oxidative stress and inflammation-related disorders. Nevertheless, a common problem for many natural extracts is their low solubility in water, which leads to decreased bioactivity. Studies have suggested that glucan particles can increase the bioavailability of an active compound by (i) possibly reducing the particle size of the compound, which increases its surface area and thereby improves its solubility and dissolution rate (ii) triggering an immune response that enables the transport of the active compound. Thus, to enhance the bioactivity of propolis, we encapsulated the extracts in yeast glucan particles (porous hollow shells of *Saccharomyces cerevisiae*). Propolis from different sources was extracted using a modified sonication method with 70% ethanol and encapsulated in glucan particles derived from baker´s yeast via spray drying, achieving a 10 wt% propolis content.

Complete encapsulation was confirmed using ATR-FTIR spectroscopy and SEM. Dissolution tests revealed that encapsulated propolis exhibited significantly improved solubility and dissolution kinetics even reaching supersaturation compared to non-encapsulated propolis. The encapsulated propolis significantly improved antioxidant and anti-inflammatory activity, with reduced levels of pro-inflammatory cytokines (TNF-α, IL-6), and inhibition of COX-2 and NF-κB pathways in LPS-stimulated macrophages. These results indicate the potential of encapsulation into glucan particles to enhance dissolution kinetics, as well as antioxidant and anti-inflammatory activities of propolis.

Poster No. 13

Development of intestinal biosimilar mucus to study drug transport through the mucus barrier

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The gastro-intestinal mucus is a hydrogel that covers the epithelium in the gastrointestinal wall, acting as a selective barrier to foreign microorganisms as well as the drug particles. It has proven to be the rate-limiting step of drug absorption. To speed up the formulation process, the mucus barrier can be artificially designed *in vitro* to serve as an applicable predictive tool during drug development. The current permeability assays fail to account for the mucus layer, or the drug transport studies are conducted in commercially available mucins. Although commercial mucins are routinely used as a model to investigate drug transport, they fail to replicate the complex native rheological and functional behavior of mucin glycoproteins. In this study, we aim to develop a biosimilar mucus layer with functional mucins that are highly responsive to their environment such as pH change, Na^+ and Ca^{2+} ions, water intake etc. causing the mucus to shrink or expand, which in return affect the mucus pore size, surface chemistry and penetration rate. To do so, the mucus was extracted from animal sources such as pigs with a mild NaOH solution and went through a several steps of purification processes such as multiple centrifugation cycles, dialysis and finally freeze-drying. The lyophilized material was successfully tested for pH responsiveness, FTIR and UV-VIS analysis was utilized to compare the spectra between commercial and self-extracted mucins, as well as confocal microscope to confirm present mucins via a staining protocol. SDS-PAGE analysis was utilized to confirm the presence of mucins in the lyophilized samples. Self-extracted mucins are currently used to recreate the barrier artificially, allowing us to fine-tune the mucus by mimicking the physiological behavior such as fasted/fed state conditions, thickness, pore size and permeation of the barrier.

Poster No. 14

Martin Krov, František Štěpánek

CONFIDENTIAL

Poster No. 15

Impact of selected probiotic bacteria on juvenile host growth upon malnutrition in gnotobiotic mouse model

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Introduction: The ability of *Lactiplantibacillus plantarum* WJL bacteria to support the growth of gnotobiotic hosts in models of chronic malnutrition is known and well-established (Schwarzer et al., 2016, 2023). In this work, it is investigated whether other bacteria also have similar capabilities and whether these traits are species- and strain-specific. Strains *of Bifidobacterium longum ssp. longum* and *Bifidobacterium adolescentis* were tested in a gnotobiotic juvenile mouse model of chronic malnutrition and their impact on the host's somatotropic axis was monitored. Concurrently, it was analyzed how malnutrition affects the bacteria in the host's gut and the level of its colonization.

Material & Methods: Germ-free C57BL/6J mice were mono-colonized with the aforementioned bacterial strains. The mice were weaned on an experimental low-protein/low-fat diet. Their growth was monitored weekly by measuring body length and weight for 5 weeks.

Results: Bifidobacterium longum (Bl) strains showed improved systemic growth compared to both Bifidobacterium adolescentis (Bad) strains and the control germ-free (GF) group, with Bl 372 strain exhibiting significantly enhanced growth. This was accompanied by elevated levels of IGF-1 in the serum of mice. Histological analysis of the architecture of the small intestine revealed the highest effect of the Bl 372 strain. Colonization levels of all strains drop after malnutrition.

