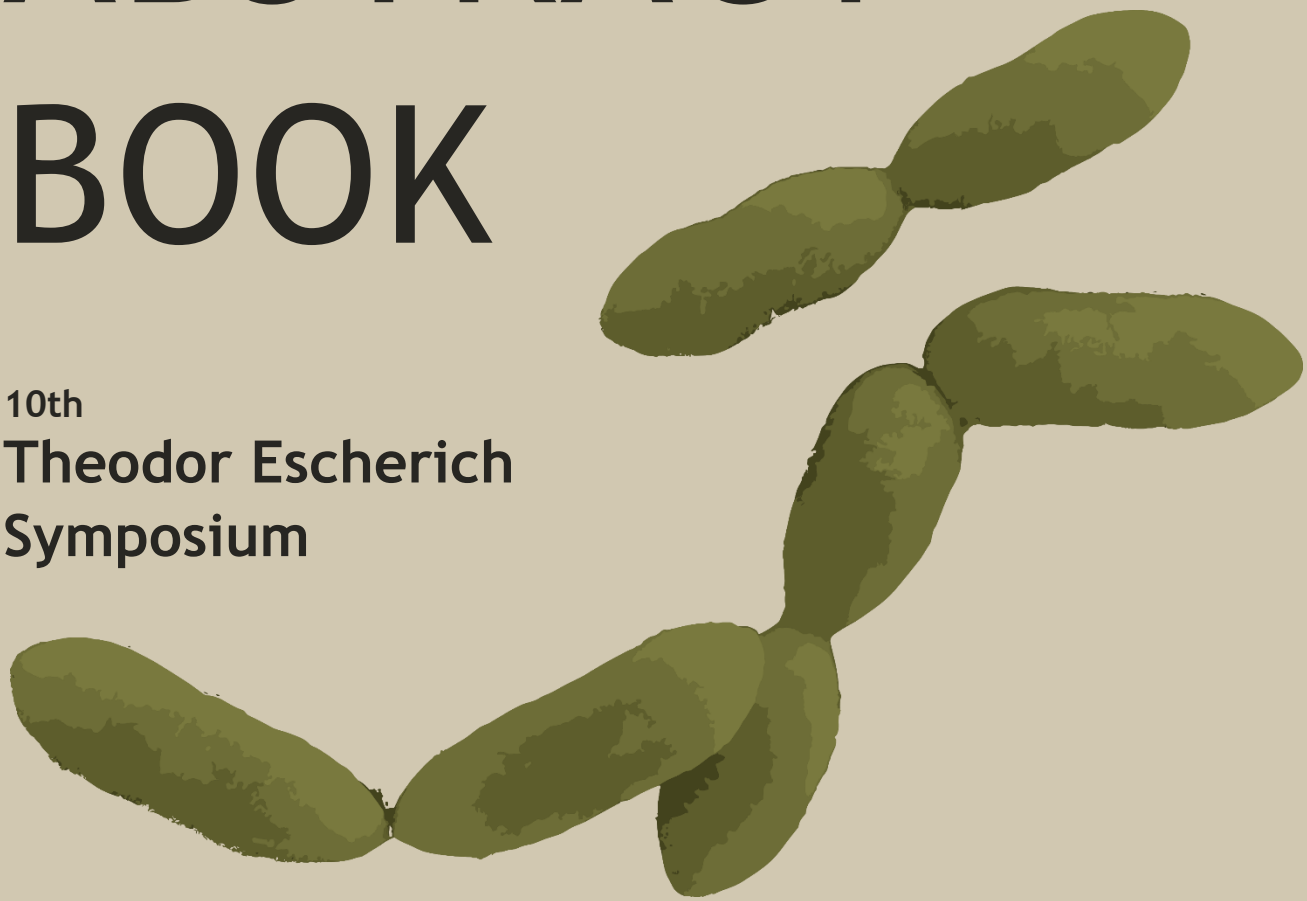
ABSTRACT BOOK



10th
Theodor Escherich Symposium

Med Uni Graz

Med Uni Graz & hybrid
18. & 19. January 2024

Co-organising societies

ÖGIT • ÖGHMP • AMICI • VAAM • ÖPATH FWF • ÖGGH • Cluster of Excellence: MicroPlanet • BioTechMed-Graz

BRONZE SPONSORS

GERMANIA PHARMAZEUTIKA

janssen PHARMACEUTICAL COMPANIES OF Johnson & Johnson

Institut AllergoSan

QIAGEN

SPONSORS

BioTechMed GRAZ

TABLE OF CONTENTS

SESSION 1 (Uni Graz): Keynote Talk

INVITED SPEAKER:

Commensal Genotoxins: Microbial Metabolites Damage DNA

Ellen L. Zechner
Institute of Molecular Biosciences, University of Graz, Austria; BioTechMed-Graz

The human intestinal tract is home to an immensely complex microbial ecosystem that is in a state of fierce competition. Similar to every other environment, bacteria that reside in the gut seek to eliminate their competitors with toxic proteins and small molecule inhibitors including metabolites that directly damage DNA. The canonical enterotoxins colibactin and tilimycin, and the newly discovered family of indolimines generate mutations and genome instability by direct DNA alkylation. Given the expected toxicity of an enteric mutagen to microbes and host cells alike, much attention has been focused on the etiological role of commensal genotoxins in pathologies affecting intestinal development, regeneration, and the earliest stages of tumorigenesis.

Genotoxic bacteria are among the first organisms to colonize the infant gut, which accentuates the clinical relevance. Multiple species of Gram-negative gut residents, including E. coli express the colibactin biosynthetic gene cluster (pks). Tilimycin synthesis genes (til) are carried by Klebsiella oxytoca and a complex of closely related species (KoSC) that become established within the first days of life. Unlike colibactin, tilimycin and the indolimines are secreted in stable, active forms that are pooled and concentrated in the colon. Toxigenic Klebsiella spp are associated with devastating cases of necrotizing enterocolitis (NEC) in newborns with significant mortality and morbidity 1 2. Current work is revealing a mechanistic basis for til-driven pathology in NEC and aims to define dysbiotic states that trigger til expression in the infant gut.

Maturation of the microbiota during childhood shifts the KoSC to low abundance. Nonetheless, children frequently take antibiotics that drive KoSC expansion. We have shown in mouse models that til metabolites in the dysbiotic gut deplete the microbiome, alter microbial function and drive mutational emergence of antibiotic resistance in gut residents 3. Moreover, til peptides induce apoptotic erosion of the epithelium and active phases of antibiotic-associated colitis in children and adults. Renewal of the intestinal lining and response to injury requires the activities of stem cells, thus gut genotoxins have the potential to induce lasting genetic variation by targeting the stem cell niche. We showed that mice carrying til+ bacteria displayed both higher frequencies of colorectal somatic mutation and more mutations per affected individual than animals carrying a non-producing mutant 4. Similarly, patient isolates of Morganella morganii associated with intestinal bowel disease (IBD) and colorectal cancer (CRC) produce DNA-damaging indolimines 5. Infection of mice with either indolimine-producing bacteria or pks+ E. coli was shown to promote colon tumorigenesis. A survey of somatic mutations at colibactin target sites of several thousand cancer genomes revealed marked enrichment in CRC 6 7. Notably these SBS-pks and ID-pks signature mutations are thought to occur during early childhood.

These data support the general view that microbiota-derived genotoxins add to the range of mutational processes that drive somatic genetic change in the colon, confer these gut residents with a role in bacterial oncogenesis and increase disease susceptibility in human hosts.

1. Paveglio S, Ledala N, Rezaul K, et al. Cytotoxin-producing Klebsiella oxytoca in the preterm gut and its association with necrotizing enterocolitis. Emerg Microbes Infect. 2020;9(1):1321-1329.

2. Coleman S, et al. High-resolution microbiome analysis reveals exclusionary Klebsiella species competition in preterm infants at risk for necrotizing enterocolitis. Sci Rep. 2023;13(1):7893.

3. Kienesberger S, Cosic A, et al. Enterotoxin tilimycin from gut-resident Klebsiella promotes mutational evolution and antibiotic resistance in mice. Nat Microbiol. 2022;7(11):1834-1848.