Discussion: Growth-promoting properties of Bifidobacteria are species-specific and strain-specific. Drop in bifidobacterial levels seen in atopic dermatitis, asthma, and inflammatory bowel diseases, can be seen in chronic undernutrition as well. The decrease in numbers is accompanied by a change in bacterial cell shape induced probably by nutritional stress.

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Poster No. 16

Anti-neuronal antibodies and the gut microbiota in patients with central hypersomnolence disorders

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The gut microbiota represents an extensive source of antigens that can structurally mimic host neuropeptides and neurohormones and therefore trigger the production of autoantibodies that cross-react with these compounds. These basically autoimmune antibodies may attribute to the pathophysiology of sleep disorders such as central hypersomnolence disorders. We hypothesize that patients suffering from central hypersomnolence disorders might show differences in gut microbiota composition compared to healthy controls and have higher levels of autoantibodies to various neuropeptides involved in the regulation of sleep and food intake.

To test this hypothesis, we determined the fecal bacterial composition of patients with central hypersomnolence disorders (91 with type 1 narcolepsy, 46 with idiopathic hypersomnia, 27 with type 2 narcolepsy) and healthy controls. To compare differences in gut microbiota composition we applied the 16S rRNA sequencing methodology. Additionally, we tested the presence of autoantibodies directed at assorted neuropeptides using ELISA and measured levels of various cytokines. Next, we measured levels of calprotectin and sCD14 in patients' sera to assess gut barrier permeability and possible penetration of microbial antigens into the bloodstream. We observed that narcoleptic patients exhibit signs of microbial translocation across the gut barrier. The results of gut microbiota sequencing showed changes in the beta diversity according to Jaccard dissimilarities between the groups of healthy controls and patients with type 1 narcolepsy and idiopathic hypersomnia. Importantly, these changes did not remain significant after adjustment for BMI, age, gender, and diet composition. We observed higher levels of autoantibodies against alpha-melanocyte-stimulating hormone (α-MSH) and neuropeptide glutamic acid-isoleucine (NEI) in all patient groups.

Our results suggest that changes in gut microbiota composition did not arise due to sleep disorders. Further, the prevalence of anti-neuronal autoantibodies was rare, indicating that their presence likely does not play a significant role in the pathophysiology of central hypersomnolence disorders.

Poster No. 17

Fast FluoroAlkylation of Proteins as a Novel Strategy for Characterization of Membrane Protein in Bacteria

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Covalent labeling and chemical cross-linking in combination with mass spectrometry have been developed into powerful methods for mapping protein structures, dynamics and interaction networks including molecular interfaces in protein-protein and protein-nucleic acid complexes. Although many probes have been developed in last two decades, majority of them still targeting lysins or cysteines. Other cross-linking reagents have only limited use for various reasons such as low reactivity, low occurrence of targeted residues and/or unexpected side products. In this study we show new generation of probes based on recently published Fast FluoroAlkylation of Proteins (FFAP) technology that enables targeting aromatic amino acid side chains. Access to aromatic residues makes this technique attractive for bacterial membrane associated and transmembrane proteins, where aromatic side chains fulfil important anchoring roles and in transmembrane protein cluster mostly at the membrane interface.

Reaction is based on two step FFAP mechanism which includes activation of hypervalent iodine reagents using metal ion or lewis acid and subsequent attack of aromatic residues within the protein sequence by the resulting radicals. Since the induction of fluoroalkyl radicals is triggered by ascorbic acid and the labeling pulse is stopped by tryptophan, it enables to perform protein labeling experiments in quench flow system in short time range (3s). The studied proteins were analyzed by bottom-up approach using high resolution mass spectrometry (solariX XR 15T, Bruker Daltonics) where samples were digested by trypsin protease, separated on reverse phase column (Luna Omega Polar C18, 100Å) online coupled to mass spectrometer.

The results on protein model bovine rod outer segment (ROS, isolates from bovine retina) demonstrate the potential of our novel labeling strategy. The modification of aromatic residues generated by fluoroalkyl radicals provide structural information not attainable by labeling of lysins. Our data lead to an assumption that the fluoroalkyl radicals will be utilized for fast cross-linking experiments studying structure and dynamics of membrane proteins and protein assemblies in bacteria.

Poster No. 18

Development of Software for LC-MS Data Processing of Oligonucleotides

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The development of advanced software for processing LC-MS data, particularly for oligonucleotides, represents a critical step forward in biomedical research and therapeutic applications. Our work focuses on creating a Python-based tool designed to streamline the analysis of oligonucleotide sequences. Upon input of an oligonucleotide sequence, the algorithm generates a library of monoisotopic masses for the sequence fragments across all charge states. These theoretical masses are then matched with experimentally measured masses within a defined ppm range using LC-MS data exported from Bruker Data Analysis software.

The algorithm has theoretical applications in inflammation and cancer diagnostics through the measurement of miRNA from the liquid body fluids. This could lead to early and precise detection of inflammation or cancer progression, offering a promising avenue for non-invasive diagnostic techniques. Moreover, the significance of this software extends beyond basic research since the oligonucleotide therapeutics have emerged as a vital class of drugs, offering targeted therapeutic strategies. However, the accurate interpretation of complex mass spectra remains a challenge in the field. This software will address these challenges by providing reliable and efficient tools for mass spectrometry data analysis, potentially improving the accuracy of oligonucleotide characterization.

Our methodology will enable quantitative and qualitative analysis of the complex biological matrix. The software's graphical user interface (GUI) will simplify interaction, making it accessible to researchers with varying levels of computational expertise.

In support of the INTER-MICRO project, our software can aid ongoing efforts in oligonucleotide research and demonstrate the integration of computational tools in modern biomedical sciences, thereby fostering innovation and improving healthcare outcomes.

Poster No. 19

Gut microbiota protects mice from acute colitis by improving intestinal barrier function

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Purpose: Gut microbiota drives inflammation by shaping the function of intestinal barrier and stimulating the immune system of intestinal mucosa. Our aim was to analyze the mechanisms of how microbiotas from two different animal facilities influence the development of acute experimental colitis.

Methods: We induced acute colitis in conventional BALB/c mice originating from the animal facility of the Institute of Microbiology (IMIC) or the First Faculty of Medicine (FoM), and in ex-germ-free mice colonized with intestinal content from either facility. Colitis was induced by intrarectal administration of 2,4,6-trinitrobenzenesulfonic acid (TNBS) dissolved in 50 % ethanol, with (hapten-type) or without (toxic-type colitis) skin presensitization. The severity of colitis was assessed by Wallace's score and measurement of colon length. Intestinal microbiota was analyzed by 16S rRNA sequencing, gut barrier function was analyzed by a fluorescein isothiocyanate dextran (FITC-Dx) permeability assay, and immune system activity was measured by flow cytometry in cells from the spleen and mesenteric lymph nodes.

Results: IMIC mice were significantly more resistant to both types of TNBS colitis and had more bacteria of the Verrucomicrobiae and Campylobacteria classes in their feces. IMIC mice had significantly less permeable gut barrier, and significantly fewer effector T cells and lower percentages of IFNγ-, IL-17A- and TNFα-producing CD4+ T cells in both the spleen and mesenteric lymph nodes even before the colitis induction. While microbial consortia in the colonized ex-germ-free mice resembled these of the conventional mice, there were no significant differences in either sensitivity to colitis or T cells subsets.

Conclusion: Early-life colonization with Verrucomicrobiae and Campylobacteria supports gut barrier development and reduces immune system reactivity, increasing resistance to intestinal inflammation in mice. These findings will help to guide future WP3 research on microbiota's impact on gut barrier integrity in health and disease using mice as model organisms.

Poster No. 20

DIETARY FIBER PECTIN FROM PAPAYA FRUIT (Carica Papaya) MODULATES GUT MICROBIOTA IN A MOUSE MODEL OF COLON CARCINOGENESIS

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Introduction: Growing evidence suggests that the gut microbiota, mainly modulated by diet, is involved in CRC development. Pectins, a class of highly fermentable dietary fibers, can modulate the gut microbiota which depends upon the ripening stages of the fruit.

Objective: This work aimed to investigate the differential effects of ripe and unripe papaya pectins on gut microbiota composition in a mouse model of colon carcinogenesis.