4. Pöltl L, Kitsera M, Raffl S, et al. Microbiota-derived genotoxin tilimycin generates colonic stem cell mutations. Cell Rep. 2023;42(3):112199. doi:10.1016/j.celrep.2023.112199

5. Cao Y, Oh J, Xue M, et al. Commensal microbiota from patients with inflammatory bowel disease produce genotoxic metabolites. Science. 2022;378(6618):eabm3233. doi:10.1126/science.abm3233

6. Dziubańska-Kusibab PJ et al. Colibactin DNA-damage signature indicates mutational impact in colorectal cancer. Nat Med. 2020;26(7):1063-1069. doi:10.1038/s41591-020-0908-2

7. Pleguezuelos-Manzano C, et al. Mutational signature in colorectal cancer caused by genotoxic pks+ E. coli. Nature. 2020;580(7802):269-273. doi:10.1038/s41586-020-2080-8

SESSION 2 (Med Uni Graz): Microbiome & Infection

INVITED SPEAKER:

The intra- and peritumoral microbiome associated with treatment responses in pancreatic and colon cancer

Christoph Stein-Thöringer, University of Tuebingen, Germany

Abstract not available.

INVITED SPEAKER:

Antigen discovery and spatial microbiome sequencing tools dive deep in the mucosa

Martin Strazar, Broad Institute of MIT and Harvard, USA

Abstract not available.

SELECTED SPEAKER:

Virulome analysis of Enterobacteriaceae from colorectal cancer patients at a tertiary hospital in Ghana

Vincent Amarh, Sarah Bachelle, Saikou Y. Bah & Bartholomew Dzudzor

University Clinics Muenster, Germany

Globally, colorectal cancer is the third most common cancer phenotype and it is the second leading cause of cancer-related deaths. Bacterial genotoxins and dysbiosis of the gut microbiota can induce intrinsic cellular response in humans, which can ultimately lead to colorectal oncogenesis. Even though the role of bacterial-encoded genotoxins have been extensively reported, we reckon that identification of novel genetic signatures in the microbiome of colorectal cancer patients would provide novel insights on bacterial pathogenesis in the context of colorectal oncogenesis. In this study, whole genome sequencing was used to characterize Enterobacteriaceae from colorectal cancer patients and a healthy control group at the Surgical Unit of the Korle Bu Teaching Hospital in Ghana. Escherichia coli was the predominant Enterobacteriaceae (77%) isolated from the samples of both the cancer and control groups. The other isolates were Klebsiella pneumoniae, Proteus mirabilis, Morganella morganii, Raoultella ornithinolytica, Alcaligenes faecalis, Providencia Stuartii, Enterobacter cloacae and Enterobacter hormaechei. Genes encoding notable genotoxins and virulence factors including colibactin (clbA-clbS), cytolethal distending toxin (cdtABC), cytotoxic necrotizing factors (cnf123) and cycle inhibiting factor (cif) were absent from the genome of the Enterobacteriaceae from the cancer patients and healthy control group. Virulome analysis demonstrated virulence genes were modestly over-represented in Escherichia coli from the healthy control group compared to the cancer patients. Genes involved in the general secretory pathway (gspC-gspM), activation of the type II secretory system (yghG) and type I pili (fimA-fimI) were significantly associated with

the healthy control group. Conversely, an integrase (int) gene was over-represented in the genome of Escherichia coli from the cancer patients, highlighting a potential role of site-specific recombination in colorectal oncogenesis.

SELECTED SPEAKER:

Role of Methanogenic Archaea in Gastrointestinal Disorders

Christina Kumpitsch, Alexander Mahnert, Stefanie Duller, Marcus Blohs, Laura Schmidberger, Simone Vrbancic, Tobias Madl, Hansjörg Habisch, Sonja Lackner, Christoph Högenauer, Adrian Moser, Tamara Zurabischvili, Christine Moissl-Eichinger

Medical University of Graz, Austria

Gastrointestinal disorders (GD) represent a diverse array of conditions impacting the digestive system, affecting about 40% of the global population. This encompassing category includes common disorders like Irritable Bowel Syndrome(IBS), and Small Intestinal Bacterial Overgrowth (SIBO), characterized by symptoms such as abdominal pain, bloating, constipation, and diarrhea. Recent research suggests a potential link between specific members of the human gut, known as methanogenic archaea, and GD. These microbes produce methane (CH4) by reducing bacterial fermentation products, and their overabundance is associated with altered intestinal motility patterns leading to GD symptoms like bloating or constipation.

This study aims to unravel the role of methanogenic archaea in GD, emphasizing their impact on microbial communities, functional profiles, and clinical manifestations.

Volunteers from two cohorts, one comprising healthy individuals and the other with GD subjects, were categorized based on their CH4 emission. A multiomics approach was employed to answer a variety of key questions regarding CH4 overproduction and health status: Which methanogenic archaeon is responsible for high CH4emission, do they have bacterial associates, what are the metabolic capacities of these microbial networks, and can they impact disease outcome?

First results show an increased abundance of methanogenic archaea was linked to elevated CH4emission independent of health status. Although microbial communities of the CH4 groups overlap, they differ significantly, suggesting that methanogens are associated with a specific and more diverse microbial community.

Despite the widespread prevalence of GD, effective cures often remain elusive, with current treatment strategies focusing on symptom alleviation. This study aims to provide mechanistic insights into whether and how methanogens contribute to the nuanced spectrum of GD symptoms and if they represent a potential target for the treatment of GD.

SESSION 3a (TU Graz): Plant/Exposome/Food Axis

INVITED SPEAKER:

The central role of the soil microbiome in One Health policy

Ahmed Abdelfattah

Leibniz-Institut ATB Potsdam, Germany

Abstract not available, reference: https://www.nature.com/articles/s41564-023-01386-y

INVITED SPEAKER:

Harnessing the potential of the plant microbiota to control prevalent phytopathogens

Tomislav Cernava

University of Southampton, UK

Abstract not available.

INVITED SPEAKER:

Soil and plant microbiomes under changing environment

Magdalena Frąç

Institute of Agrophysics, Polish Academy of Sciences, Lublin, Poland

The soil microbiome performs many functions important to agroecosystems and also interacts with the plant microbiome through soil-plant-microbiome interactions. These impacts are crucial for the provision of ecosystem services by given microbial communities and the formation of plant holobionts. Changing environmental conditions have a major impact on the formation of the soil and plant microbiome, structure, functions and processes occurring in the environment involving microorganisms. It is worth emphasizing that the microbiomes of agroecosystems are extremely important for maintaining the quality of the agricultural environment and are used to develop biotechnological solutions for sustainable and ecological agriculture. Many strategic documents raise issues of microbiomes and biodiversity loss, as well as the need to improve and reverse this negative situation. The report “Caring for soil is caring for life” emphasizes the need to reverse the negative trend of biodiversity loss, and the latest report on the world of microbiomes deals with the need to improve still poor knowledge and many challenges in the area of microbiome research.

Interactions between plants and microorganisms occur in many environments. Some of the most interesting objects with complex interactions and relationships include intercropping, including intercropping of cereals and legumes. In intercropping, root secretions stimulate microorganism communities, and the mycorrhizal network is transferred and tightened between plant species. We assume that a greater diversity of metabolites leads to the

creation of more complex microbial communities, ensuring the health and balance of agroecosystems. In addition, root secretions and metabolites produced by microorganisms can stabilize interactions between the plant and the microbiome, and are also important for rhizosphere colonization, increased stress tolerance and reduced threats from pests and pathogens.

In the era of observed environmental changes, the content of soil organic matter and its stability are important, as they can have a huge impact on agroecosystems biodiversity. Therefore, understanding the interactions in microbial communities through long-term experiments may contribute to identifying the best practices, methods and production systems for preserving biodiversity.

Observed environmental changes and public awareness increase interest in vertical urban farming, which also includes the cultivation of microgreens. It is known that microgreens contain a significant pool of nutrients and vitamins, but there is little data on micgrogreens-microbiomes interactions. The use of microbial inoculation in microgreens cultivation is poorly researched.

Therefore, the topics in the above-mentioned areas should be explored in depth, providing new solutions based on soil-plant-microbiome interactions, and the new vision of agriculture should also include the close relationships between plant-soil-microbiome interactions for harnessing sustainable microbiome-based strategies for future resilient agriculture development.

This work was supported in the frame of Horizon Europe Programme, agreement no. Project 101082289 — LEGUMINOSE, by The National Centre for Research and Development in Poland in frame of the project EJP SOIL, Project SOMPACS, contract number EJPSOIL/I/78/SOMPACS/2022 and by National Science Center in Poland within OPUS 23 Call, Project MICROGREENS, contract number UMO-2022/45/B/NZ9/04254.

INVITED SPEAKER:

The Agri Microbiomes in a Food Security Context

Lise Korsten

University Pretoria, South Africa

Abstract not available.