Methods: Male Wistar rats were divided into 4 groups fed ripe and unripe papaya pectins and subjected to AOM injections. Animals were euthanized after 10 weeks. Aberrant crypt focus (ACF) and SCFA production were analyzed in the colon. Metagenomic sequencing was done using Nanopore sequencing technology.

Results: Ripe papaya pectin intake reduced the number of ACF with 1 and 2 crypts. Acetate production was higher in animals fed ripe and unripe papaya pectin. Ripe papaya pectin increased richness in microbiota alpha diversity. The ripe papaya pectin group and the negative control group shared a similar bacterial profile. The intake of ripe papaya pectin increased the abundance of *Lactobacillus* and *Limosilactobacillus* while the intake of ripe papaya pectins increased the abundance of *Rombutsia.* The AOM experimental group had an increase in the abundance of *Mediterraneibacter*.

Conclusion: These results suggest that the intake of ripe papaya pectin had a potential preventive effect on colon carcinogenesis development by decreasing the number of ACF, increasing SCFA production, and increasing the abundance of *Lactobacillus*, a well-known probiotic strain that promotes health benefits to the host. This could be beneficial in future preventive measures to reduce CRC development using a natural component of the diet rich in dietary fiber.

Poster No. 21

High-protein diet accelerates the initial stage of colorectal cancer

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Colorectal cancer (CRC) is driven by tumor-promoting inflammation and genomic instability, both of which can be modulated by diet. Here, we investigated how dietary protein affects CD8+ T cell response as an anti-tumor defense and cancer severity in a mouse model of colitis-associated cancer.

Colitis-associate cancer was induced by azoxymethane and dextran sodium sulfate in BALB/c or RAG2 KO mice fed a synthetic control diet (CD) or high-protein diet (HPD). The role of cytotoxic T cells in the stage of carcinogenesis was analyzed by either depleting CD8+ cells with antibodies in vivo or by adoptively transferring RAG2 KO mice with cells containing or lacking CD8+ lymphocytes. Cytokine levels were measured in the supernatants after colon cultivation by ELISA.

BALB/c mice on HPD had more tumors, which was not altered by CD8+ depletion. However, RAG2 KO mice on HPS reconstituted with lymphocytes without CD8+ had even more tumors than those reconstituted with all B and T cells. These mice produced less IL-2 and TNF-α in the spleen, and more S100A8 in their gut. In RAG2 KO mice, CD8+ depletion itself had no effect on tumor growth, although the levels of IL-10, IL-23 and TNF-α were reduced.

We conclude that HPD alters the inflammatory milieu in the gut and accelerates the progression of cancer. Cytotoxic T cells play a crucial role in cancer control but the approach to study their role in the procarcinogenic effects of HPD should be further investigated. Within the INTER-MICRO project, these findings may allow a better understanding of whether anti-tumor effector immunity may be influenced by diet, an environmental factor causing dysbiosis.

Poster No. 22

Oral metronidazole reduces delayed-type hypersensitivity by modulating mucosal immunity

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The gut microbiota shapes individual susceptibility to inflammation. Here, we investigated how these immunomodulatory properties can be altered by oral antibiotics (ATB) and whether they are microbiota-dependent or -independent.

We fed BALB/c mice with specific ATB (metronidazole (M), colistin or vankomycin) or placebo for 2 weeks and induced delayed-type hypersensitivity (DTH) immediately, 1 or 2 weeks after the end of treatment. The gut microbiota and immune system were tested by using germ-free mice and transferring the antibiotic-altered microbiota or leukocytes to germ-free or immunodeficient mice. The effect of ATB on mucosal or systemic immunity was analyzed by intestinal fragment culture or flow cytometry in vivo and on anti-CD3/CD28 stimulation in vitro.

All ATBs tested reduced DTH, with oral M having the greatest effect, which was still observed 2 weeks after the end of M treatment. M decreases the production of pro-inflammatory cytokines in Peyer's patches and decreases the ability of T cells to respond to TCR triggers, but has no effect on T cells in vitro. M also decreases DTH in the absence of microbiota and its effect can be transmitted by leukocytes. Interestingly, transfer of the microbiota from M-treated mice has the opposite effect.

Orally administered M reduces the inflammatory tuning in Peyer's patches and lowers DTH for a long time, independent of the microbiota, but transfer of the dysbiotic microbiota from M-treated mice increases DTH.