SELECTED SPEAKER:

Indigenous seed endophytic bacteria of millets improve seedlings developments and protect from fungal diseases

Satish. K. Verma, Kanchan Kumar, Gaurav Pal, Anand Verma, James White

AIT- Austrian Institute of Technology, Austria

Seed endophytic bacteria (SEB) are considered as primary endo-symbionts of plants which play crucial roles in early stages of development of seedlings. Present study was aimed to investigate removal and further re-inoculation effect of inherent SEB on millet crops seedlings establishment and protection against fungal infection. This study reports that seed inhabiting bacterial endophytes are responsible for greater growth and development of seedlings. Bacterial endophytes also improved the resistance against soil borne fungal diseases. Total four endophytic bacteria were isolated from surface sterilized seeds (brown top millet) and identified by 16S rDNA sequencing as Curtobacterium sp. (M1), Microbacterium sp. (M2), Methylobacterium sp. (M3), Bacillus amyloliquefaciens (M4). Surface sterilized seeds were disinfected with streptomycin to remove bacteria from seeds. Removal of bacteria from the seeds compromised the seedling development in terms of root-shoot length, root hairs formation and photosynthetic pigments. When endophytes were re-inoculated, seedlings recovered their development significantly. More or less similar results were observed in pearl millets, finger millets and sorghum with their own SEB. M3 and M4 were found most potent in promoting growth of seedlings. Bacteria were produced auxin (M2, M4), solubilised phosphate (M1, M2, M4) and inhibited all tested fungal pathogens. Significant protection from F. oxysporum infection on seedlings was found by M4 in microcosm assay. Antifungal lipopeptides genes for surfactins and iturin were detected in M4 and it also showed positive drop collapse assay for surfactins. Present study demonstrates that millet seeds inhabit indigenous endophytes which responsible for greater developments and protection of seedlings.

SELECTED SPEAKER:

The Ecological Niche of Entomopathogenic Fungi: Global and Local Aspects

Adrian Wolfgang

Graz University of Technology, Austria

Non-target effects of agrochemicals have raised considerable concerns regarding human and ecosystem health. Entomopathogenic fungi (EPF) are naturally occurring antagonists of insects and a promising approach to complement and replace synthetic insecticides. However, the ecological niches of these fungi need to be considered for successful field application and for the isolation of climatically adapted strains. So far, no global or local occurrence probability map exists that could be used as a basis to recommend a certain EPF in a given agroecological setting. Using a global dataset containing EPF occurrence data, global cropland suitability was modeled for two EPF genera, Metarhizium and Beauveria, as a function of soil and climatic properties. In addition, this data was compared to a projection for global insect occurrence changes. Furthermore, the ecological inferences obtained by using global data were compared to inferences obtained when

analyzing a local dataset of Styrian agricultural soils. The global dataset revealed Metarhizium to occur in temperate and tropical regions with high primary productivity in soil, while Beauveria naturally occurs in more mountainous regions. Similar results can be obtained using local soil microbiome datasets: Beauveria abundance was positively correlated with slope and exposition of the test field to the south, and negatively correlated with soil water content. Croplands potentially threatened by increasing insect abundances are suitable for the application of EPF, while regions with already declining insect populations may benefit from the more targeted approach of biological pest control. These findings may guide future isolation strategies for locally available EPF strains, demonstrate the biocontrol potential in croplands on a global scale, highlight regions expected to be increasingly pressured by growing insect pest populations, and thus promote biological pest control on both local and global scales.

SESSION 3b (TU Graz): Plant/Exposome/Food Axis

INVITED SPEAKER:

Plant growth promoting or detrimental: The role of of the flagellar transcriptional regulator flhC of Acidovorax for plant-microbe interactions

Siani R.[1-2], Si Y.[3], Niedermaier S.K.[1-2], Ishola O.A.[1], Stabl G.[3], Mahmoud F.[1], Gutjahr C.[3], Radl V.[1], Schloter M.[1-2]

[1.] Research Unit for Comparative Microbiome Analysis, Helmholtz Munich, Germany
[2.] Chair of Soil Science, Science Center Weihenstephan, Department of Ecology and Ecosystem Management, Technical University Munich, Germany
[3.] Department of Root Biology and Symbiosis, Max Planck Institute of Molecular Plant Physiology, Potsdam-Golm, Germany

The beta-proteobacteria *Acidovorax,* which can be found in a variety of environments, is mostly known as a plant pathogen, although some species (eg. *A. radicis*) can promote plant health. Most isolates fall in a twilight zone of opportunism/mutualism. We found that Type III (T3SS) and VI secretion systems (T6SS) delineate the border between plant-associated and free-living strains, being present in most of the former and almost absent of the latter (1). Here, we studied the role of the FlhCD transcriptional regulator, which consists of FlhD and its allosteric activator FlhC, in moderating the response of *Acidovorax* to plant-molecular pattern. Beside activating the flagellar class II operon, FlhCD has been shown to participate in the regulation of traits such as biofilm formation, metabolism of various carbon sources, and different aspects of virulence (2, 3, 4). Unlike for the flagellar T3SS, little is known about the interaction of FlhCD with T6SS. Consistently with the spectrum of ecological processes under FlhCD regulation, we hypothesize that T6SS as well might be connected to the same regulatory network. From a collection originally isolated from healthy *Lotus japonicus* plants, we selected two clonal *A. delafieldii* strains, only differing by the presence of *flhC*. To monitor the differential regulatory processes in a controlled yet realistic environment, we cultivated the strains in a media supplemented with an extract from *L. japonicus* roots. Comparing the strain missing *flhC* to its relative, transcriptome analyses detected an increased expression of several genes coding for

elements of T6SS. Interestingly, along with flagellar assembly and chemotaxis, also T3SS genes were expressed in the *flhC*-carriying strain. Given the constitutive costs and antigenic potential of the flagella, and the ecological importance of T6SS, we hypothesize that losing *flhC* might confer selective advantage to host-associated bacteria. To test this, we collected 4163 reference genomes, spanning 38 phyla, of which 1640 and 1495 were isolated respectively from hosts and the environment, and mined the collection for *flhC* and *flhD* sequences. Both sequence alignments were evaluated in an evolutionary phylogenomic framework, and we found evidence of positive diversifying selection acting on *flhC*, but not *flhD*, in the host-associated genomes. Based on our results, we believe *flhC* change or loss of function to generally benefit bacterial-host association, as in the case of *Acidovorax delafieldii* where it modulates multiple host-related traits.

References:

1. Siani, Roberto, et al. Microbial Genomics 7.12 (2021).
2. Chaban, Bonnie, H. Velocity Hughes, and Morgan Beeby. Seminars in cell & developmental biology. Vol. 46. Academic Press, 2015.
3. Sule, Preeti, et al. Applied and environmental microbiology 77.11 (2011): 3653-3662.
4. Guan, Wei, et al. Molecular plant pathology 21.4 (2020): 489-501.

INVITED SPEAKER:

The edible plant microbiome and its applications to develop medical foods for women in menopause and people with rheumatoid arthritis

Gerardo V Toledo

Solarea Bio Boston, USA

Abstract not available.

SESSION 4 (VAAM): Innovative Methods in Microbiome research

INVITED SPEAKER:

Expanding the microbiome toolkit in genomics and microscopy

Alexander Probst

University of Duisburg-Essen, Germany

Abstract not available.

INVITED SPEAKER:

Exploring links between the human gut microbiota and health using large multi-omics population cohorts

Guillaume Meric

Baker Heart & Diabetes Institute Melbourne, Australia

Abstract not available.

SELECTED SPEAKER:

Development of a genome-scale metabolic model for the murine gut microbiome (McMurGut)

Torben Kühnast, Isabella Pototschnig, Alexander Mahnert, Martina Schweiger, Thomas Weichhart, Christine Moissl-Eichinger

Medical University of Graz, Austria

Current insights into the microbiome's role in human health and disease have primarily evolved from correlational studies and extensive multiomics analyses of microbiota across various health states. To deepen our comprehension of metabolite fluxes and their potential transfer from the microbiome to the host, novel bioinformatic tools are used to simulate microbial metabolite production and consumption using flux balance analysis. However, the predictive accuracy of these simulations hinges upon detailed genome-based, enzymatic models specific to each microbial entity. For the human gut, such metabolic model collections are available to the public, however, despite the extensive use of mice in microbiome research, a dedicated catalog specifically for the murine model is notably absent. To address this gap, we developed a genome-scale metabolic model catalog for the murine gut microbiome. In a first step, a comprehensive, taxonomic inventory of mouse fecal samples based on 16S rRNA gene profiling was performed. Representative microbial genomes were then sourced from various databases and used for metabolic model generation using gapseq. The obtained catalog (McMurGut) was then the basis for MICOM metabolic modelling experiments, which allowed the prediction metabolite fluxes in murine healthy and disease models. McMurGut showed a substantially elevated genus-coverage compared to the mouse genome bacteria catalog (MGBC), which contains over

26,000 murine gut genomes, or AGORA2, the largest human gut model catalog with over 7,302 microbial strains. Thus, this novel approach provides a robust foundation for metabolic modeling, enhancing the accuracy of predictions and expanding our understanding of the murine gut microbiome. The established pipeline to create McMurGut is customizable to any mouse microbiome composition, and requires only the microbial profiling data (amplicon, metagenomic sequencing) as input.

SESSION 5 (BioTechMed): Microbiome Dynamics

SELECTED SPEAKER:

Exploring trade-offs in carbon utilization at multiple levels of differentiation in the rumen microbiome

Cameron R. Strachan, Xiaoqian A. Yu, Tea Movsesijan, Viktoria Neubauer, Anna J. Mueller, Martin Wagner, Qendrim Zebeli, Martin F. Polz, Evelyne Selberherr

VetMedUni Vienna, Austria

The microbial utilization of organic acids in the rumen has been shown to modulate the host's ability to utilize plant biomass, which is tightly linked to both host health and environmental impact. However, our metabolic understanding of the rumen microbiome is mostly coarse-grained, and little is known about how individual microbial lineages partition specific organic acids. Here, we focus on two key groups of organic acid utilizers, determine how they are genotypically structured and then relate this structure to metabolic function. The first group includes members from the Campylobacteraecae that are particularly abundant and active on the rumen epithelia. We find that closely-related Campylobacteraecae populations, which were structured by recent genome-wide sweeps, have partitioned acetate. In this case, metabolic differentiation appears to have resulted from a trade-off with propionate inhibition. The second microbial group includes lactate-utilizing Megasphaera, which are less abundant but thought to prevent acidosis and lower methane emissions. We observed that the rumen Megasphaera are comprised of highly divergent sister clades that have specialized in different lactate utilization pathways, which, based on growth dynamics and pathway regulation, is likely the result of a gleaner-opportunist trade-off. Together, we provide examples of metabolic differentiation resulting from micro-evolutionary processes structuring populations and long-standing ecological roles. In both cases, co-existing microbial lineages specialize in organic acid utilization and are constrained by different types of metabolic trade-offs.

SELECTED SPEAKER:

Genome-wide sweeps are fundamental adaptive units in the human gut microbiome

Xiaoqian Yu, Cameron Strachan, Craig Herbold, Michaela Lang, Christoph Gasche, Athanasios Makristathis, Shaul Pollak, Adrian Tett, Martin Polz

University of Vienna, Vienna, Austria

The human gut microbiome is shaped by diverse selective forces originating from the host and associated environmental factors, and in turn profoundly influences human health and disease. Although associations of microbial lineages with several diseases have been shown at different levels of phylogenetic differentiation, it remains poorly understood to what extent unifying adaptive mechanisms sort microbial lineages into ecologically differentiated populations. Here we show that a pervasive mechanism differentiating microbiome bacteria are genome wide selective sweeps leading to population structure

akin to epidemics, where closely related strains spread across geographically and ethnically diverse human populations. Such sweeps arise when an adaptation allows a clone to outcompete others within its niche followed by re-diversification, and manifest as clusters of closely related genomes on long branches in phylogenetic trees. This structure is revealed by constraining recombination events that mask the clonal descent, and we find that genome wide sweeps originate under a wide regime of recombination rates in at least 45 taxa from 16 bacterial families. Such sweeps can affect populations repeatedly with estimated ages ranging from tens to thousands of years. We provide further evidence that these populations are ecologically differentiated by showing that they are differentially associated with age and colorectal cancer. Our analysis provides an evolutionary mechanism for the observation of strains within metagenomes that are stably inherited and differentially associated and provides a theoretical foundation for analyzing adaptation among co-occurring strains.

SELECTED SPEAKER:

Age-Related Dynamics of Methanogenic Archaea in the Human Gut Microbiome: Implications for Longevity and Health

Rokhsareh Mohammadzadeh, Alexander Mahnert, Tejus Shinde, Christina Kumpitsch, Viktoria Weinberger, Helena Schmidt, Christine Moissl-Eichinger

Medical University of Graz, Austria

Methanobrevibacter smithii is considered as a dominant colonizer early in human life with elevated abundances in centenarians. However, the specific changes in the archaeome during extreme aging and its interactions with gut bacteria remain unclear.

Our findings revealed significant age-related alterations in the archaeome, with an increase in the presence of high methanogen phenotype and a decline in overall archaeal diversity. Centenarians, particularly, exhibited higher Methanobacteriaceae, driven primarily by an increase in Methanobrevibacter smithii. Surprisingly, the archaeal composition in centenarians resembled that of younger adults more than their older counterparts.

The transition phase of elderly adults revealed a surge in Thermoplasmatales and Ca. M. intestini. Notably, Ca. M. intestini played a crucial role in stabilizing microbial networks in young adults, paving the way for M. smithii in older adults and centenarians.

Notably, centenarians exhibited highly complex archaeal networks, characterized by numerous positive and negative co-occurrences with bacteria, reminiscent of high methanogen individuals across all age groups. Furthermore, the co-occurrence of Methanobrevibacter with Oscillospiraceae as a butyrate producing bacteria taxa throughout all age groups suggests that these archaeal communities may compensate for the reported age-related drop in other butyrate producers such as Lachnospiraceae. The presence of archaea in the gut appeared to correlate negatively with dysbiosis, notably in terms of their negative co-occurrence with Streptococcus. This finding implies that archaea may play a role in maintaining gut microbiome health.

In conclusion, disturbances in the formation of high methanogen phenotype during aging are critical for maintaining butyrate levels in the gut. This study highlights the dynamic nature of the presence of high methanogen phenotype and the impact of methanogens on gut health throughout the aging process.

SELECTED SPEAKER:

Microbiome research tools beyond 16S rRNA and shotgun sequencing

Jasmin Huber, Gabriel Vignolle, Iqra Yousaf, Irena Yordanova, Nina Gruber, Michaela Hendling, Lucia Ciglar, Silvia Schönthaler, Manuela Hofner, Walter Pulverer, Andreas Weinhäusel, Klemens Vierlinger and Christa Nöhammer

AIT Austrian Institute of Technology GmbH, Vienna, Austria

Our research group focuses and aims for the identification of minimally invasive biomarkers for disease diagnostics and therapy response stratification. To achieve in this goal we typically apply a multi-omics approach by investigating mRNA and miRNA expression, DNA methylation, protein- and antibody profiles in parallel taking advantage here of high throughput technologies such as microarrays and next generation sequencing. Multi-omics strategies are at the forefront of personalized medicine and are grounded in the understanding that complex diseases cannot be fully characterized by isolate bio measures and that rather, interactions between genes, transcripts, proteins, and the environment determine the development and trajectory of complex diseases. Not least multi-omics profiling is perfectly suited to study host-microbiome/pathogen interactions. Along these lines we recently added antibody reactivities directed against microbes as an additional layer of biological and potentially disease-related information which we interrogate in plasma via an in-house produced microarray containing crude protein lysates obtained from 253 bacterial and 7 fungal strains. We will showcase a multi-omics study aiming for minimally-invasive biomarkers able to predict the advent of atherosclerotic plaque formation in coronary arteries (stenosis) and report here among others on significant differences detected in antibody reactivities against certain bacteria when comparing stenosis and control patients. Further, we will introduce phage immunoprecipitation sequencing (PhIP-Seq) as a novel approach to study antibody responses against specific, predefined peptides. PhIP-Seq utilises the T7 phage to express antigenic peptides on its surface. After contact with patient plasma, antibody-reactive phage peptides can then be identified by NGS, as the identity of the reactive peptide in the phage genome is reflected by the prior generation of corresponding oligonucleotide pool phage libraries.

SELECTED SPEAKER:

Decoding FMT Dynamics in UC: Early Assessment of Functional, Metabolic, and Taxonomic Predictors

Marija Durdevic, Daniel Podlesny, Patrizia Kump, Christoph Högenauer, Marinka Zitnik, Gregor Gorkiewicz

Medical University of Graz, Austria

Ulcerative colitis (UC) is a significant problem in gastroenterology, necessitating innovative therapeutic approaches. By leveraging the intricate relationships between gut microbial ecosystems and host immunity, fecal microbiota transplantation (FMT) has emerged as a promising alternative treatment.

Our study underscores the pivotal role of early assessments of taxonomic, metabolic, and functional changes as reliable predictors of FMT efficacy in UC. Notably, we highlight the significance of a specific microbial community, with Akkermansia playing a crucial role in establishing the metabolic niche and alleviating the symptoms of ulcerative colitis. We also explore the origins of this community—whether from the patient baseline, donor, or a combination—through strain tracking.