Poster No. 23

Convergence of gut phageomes but not bacteriomes after experimental transplantation of wild mouse bacteriophages into captive house mice

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Introduction: Bacteriophages are abundant components of vertebrate gut microbiota, impacting bacteriome and directly interacting with superhost. Widely used specific pathogen-free (SPF) mouse models of host-microbiota interactions have altered gut bacteriomes compared to wild mice, and data on wild mouse phageomes are lacking. Experimentally induced bacteriome dysbiosis in mouse model can be treated by transplanting healthy phageomes, but the effects of phageome transplants on healthy adult gut microbiota are unknown.

Material & Methods: We compared gut bacteriome (16S metabarcoding) and phageome (metagenomics) between wild-living and captive non-SPF mice. We transplanted phageomes from wild to captive mice and evaluated post-transplantation microbiota changes.

Results: We demonstrate divergent gut phageomes and bacteriomes in wild and captive non-SPF mice. Phageome and bacteriome structuring mirrored each other, correlating at the individual level. Transplantation of phageomes from wild to captive mice did not cause major shifts in recipient phageomes. However, the convergence of recipient-to-donor phageomes confirmed that wild phages can integrate into recipient communities. Differences in integrated phages between the two recipient mouse strains illustrate context-dependent effects of phage transplantation. The transplantation did not impact recipient gut bacteriomes.

Discussion: Compared to our non-SPF mice, re-analysis of previous data from SPF mice revealed an enrichment of Suoliviridae crAss-like phages, suggesting differences in bacteria-phage interactions that may contribute to distinct microbiota functions in SPF mice. The resilience of healthy adult gut microbiomes to the intervention has implications for phage allotransplantation safety.

Poster No. 24

The gut microbiome and diet composition of Papua New Guinea Rodents among different altitudes

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Introduction: The gut microbiome plays a vital role in numerous physiological functions of the host. Unlike captive animal models, free-living animals offer a great opportunity to study the effect of the microbiome on host phenotype under natural conditions. The tropical island of New Guinea has extreme natural diversity, including rodents of the tribes Hydromyini and Rattini, which inhabit almost all ecosystems on the island. These two rodent radiations represent a unique model to study the role of the microbiome in rodent evolution and ecology.

Material & Methods: We sampled 416 New Guinea rodents from two tribes and 26 putative species at five altitudes (from 200 to 3700 m a.s.l.). Using 16S rRNA and 18S rRNA metabarcoding we profiled the gut bacteriome and diet of the rodents and analyzed the effects of altitude, host taxonomy, and diet on microbiome diversity and composition.

Results: We found no significant difference in alpha diversity between the two tribes, while beta diversity showed significant differences. The genus Pogonomys exhibited the highest alpha diversity despite a relatively low sample size. Both host taxonomy and altitude significantly affected microbiome diversity and composition. Additionally, we found a significant correlation between microbiome composition and diet.

Discussion: Our initial results suggest that host taxonomy, diet, and altitude have a strong effect on shaping the microbiome of New Guinea rodents.

Poster No. 25

Selective cutting preserves the health of temperate forest soil microbiome

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Harvesting of forest trees has important consequences not only for the forest stands but also for the microbiome of forest soils. Total tree removal (clearcutting) was shown to seriously decrease soil activity and lead to the disappearance of symbiotic tree root-associated microorganisms: the ectomycorrhizal fungi. Here we compared the microbiome as well as ecosystem response to clearcutting and selective tree harvesting in a temperate forest. While clearcutting led of higher water and nutrient availability in soil, it significantly decreased the abundance and activity of ectomycorrhizal root symbionts and led to limited tree regeneration. In contrast, selective harvesting showed several positive effects including partial increase of water availability, fast recovery of the ecosystem productivity measured as tree growth and, importantly, retained the diversity, abundance and functioning of the ectomycorrhizal symbiosis between fungi and tree roots. We show that selective harvesting represents and alternative that retains forests as C sinks and has no deleterious effects on microbial symbionts and tree regeneration.