Moreover, we explore their collaborative potential, revealing high acetate production as a key player in immune regulation and mucosal healing. Our investigation further extends to community functional activities involved in immune and inflammatory regulation, as well as functions related to barrier integrity.

By closely monitoring these signals right after the FMT, our findings can serve as a robust predictor of the FMT response in UC. Early identification of microbial biomarkers coupled with baseline immunomodulation treatment enables a proactive approach to tailor FMT interventions, optimize treatment strategies, and enhance overall patient outcomes.

INVITED SPEAKER:

Parsing and interpreting the various dynamical regimes of the human gut microbiota

Sean Gibbons

Institute for Systems Biology, Seattle, USA

Abstract not available.

SESSION 6 (ÖGHMP): Microbial Interplay and Vesicles

INVITED SPEAKER:

The development of the gut-brain axis in premature neonates

David Berry

University of Vienna, Austria

Abstract not available.

INVITED SPEAKER:

Unveiling the invisible: IgA-coated bacterial membrane vesicles drive pro-inflammatory responses in ulcerative colitis

Himadri Bahadur Thapa

University of Graz, Austria

The intestinal microbiome is thought to drive chronic inflammatory responses in ulcerative colitis (UC), but molecular mechanisms and disease-relevant effectors remain to be elucidated. Here we analyzed the pro-inflammatory properties of colonic luminal samples obtained from UC and control patients during colonoscopy. We found that membrane vesicle (MV) fractions of UC patient samples, consisting mainly of bacterial MVs and host-derived exosomes, exhibit high IgA levels and strong pro-inflammatory potency in IgA-receptor-positive (CD89+) immune cells. Additionally, UC patients show a pronounced infiltration of CD89+ immune cells in the colonic mucosa. Further analyses revealed that IgA-coated bacterial MVs, but neither host-derived exosomes nor soluble IgA, are potent activators of pro-inflammatory responses in CD89+ cells. IgA-coated bacterial MVs also exacerbated intestinal inflammation in a DSS colitis model using human CD89-expressing mice. Collectively, our results link colonic bacterial-derived MVs with host-derived IgA and CD89+ immune cells, which cooperatively drive inflammatory responses in UC patients.

INVITED SPEAKER:

Gut bacteria nanovesicles: their potential role in mediating cross-kingdom communication within and beyond the gut

Régis Stentz, Emily Jones, Dimitris Latousakis, Arlaine Brion, Aimee Parker, Simon Carding

Quadram Institute, Norwich, UK

It is becoming clear that bacterial extracellular vesicles (BEVs) produced by members of the intestinal microbiota contribute to microbe-host cell interactions. The questions that still need to be clarified are, what is the nature of the cargo packaged into BEVs and how do they impact on host cell functions?

Here we analysed and compared the proteome of BEVs produced and released by the major human gut symbiont Bacteroides thetaiotaomicron (Bt) in the mouse intestine and in vitro and identified proteins that are exclusively enriched in BEVs produced in vivo suggesting that their increased abundance is induced by host-related factors. These abundantly BEV-secreted proteins included three peptidases annotated as dipeptidyl-peptidase IV (DPP-4). In humans, DPP-4 is a key determinant of blood glucose homeostasis since it is responsible for the degradation of incretins such as GLP-1. We show that among the three enzymes, only BT_4193 is a true DPP-4. Furthermore, the highly efficient degradation of substrates by intact BEVs derived from the mouse caecum was demonstrated. The potential significance and impact of DPP-4 and other enzyme activities carried by BEVs on host physiology will be discussed.

We also demonstrate that following oral administration, Bt BEVs can be detected in systemic tissues and in particular, the liver. Our findings raise the intriguing possibility that BEVs, as well as playing physiological roles in the gut lumen, may act as a long-distance microbiota–host communication system.

Overall, these findings provide new insights into the role BEVs play in microbiota-host interactions with their contents capable of playing key roles in the maintenance of intestinal homeostasis and could potentially have an impact on other organs, including the brain.

SESSION 7 (CoE Microplanet): We're living on a micro-planet!

INVITED SPEAKER:

Next generation chemical imaging of the gut microbiome-drug interaction network

Michael Wagner

University of Vienna, Austria

Abstract not available.

INVITED SPEAKER:

Understanding emergent phenomena of microbial decomposer communities using individual-based modelling

Christina Kaiser

University of Vienna, Austria

Abstract not available.

INVITED SPEAKER:

Personalized engraftment prediction with metabolic models of the human gut microbiota

Christian Diener

ISB, Seattle, USA/ Medical University of Graz, Austria

The human gut microbiota acts as the primary gatekeeper to incoming bacterial strains in the large intestine. Complex environment- and host-dependent bacterial interaction networks dictate whether probiotics or pathogens can engraft in the human gut. Thus, there does not seem to be a one-size-fits-all strategy for building a microbiome that allows the entry of health-promoting bacteria while providing resistance to pathogen invasion.

Here, we propose a strategy that combines microbial community-scale metabolic models (MCMMs) with ecological principles in order to estimate metabolic fluxes across the entire gut microbiome of an individual. This approach recapitulates empirically-measured bacterial growth rates in the human gut and quantitatively predicts the production rates of microbial metabolites ex vivo. We show that MCMMs can predict the susceptibility of an individual's gut microbiome to invasion by C. difficile and the return to a resistant state after a fecal microbial transplant. By mapping metabolite-level cross-feeding and competition in personalized models, we identified three distinct realized niches for C. difficile which were consistently observed across 14,862 individuals from four independent cohorts. Personalized MCMMs also correctly recapitulated C. difficile growth suppression

by the VE303 probiotic cocktail, which has been deployed successfully in the treatment of recurrent C. difficile infection.

Our results show that the personalized mechanistic models of the gut microbiome may be an effective tool in predicting personalized pathogen invasion risk and to assess the individual-specific effects of probiotic or dietary interventions. This work may provide the basis for large scale screening and the rational design of personalized interventions based on the composition of the gut microbiota.

SESSION 8 (ÖGGH, ÖGPATH and ÖGIT): Pathogens and Solutions

INVITED SPEAKER:

C. difficile: Breaking the Cycle of Spore to Vegetative to Spore in 2024

Paul Feuerstadt

Yale University School of Medicine, Hamden, CT, USA

Abstract not available.

INVITED SPEAKER:

The gut-lung microbiome: a new organ to ICU-revive?

Sebastian Imbert

Bordeaux University Hospital, France

Abstract not available.

SELECTED SPEAKER:

Exploring microbial competition and warfare to therapeutically utilize the microbiome

Lisa Osbelt, Éva d. H. Almási, Marie Wende, Sabine Kienesberger, Marc Erhardt, Ellen L. Zechner, Till Strowig

Helmholtz Center for Infection Research, Braunschweig, Germany

The Klebsiella oxytoca species complex (KoSC) is a component of the human microbiome and particularly prevalent during infancy and childhood. KoSC strains can produce two enterotoxic natural products, tilimycin and tilivalline, while also contributing to colonization resistance (CR) against pathogenic Enterobacteriaceae. The relationship between these seemingly contradictory roles has remained underexplored. In this project, we demonstrate that K. oxytoca provides CR against lethal Salmonella Typhimurium infections. Genetic disruption of toxin production showed that tilimycin exhibits antimicrobial activity against various Salmonella strains in vitro. Notably, CR against Salmonella depended on toxin production in germfree mice, while it was largely toxin-independent in mice with a residual microbiota. Further, we found that availability of abundant carbohydrate availability induced toxin production, and that nutrient competition not only limited toxin production, but also was critical for CR. Thus, mutual interactions occurring between KoSC members and the microbiota impact both gut community composition and function. While this is in itself an interesting biological observation, it might also be a relevant finding for future interventions. It could aid in mitigating the adverse consequence of acute toxin production, and in designing well-defined cocktails containing a “predator strain” like K. oxytoca and other broad substrate consumers that prevent potential toxicity.