Poster No. 26

A comparison of the realized and fundamental niches of selected arbuscular mycorrhizal fungal species along a nitrogen gradient

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Arbuscular mycorrhizal fungi (AMF) responses to environmental change are often predicted based on realized niches (observed distributions). However, these niches may not capture the full potential of AMF species due to limitations from biotic interactions.
This study investigates the fundamental niches (full growth potential) of AMF species compared to their realized niches along a nitrogen gradient, a factor highly impacted by human activity.
We utilized the GlobalAMFungi database to estimate realized niches of selected AMF taxa and conducted controlled greenhouse experiments to assess their fundamental niches along a nitrogen gradient. After eight weeks of continuous application of nutrient solution with six different nitrogen concentrations (in form of ammonium nitrate), we monitored root colonization and other structural traits of AMF and also host plant growth.
Preliminary results suggest that AMF species exhibit varying preferences along the nitrogen gradient. For instance, *Rhizophagus irregularis* thrived even at high nitrogen levels detrimental to host plants and other AMF species. However, only certain AMF species, under specific nitrogen treatments, showed positive effects on host plant growth.
Understanding the fundamental niche of AMF along the nitrogen gradient and comparing it with realized niches can enhance predictions of how global changes, such as nitrogen deposition, affect AMF distribution and community composition.
This study sheds light on the intricate dynamics between AMF, host plants and nitrogen availability, crucial for ecosystem management in the face of anthropogenic impacts.

Poster No. 27

GlobalFungi and GlobalAMFungi Databases: Powerful tools to explore fungal mycobiomes

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The introduction of high-throughput sequencing has significantly advanced our ability to monitor microbial distribution in a wide range of ecosystems and geographical areas. The creation of the GlobalFungi databases (Větrovský et al., 2020) aims to improve access to global data on fungal occurrences in the environment, facilitating the study of fungal community compositions and biogeography. The need for GlobalAMFungi (Větrovský et al., 2023) arose due to the bias of general fungal primers against arbuscular mycorrhizal (AM) fungi in the GlobalFungi database, prompting the development of a dedicated platform focusing on the key barcoding regions (SSU, ITS2, and LSU) for AM fungi. The GlobalFungi database compiles over 4.5 billion observations of fungal ITS1 and ITS2 marker sequences from 846 studies, covering almost 85,000 samples globally, while GlobalAMFungi contains nearly 50 million observations of AM fungal DNA sequences from approximately 8,500 samples, incorporating geographical metadata from 100 studies. GlobalFungi enables the exploration of fungal communities across different terrestrial environments using general fungal primers, while GlobalAMFungi provides an extensive overview of AM fungal distribution. Both databases feature web interfaces for data searching and visualization and promote community contributions to expand their collections, they represent a significant advancement in mapping the global diversity and distribution of fungi. By integrating these resources, we offer a comprehensive perspective on fungal ecology, biogeography, and the environmental factors that influence fungal distribution, encompassing all ecologically relevant fungal groups.

Větrovský T. et al. (2020) GlobalFungi. Scientific Data 7, 228.

Větrovský T. et al. (2023) GlobalAMFungi. New Phytologist 240, 2151-2163.

Poster No. 28

Soil mycobiome diversity peaks in the mountains

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For macroorganisms, global distribution patterns revealed mountains to harbour striking biodiversity in relatively small areas. In fungi, the relevant knowledge is still largely lacking: While previous studies have assessed the role of highlands indirectly via climate effects, they have not focused on montane diversity of fungi per se or on the environmental factors that might drive montane biodiversity.

Here, we assessed global fungal species diversity and focused on mountain ranges in comparison to lowlands using GlobalFungi, a global dataset of geolocated records of fungal taxa. We assessed local diversity describing the species abundance within samples, and used different methods to predict this diversity globally, such as regression analysis and Random Forest modeling using climatic, soil and topographical properties, and environmental productivity. We then specifically focused on the diversity within montane and lowland regions, which were compared globally and, separately, within each of the world's biogeographic realms. To ensure comparability across mountains, lowlands and realms, we accounted for the potentially confounding effects of sampling and geographic area.

We present the first comprehensive study of fungal diversity in mountain ranges and its potential implications. With GlobalFungi, we can assess fungal diversity on a global scale and assess distribution and drivers in mountains as hyperdiverse areas, which may serve as refuges for fungal diversity on the global scale in the wake of climate change.

Poster No. 29

Nitrile synthesis catalysed by aldoxime dehydratase immobilized on metal affinity resins.