SELECTED SPEAKER:

Identification of the bacterial metabolite aerugine as potential trigger of human dopaminergic neurodegeneration

Nathalie C. Wörz, Anna-Katharina Ückert, Sina Rütschlin, Simon Gutbier, Mahfuzur R. Miah, Airton C. Martins, Isa Hauer, Anna-Katharina Holzer, Birthe Meyburg, Ann-Kathrin Mix, Felix Anderl, Christof Hauck, Michael Aschner, Marcel Leist, Thomas Böttcher

University of Vienna, Austria

Parkinson's disease (PD) is one of the most prevalent neurodegenerative disorders, affecting millions of people worldwide. [1] However, its causes are largely unknown. Although some genes are known to be associated with PD, at least 90% of all cases are of sporadic origin. Age is clearly the major risk factor, but environmental factors, like agricultural chemicals and bacterial metabolites, play an important role as well. [2, 3] Furthermore, in experimental animals, a link between gut microbiota and PD pathology has been established. [4]

Following up suggestions that extracts form Streptomyces venezuelae cause selective cytotoxicity to dopaminergic neurons, we identified a bacterial metabolite known as aerugine and confirmed this finding by re-synthesis. Aerugine was previously shown to be a product of a wide-spread biosynthetic cluster also found in the human microbiome and several pathogens. It triggered half-maximal dopaminergic neurotoxicity at 3-4 µM, was less toxic for other neurons (10-20 µM), and non-toxic (at <100 µM) for common human cell lines. In the C. elegans model organism, general survival was not affected by concentrations up to 100 µM. When transgenic worms were exposed to aerugine, specific neurodegeneration was observed. It also exerted functional dopaminergic toxicity in nematodes. Thus, our research has unveiled a bacterial metabolite with a remarkably selective toxicity toward human dopaminergic neurons in vitro and for the dopaminergic nervous system of C. elegans in vivo. These findings suggest that microbe-derived environmental chemicals should be further investigated for their role in PD pathogenesis. [5]

[1] Deuschl, G., The Lancet Public Health, 2020. 5(10): p. e551-e567.
[2] Obeso, J.A., et al., Movement Disorders, 2017. 32(9): p. 1264-1310.
[3] Caldwell, K.A., PloS one, 2009. 4(10): p. e7227-e7227.
[4] Sampson, T.R., et al., Cell, 2016. 167(6): p. 1469-1480.
[5] Ückert, A.-K., et al., Environment International, 2023. 180: p. 108229.

SESSION 9 (AMICI): Microbiomes and Human Surfaces

SELECTED SPEAKER:

Microbiota-associated metabolites: methylphenols and the blood–brain barrier

Lesley Hoyles, Simon McArthur

Nottingham Trent University, UK

Microbial fermentation of amino acids in the large intestine generates metabolic end-products that can interact with the host at the intestinal and systemic levels. End-products of tyrosine and phenylalanine fermentation include p-cresol. This microbiota-derived metabolite undergoes conjugation in enterocytes and the liver, reaching the systemic circulation as p-cresol sulfate (pCS) and p-cresol glucuronide (pCG). Healthy people can clear pCS and pCG efficiently, but both these metabolites accumulate in the blood of patients with kidney disease. pCS may contribute to the impaired cognitive function frequently observed in those with chronic kidney disease. In vitro and in mice, physiologically relevant levels of pCG prevented the blood–brain barrier (BBB)-permeabilizing effects of endotoxin, acting by antagonizing the LPS receptor TLR4. In contrast, physiologically relevant levels of pCS increased paracellular permeability and disrupted intercellular tight junctions. pCS changed the whole-brain transcriptome, suppressing neuronal activity, transcription and mitochondrial respiration pathways. It also stimulated the epidermal growth factor receptor (EGFR), leading to mobilzation of matrix metalloproteinase (MMP)-2/9. In vivo, the deleterious effects of pCS on the BBB were prevented by the EGFR antagonist erlotinib or the MMP2/9 inhibitor SB-3CT. Human hCMEC/D3 endothelial cells exposed to serum from haemodialysis patients, but not from healthy donors, showed an erlotinib-sensitive increase in paracellular permeability that correlated with the total serum pCS content. These data demonstrate the complexity of microbial metabolite–host communication pathways underlying the gut–brain axis, and identify means by which microbiota-associated metabolites can be targeted to improve brain function.

SELECTED SPEAKER:

Widespread gut commensals of the family Sutterellaceae perform fumarate and nitrate respiration

Nataliia Solntseva[1,2], Songcan Chen[1], Jay Osvatic[3], Marc Mussmann[1], Alexander Loy[1]

University of Vienna, Austria

Members of the family Sutterellaceae (Pseudomonadota) are ubiquitous in the intestinal tract. The genera Sutterella and Parasutterella are core taxa of the human gut microbiome, but also present in the gut of various animals, including mice, dogs, cows, and birds. They are associated with different health, disease or lifestyle states of their hosts. Yet, reports on the relationship of Sutterellaceae with human diseases such as irritable bowel syndrome and depression are conflicting. Sutterellaceae type strains are asaccharolytic, some are capable of nitrate reduction and microaerophilic growth. Despite their wide distribution

and associations with various health conditions, their specific metabolic niches and functions in the gut are poorly characterized. Here, we studied the fundamental physiology and energy metabolism of Sutterellaceae by growth tests with gut-derived strains of the Sutterella, and Turicimonas, metabolite analysis, single-cell mass measurements, and comparative genomics and transcriptomics. All strains encode a periplasmic nitrate reductase (NapAB), a fumarate reductase complex (FrdABC), and a putative octahaeme cytochrome c sulfite reductase (MccA), which suggests they can utilize nitrate, fumarate, and sulfite for anaerobic respiration. Addition of 5-50 mM fumarate or the fumarate precursor aspartate to S. wadsworthensis and S. parvirubra cultures significantly increased cell density, growth rate and led to a succinate accumulation. Moreover, the addition of fumarate, but not formate, caused significant increase in single cell mass in the exponential phase. Both species constitutively expressed frdA, aspA (aspartate ammonia-lyase), napA and mccA at high levels, suggesting that they depend on fumarate and nitrate reduction. Our work shows previously unrecognized metabolic capabilities of members of the ubiquitous gut family Sutterellaceae that make them potential competitors for nutrients with enteropathogens such as Salmonella enterica.

SELECTED SPEAKER:

Nanomechanical mechanisms of *Borrelia* interactions with extracellular matrix

Yoo Jin Oh

Johannes Kepler University Linz, Austria

As opposed to pathogens passively circulating in the body fluids of their host, pathogenic species within the Spirochetes phylum are able to actively coordinate their movement in the host to cause systemic infections. Based on the unique morphology and high motility of spirochetes, we hypothesized that their surface adhesive molecules might be suitably adapted to aid in their dissemination strategies. Bio-AFM provides the ideal condition for nano-scale characterization of the microbial surface in their native, physiological environment and for elucidating processes occurring at the interface between microorganisms and cells. In this study, we probed the interaction forces between decorin binding protein A/B (DbpA/B) from different genospecies and various ECM proteins. Using single-molecule force spectroscopy, we disentangled the mechanistic details of DbpA/B and decorin/laminin interactions. Our results show that spirochetes, depite a limited number of adhesive molecules, are able to leverage a wide variety of adhesion strategies through force-tuning transient molecular binding to extracellular matrix components, which concertedly enhance spirochetal dissemination through the host.

SELECTED SPEAKER:

Immediate targeting of host ribosomes by jumbo phage encoded proteins

Milan Gerovac, Laura Wicke, Kotaro Chihara, Bettina Böttcher, Rob Lavigne, Jörg Vogel

University of Würzburg, Germany

Bacteriophages must seize control of the host gene expression machinery to promote their own protein synthesis. Since the bacterial hosts are armed with numerous anti-phage defence systems, it is essential that mechanisms of host take-over act immediately upon infection. Although individual proteins that modulate components of the bacterial gene expression apparatus have been described in several different phages, systematic approaches which capture the phage's arsenal for immediate targeting of host transcription and translation processes have been lacking. In particular, there are no known phage factors that associate directly with host ribosomes to modulate protein synthesis. Here, we take an integrative high-throughput approach to uncover numerous new proteins encoded by the jumbo phage ΦKZ that target the gene expression machinery of the Gram-negative human pathogen Pseudomonas aeruginosa immediately upon infection. By integrating biochemical and structural analyses, we identify a conserved phage factor that associates with the large ribosomal subunit by binding the 5S ribosomal RNA. This highly abundant factor is amongst the earliest ΦKZ proteins expressed after infection and stays bound to ribosomes during the entire translation cycle. Our study provides a general strategy to decipher molecular components of phage-mediated host take-over and argues that phage genomes represent a large discovery space for proteins that modulate the host gene expression machinery.