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Nitriles are important compounds widely used in various industries such as fragrance, pharmaceutical or rubber industries. Conventional methods of nitrile synthesis require hazardous chemicals (CCl_4, cyanide) or high temperatures. Nitriles can be produced under mild conditions (aqueous media, near-neutral pH and moderate temperatures) by their dehydration using aldoxime dehydratase (Oxd). Oxds are heme-containing enzymes found in bacteria and fungi. In this study, we used the bacterial OxdBr1 (from *Bradyrhizobium* sp.) overproduced in *Escherichia coli*. This enzyme shows a high activity and stability and does not require anaerobic conditions unlike some other Oxds. The activities and stabilities of the immobilized Oxd catalysts were investigated using a model reaction of (*E*,*Z*)-phenylacetaldoxime to phenylacetonitrile. The immobilization of the enzyme (in form of a crude extract) on a metal affinity resin (Ni-NTA) allowed a reuse of the catalyst and a flow reaction. This approach can be used for preparative reactions, and increases cost efficiency.

1. Křístková et al, J. Biotechnol. 384, 12–19 (2024).

Poster No. 30

NITRILES AND CYANIDES IN MICROBE-PLANT COMMUNICATION

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Nitriles are widespread plant metabolites and function principally as defense compounds against bacteria, fungi, and herbivores. They involve simple and complex nitriles, the latter consisting first of all of cyanogenic glycosides. The synthesis of nitriles in plants starts from a few amino acids and requires cytochrome P450 (CYP) enzymes. Common intermediates of this route are aldoximes, which are also defence compounds or serve as precusors of glucosinolates.

Bacteria and fungi developed mechanisms to cope with the presence of nitrile defense compounds and HCN in plants. The general strategy is to hydrolyze the nitriles or HCN to less toxic metabolites (carboxylic acids and amides), while also utilizing the liberated ammonia. This pathway is common in many bacteria and fungi and is mainly catalyzed by nitrilase. The microorganisms also mimick some nitrile production and nitrile degradation pathways that exist in plants – thus some of them degrade the neurotoxic plant metabolite b-cyano-L-alanine or synthesize nitriles from aldoximes via an alternative route with aldoxime dehydratases instead of CYP enzymes.

The group of nitrilases is highly diverse in terms of sequence, molecular weight, substrate specificities and optimum reaction conditions. The investigation of nitrilases in wild-type bacteria and especially fungi may be difficult due to low levels of the enzymes.

Therefore, we have expressed the nitrilase genes in *Escherichia coli* and characterized a number of new nitrilases, especially from Ascomycota and Basidiomycota fungi. Furthermore, we have mapped the distribution of the different nitrilase types in plant-associated bacteria and fungi, aiming to shed light on the relationships between the nitrile/cyanide metabolism in plants and in their microbiomes. Additionally, the impact of nitrilases lies in bio- and environmental technologies[1], and bioanalytic chemistry, e.g., determination of HCN in plant tissues.

1. Martínková et al, Proc. Biochem. 142, 62-67 (2024).

Poster No. 31

The role of soil prokaryotic communities in the biodegradation of lignin

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Lignin is a complex phenylpropanoid polymer found in the cell walls of higher plants. It accounts for approximately 30% of the organic carbon in the biosphere, making it a significant carbon sink and, on the other hand, once deposited into soil, as a C source for indigenous microbiota. Lastly, it potentially represents an omnipresent renewable and sustainable source of e.g. specialty liquid fuels and chemicals. Although lignin is traditionally believed to be predominantly biodegraded by fungi, the role of prokaryotic organisms in lignin depolymerization and the subsequent degradation of its fragments has been largely overlooked. This study aims to explore the soil prokaryotic communities involved in the degradation of lignin and its structural constituents, as well as the enzymatic mechanisms underlying this process.

To obtain lignin-degrading microbial communities, compost soil (20% organic carbon) was used as the inoculum for enrichment cultures with lignin and individual lignin structural proxy molecules: 5,5'-dehydrodivanillate, pinoresinol, guaiacylglycerol-β-guaiacyl ether (GGE), and acetosyringone (AS). Stabilized consortia were then subjected to metataxonomic analysis of the prokaryotic community structure and Nanopore sequencing to obtain metagenome-assembled genomes (MAGs). These MAGs were analyzed to identify the genetic determinants responsible for the utilization of each respective substrate. The activity of the proteins encoded by the candidate genes was further characterized through heterologous expression in *Escherichia coli*.

The microbial community structure of obtained consortia will be presented in detail. So far, MAGs from AS- and GGE-utilizing consortia were analyzed, and the scenario of GGE and AS utilization was reconstructed including the identification of crucial genetic determinants. The AS-utilizing consortium was dominated by the genera *Pseudomonas* and *Methylotenera*. Importantly, a brand-new pathway of AS degradation was revealed in the dominant *Pseudomonas* MAG. *Acinetobacter*, *Altererythrobacter*, and *Pseudomonas* populations were found to dominate GGE-based enrichment cultures. In the GGE-utilizing consortium, a *Croceibacter* sp. strain was revealed, possessing an oxidoreductase initiating the first step of GGE oxidation. Proposedly, the *Methylotenera* sp. MAG was found across multiple consortia and might act in the demethylation of aromatic moieties, thus exposing them to further oxidation and cleavage.

We identified novel genetic determinants proposedly involved in the utilization of lignin fragments, contributing to our understanding of the role of prokaryotic communities in lignin degradation. These findings have significant implications for soil microbial ecology and carbon cycling. Furthermore, since acetosyringone (AS) plays a crucial role in plant-microbe interactions, we suggest that the novel AS-degradation pathway may influence these processes.

In future research, MAGs from the 5,5'-dehydrodivanillate, pinoresinol, and lignin consortia will be analyzed to further reconstruct the scenario of lignin utilization. This ongoing work aims to deepen our understanding of the microbial and enzymatic mechanisms behind lignin degradation, providing broader insights into soil ecosystems and their contributions to the carbon cycle.

Poster No. 32

Stable isotope probing to disentangle carbon for nitrogen trading in mycorrhizal hyphosphere

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There is experimental evidence for the key role of hyphosphere microbiomes in releasing mineral nutrients such as nitrogen (N) from organic moieities in soil to be accessible for the arbuscular mycorrhizal (AM) fungi. Yet whether and how the N is exchanged for reduced carbon (C) supplied by the fungus remains unclear. To disentangle whether and which microbes involved in organic N (chitin) degradation are rewarded by fungal C we undertook several stable isotope probing experiments, labeling both the AM fungus or the chitin with ^{13}C, or chitin only with ^{15}N. We followed the incorporation of the heavy isotopes into the microbial RNA in microbially complex microcosms. Our results so far indicate that microbes could utilize the chitin for both N and C resources, not necessarily relying on the fungus to supply the energy. On the other hand, evidence was obtained for specific microbial communities to be established in the AM fungal hyphosphere – whether these taxa specifically occupy the hyphoplane or are more loosely associated with the fungus still needs to be specifically addressed.

Poster No. 33

Exploring unique microbial pathways on novel prokaryotic phyla in extreme subsurface environments

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Microbial communities in extreme environments are invaluable sources of unique and potentially novel life forms, playing a critical role in ecosystem health and stability. These environments, often characterized by limited nutrient availability, select for microbes with specialized metabolic capabilities and living strategies. In this study, we explored the microbial community in subsurface water springs of the historic silver and uranium mine in Jáchymov, Czech Republic. Through metagenomic analysis, we reconstructed several nearly complete genomes of resident prokaryotes, identifying members of novel or previously uncharacterized phylogenetic groups.
This work centers on these metagenome-assembled genomes (MAGs), elucidating their precise taxonomic positions and metabolic capabilities. A notable feature of these MAGs is the presence of the Wood-Ljungdahl pathway (WLP), an ancient biochemical pathway involved in reversible CO2 reduction. The WLP is essential for carbon fixation, methanogenesis, and the oxidation of acetyl-CoA, methane, and other short-chain alkanes.
The key enzyme complex of the WLP, carbon monoxide dehydrogenase/acetyl-CoA synthase (CODH/ACS), is found in both prokaryotic domains but can be distinguished by specific subunit configurations. We discovered an unusual arrangement of the bacterial CODH/ACS gene cluster in this study, suggesting that these variations of the WLP may have evolved divergently, possibly through horizontal gene transfer in these isolated ecosystems.
Understanding these unique microbial pathways enhances our knowledge of microbial diversity and their ecological roles, contributing to the broader One Health framework by highlighting the interconnectedness of environmental and microbial health.

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