SELECTED SPEAKER:

iNKT cells immunomodulation and mucosal healing by microbiota-derived lactate

Francesco Strati

University of Milano-Bicocca, Italy

Invariant natural killer T (iNKT) cells are unconventional, CD1d-restricted, T lymphocytes playing a critical role in mucosal immune homeostasis.iNKT cells plastically adapt their functional phenotypes to the surrounding environment and are involved in IBD pathogenesis. Although iNKTcells release pro-inflammatory cytokines in response to the altered gut microbiota of IBD patients, exposure to microbiota-derived metabolitescan promote homeostatic IL10-mediated iNKT responses resulting in better clinical outcomes in Crohn's disease (CD) patients. Thus,understanding the mechanisms leading to iNKT cells' functional shaping by microbiota-derived metabolites is important to design noveltherapeutic approaches for IBD patients. We hypothesised that iNKT cells, serving as sentinels of tissue integrity, are the primary immune cellssensing microbiota-derived metabolic signals promoting the resolution of inflammation in IBD. In particular, here we show that microbiota-derived lactate can tightly control iNKT cells function promoting iNKT-mediated mucosal tolerance while preventing T-cell-mediated overtinflammation and tissue injury. We performed immunophenotyping of iNKT cells by multiparametric flow-

cytometry from surgical specimens ofCD patients and correlated NKT10 responses with intra-colonic levels of lactate, gut microbiome functions and tissue injury/healing. We studiedthe molecular pathway determining the NKT10 phenotype in a lactate-enriched microenvironment by RNA-seq and CHIP-seq. We validated therole of NKT10 responses by microbiota-derived lactate in-vivo by using experimental colitis models. On overall the present work shows i) howmicrobiota-derived lactate modulates the immunophenotype of iNKT cells, ii) the mechanisms underlying the sensing of microbiota-derivedlactate by iNKT cells, iii) iNKT cells role in inflammation resolution.

SELECTED SPEAKER:

Methanobrevibacter smithii and *Cand.* M. intestini – Similar but different

V. Weinberger, R. Mohammadzadeh, T. Kühnast, D. Strauß, K. Kalt, S. Moser, M. Cecovini, P. Mertelj, T. Zurabishvili, H. Habisch, T. Madl, D. Kolb, D. Pernitsch, K. Hingerl, E. Jones, A. Jordan, R. Juodeikis, R. Stentz, S. Carding, C. Moissl-Eichinger

Medical University of Graz, Austria

Within the gut microbiome, the estimated average of methanoarchaeal abundance is 1.2%, with Methanobrevibacter smithii prevailing as the dominant methanogen. In general, Methanobrevibacter species interact widely with other members of the gut microbiome, by subsequently facilitating the processes of digestion and fermentation and with the human host, thereby playing a significant role in the human gut. Recent findings have identified two distinct species-level clades within M. smithii: M. smithii and Candidatus M. intestini (respectively referred to as smithii and smithii_A in the GTDB taxonomy).Despite their significance, detailed characteristics of M. smithii and Ca. M. intestini remain largely unexplored. To enhance our understanding regarding the characteristics of these two species and to elucidate their differences, we employed multi-omics techniques (i.e. genomics, proteomics, metabolomics) culture-based methods, and structural analysis. Cultivation-based methods revealed similar growth conditions for both M. smithii ALI and Ca. M. intestini. Moreover, Ca. M. intestini appeared to not only have the ability of producing but also consuming formate, an ability not observed for M. smithii ALI.

Furthermore, we successfully isolated extracellular vesicles from human-derived archaeal isolates, a novel discovery. Initial measurements included concentration, hydrodynamic diameter, and content analysis encompassing protein, lipid, DNA, and RNA. Additional ongoing analyses include sequencing, proteomics, and metabolomics. To furthermore investigate the potential of M. smithii ALI, Ca. M. intestini to interact with the human host, co-incubation experiments were performed using Thp-1 cells, and murine bone marrow derived monocytes. Experiments were focused on cytotoxicity and the uptake of archaeal cells and/or their extracellular vesicles. Overall, our work provides a detailed characterization of human gut methanogens, serving as a pivotal groundwork for further research.

INVITED SPEAKER:

Taste the difference: cell biology of a multicellular bacterium inhabiting the human mouth

Silvia Bulgheresi

University of Vienna, Austria

No abstract available.

INVITED SPEAKER:

T cell regulation by bacterial metabolites

Clarissa Campbell

CeMM, Vienna, Austria

No abstract available.

INVITED SPEAKER:

10 years of the human skin archaeome

Alexander Mahnert

Medical University of Graz, Austria

No abstract available.

POSTERS

ENVIRONMENT, BIODIVERSITY, PLANT & FOOD

No. 1

Changes in microbiome composition predict variation in plant traits

Antonino Malacrinò, Alison E. Bennett

Dept. of Agriculture, Università degli Studi Mediterranea di Reggio Calabria, Reggio Calabria, Italy

Quantifying the effects of variation in microbiome composition on host traits is still an outstanding challenge in holobiont biology. Plants, for example, assemble most of their microbiome from soil, which composition is influenced by both biotic and abiotic factors, driving variation in plant-associated microbial communities (e.g., roots, leaves). The impact of this variation in plant microbiome composition on host traits and function is still little understood. We set out to address this question in a greenhouse experiment using tomato as a model. We extracted a diverse microbial community from restored prairie soil, manipulated it to have incremental changes in microbiome composition, and added the manipulated communities to tomatoes growing in sterile soil. We then characterized microbial community composition in soil, roots, and leaves, and measured a suite of plant traits to determine whether changes in soil microbiome composition influenced them. We found clear signatures of changes in microbial community composition on host traits, and interestingly we found that incremental changes in microbiomes composition altered plant fitness traits more than morphological traits, with variations across plant development and compartments (soil, roots, leaves). Under the global changes we are witnessing, our results are relevant to predict the impact of shifts in microbial communities on plants traits, or to help us to manipulate microbiomes to alter plant traits and function, which is key towards a more sustainable agriculture.

No. 2

Exploring the impact of intercropping on the rhizosphere microbial communities of cultivated lettuce

Kristina Michl, Simone Bosco, Verena Gschiel, Gabriele Berg, Tomislav Cernava

Institute for Environmental Biotechnology, Graz, Austria

The influence of microbial diversity on plant growth and health is well described as microorganisms contribute to enhanced nutrient uptake and increased resilience against various stressors. Integrating this perspective into agricultural practices has the potential to result in improved crop productivity and resistance. Intercropping, the simultaneous cultivation of multiple crops in the same field, has been explored as an alternative to monoculture, revealing benefits such as heightened microbial diversity and improved plant growth in various cropping combinations. In this study, we aimed to assess the effect of

intercropping on the microbial composition of the rhizosphere of Lactuca sativa var. capitate L. Rhizosphere samples were collected at three time points during the growing season from fields with different cultivation methods, including monoculture with and without mulching, and intercropping with narrow or wide. High-throughput sequencing of the 16S rRNA gene fragment and ITS region was done to analyze the taxonomic structure of bacterial and fungal communities, respectively. The relative abundance of both bacterial and fungal communities was primarily influenced by the sampling time point, followed by the cultivation method. Moreover, the Shannon diversity index revealed significantly higher diversity in both intercropping treatments at the first time point compared to the monoculture. PERMANOVA analysis indicated that both sampling time and cultivation system contributed similarly to the observed variance. Non-metric multidimensional scaling (NMDS) plots revealed distinct clustering of samples based on cultivation systems, illustrating a shift in bacterial and fungal communities due to different treatments. In summary, while intercropping had a pronounced effect on microbial diversity early in the cultivation season, mulching also exhibited an increase in diversity over time, although determining distinct communities compared to intercropping.

No. 3

Halophytes vs. crop plants: Unraveling Microbiome Structure and core taxa

Mohamed R. Abdelfadil[1,2], Steffen Kolb[2], Silke Ruppel[1]

1) Department of Plant Microbe Systems, Leibniz Institute of Vegetable and Ornamental Crops,Großbeeren, Germany.
2) Microbial Biogeochemistry, RA Landscape Functioning, Leibniz Centre for Agricultural Landscape Research (ZALF), Müncheberg, Germany.

Climate change and anthropogenic activities are intensifying salinity stress that significantly reduces plant productivity and biodiversity in many agroecosystems. Nonetheless, there are salt-tolerant plants (halophytes) that are adapted to under extreme salty conditions. Over years these plants along with their associated microbiome evolved various mechanisms to alleviate salt stress. Exploring the microbiome composition of different halophytic plants is an opportunity to discover a halophyte specific microbiome. Studies comparing the microbiome structure between halophytic and non-halophytic plants, such as conventional crops, are still rare. Therefore, we performed a comprehensive meta-analysis study using published bacterial 16S rRNA gene sequencing datasets to reveal microbiome structure and identify core taxa in various halophytic and non-halophytic plant hosts. Fifteen studies met our quality selection criteria to retrieve microbiome datasets, covering 40 plants, representing 10 different halophyte species and 4 different non-halophyte species from around the world. Microbiome structure analysis revealed distinct compositions for halophyte plants that face high salinity concentrations in their rhizosphere from halophytes grown at low salinity or from crop plants. Furthermore, 17 bacterial genera exclusively detected in all halophytes grown at high salt concentrations. These findings demonstrated that salinity is one of the environmental factors controlling the structure of the rhizosphere microbiome. Additionally, this advances our understanding and reinforces the "cry-for-help" theory of how stressed plant possibly assemble special microbiome to mitigate stresses. In silico identifying shared core taxa

associated with a group of plants with similar environmental conditions can help to design effective synthetic microbial communities (SynComs) that may mitigate salt stress impacts.

No. 4

Metagenomic Soil Analysis Utilizing Oxford Nanopore Sequencing

Stefan Leiner, Germana Baldi & Katrin Panzitt

Institute of Pathology, Medical University of Graz, Austria

The number of environmental microbiome studies has been rapidly increasing with the advent of advanced molecular biological techniques, especially NGS (next-generation sequencing) and improved bioinformatics analyses methods, e.g. metagenomic analyses. However, these studies often compare data generated by different laboratories using different sampling strategies, storage conditions, DNA preparation protocols and library generation protocols,-all with inherent biases.

Formerly, microbial NGS studies were mostly performed with short read sequencing (16S sequencing). But in the past years the need for longer sequencing reads improving the resolution of challenging genome regions has lead to the development of platforms such as ONT (Oxford Nanopore Technology) or PAcBio which either in combination with 16S or on their own are better suited to correctly depict the variability of microbial communities.

Our goal was to perform the most bias-free metagenomics analysis possible of an environmental sample. As environmental sample we chose grassland soil, sampled in a standardized way by the soil sampling campaign of DSMZ (German collection of microorganisms and cell cultures) in Germany in May 2023. The soil samples were evaluated in terms of their storage conditions from the time of sampling to the time of laoratory workup. Potential changes in the microbial community were evaluated with ONT sequencing in a direct, non-PCR based sequencing approach. Furthermore a bioinformatics pipeline was established, with the additional goal of suitability for in-field sequencing.

No. 5

Microbiome research meets cryptogam ecology: Selectivity of lichens for potentially symbiotic Alphaproteobacteria

Diego Leiva, Margarita Carú, Julieta Orlando

Departamento de Ciencias Ecológicas, Universidad de Chile, Santiago, Chile.

Lichen-forming fungi associate preferentially with certain photobionts (algae or cyanobacteria) depending on their specificity, availability and ecological success. This whole ecological process is called photobiont selectivity and drives the evolution and biodiversity of lichen symbionts.

Diverse bacterial groups have been detected and proposed as possible partners in the lichen symbiosis, but none has been found to generate a relevant mutualistic outcome in lichens. Sphingomonadales (Sles.) and Rhizobiales (Rles.) are two alphaproteobacterial orders

which are widespread in lichens, and as the potential functions of the former are host specific (e.g., nutrient recycling), we hypothesized that selectivity would be higher for bacteria in the Sles. To test this, we collected ten specimens of the cyanolichen Peltigera frigida and their soil substrate from two forest sites, 16S-metabarcoded their bacterial communities, and calculated a selectivity index based on the availability, specificity and phylogenetic relationships of bacterial genera among each alphaproteobacterial order.

Absolute selectivity was higher for Sles. than for Rles. (0.62 vs. 0.54) and several genera presented positive selectivity values (preference). Among Sles., P. frigida prefers the heterotrophic Sphingomonas s.s. (0.74) and the photoautotrophic Sandarakinorhabdus (0.37). In Rles., there is a preference for the methylotrophic bacteria Methylobacterium (0.68) and Methylopila (0.42), the heterotrophic Devosia (0.57), Aureimonas (0.57) and Tardiphaga (0.40), and the litoautotrophic Bosea (0.49); in contrast, only Pararhizobium (0.28) shows a positive value among the diazotrophs. In general, our results agree with the expected functions of bacteria in this cyanolichen (high preference for nutrient-recycling Sles. and rejection of diazotrophic Rles.), highlighting the need of integrative hypothesis-driven approaches to disentangle the interactions between lichens and their microbiome.

No. 6

Nurturing Indigenous Crop Health for Human Wellbeing

Jarishma Gokul

University of Pretoria, Pretoria, South Africa

The plant microbiome represents a hidden ecosystem harboring a diverse array of beneficial microorganisms that forge intricate alliances with crops. This holds the promise of revolutionizing agriculture, offering a profound pathway to enhance crop health, nutrient uptake, and overall productivity in the African landscape. Within the intricate tapestry of the plant microbiome, beneficial microorganisms, act as silent allies to their host crops. By assisting in nutrient absorption, bolstering disease resistance, and mitigating environmental stressors, the microbial community enables crops to thrive even in challenging conditions. Indigenous crop resilience not only secures food production but also reduces the need for chemical inputs, aligning agriculture with sustainable and environmentally responsible practices. Crucially, the ramifications of these microbiome-driven improvements ripple outward to human health and wellbeing. Orphan crops, often marginalized despite their nutritional richness and cultural significance, stand to gain immensely from a flourishing plant microbiome. These crops are staples in traditional African diets, possessing unique attributes that hold potential not only for improved nutrition and enhancing food security but also for agricultural diversity and resilience and bolster local economies, contributing to the overall wellbeing of our planet.

No. 7

Rapid and collective reactivation of a desert soil crust microbial community upon a water pulse

Stefanie Imminger, Dimitri V. Meier, Arno Schintlmeister, Anton Legin, Osnat Gillor, Stephanie A. Eichorst, Dagmar Woebken

Centre for Microbiology and Environmental Systems Science, Vienna, Austria

Lack of water poses a fundamental constraint on soil microbial activity, crucial for soil ecosystem health. Existing studies on microbial desiccation tolerance and reactivation upon water availability are limited to a few cultured organisms, not representative for dominant soil taxa. This knowledge gap includes dynamics and physiological aspects of reactivation, as well as the number of cells that can revive. Alternating phases of microbial activity and dormancy are most pronounced in desert soils, especially during infrequent rain events. We used biocrusts from the Negev Desert, Israel, to focus on gene transcriptional changes of individual microbial populations and single-cell biomass generation during a controlled rehydration experiment. Biocrusts were rehydrated with heavy water, and deuterium incorporation (a general marker of anabolic activity) was measured by NanoSIMS. We detected biomass production in >90% of cells only few hours after rehydration, calculated biomass generation rates and estimated replication times of individual cells. Metagenome-assembled genomes facilitated metatranscriptome normalization and split by populations. We analyzed transcriptional responses of different community members in a highly resolved time series spanning 15 minutes to 55 hours. Normalization of transcripts per population enabled the detection of hidden transcriptional patterns. Rapid and collective reactivation of microbial populations, independent of taxonomy or physiology, was evident within 15-30 minutes of hydration. Temporal phases of cellular processes, included early DNA repair fueled by storage compound oxidation, followed by external carbon source uptake and main metabolism resumption. Our integrated approach provides detailed insights into in-situ resuscitation mechanisms and dynamics of typical desert soil microbiota.

No. 8

The plant microbiome as key to understand the Kiwifruit Vine Decline Syndrome

Schena Leonardo, Mosca S., Malacrinò A.

Department of Agriculture, Mediterranea University of Reggio Calabria, Reggio Calabria, Italy

The Kiwifruit Vine Decline Syndrome (KVDS) causes a progressive decay of kiwifruit vines roots and the consequent collapse of the entire plant. The agent(s) and mechanisms behind KVDS are not yet fully understood, although spatial occurrence and symptoms suggest that this syndrome has a biotic origin and that oomycetes might play a primary role. Here, we focused on clarifying the causes behind KVDS by analysing the microbiome of soil and roots of symptomatic and asymptomatic kiwifruit plants using amplicon metagenomics targeting bacteria, fungi, and oomycetes. Results show marginal differences in the diversity and structure of microbial communities, regardless of the presence of KVDS symptoms. However, amplicon sequence variants (ASVs) identified as Phytopythium vexans were

enriched in both soil and root samples collected from symptomatic plants. These findings were confirmed by the isolation of the oomycete community using baiting. Although several other oomycetes were isolated (including Phytopythium litorale, Phytophthora cryptogea, Globisporangium sp., and Pythium sp.), P. vexans was the most frequently isolated. Taken together, our results support the role of oomycetes as causal agents of KVDS, with P. vexans likely playing a major role. Further work will elucidate the mechanisms leading to the induction of KVDS, clarifying the specific contribution of P. vexans and the other oomycetes in the wider context of the plant-microbiome-environment interactions.

No. 9

Understanding the variation in bacterial communities of tempe and its implications for food safety in Indonesia

Wisnu Adi Wicaksono, Gabriele Berg

Institute of Environmental Biotechnology, TUG, Graz, Austria

Fermented foods are gaining attention due to their potential health benefits. Research has shown that consuming fermented foods can improve the diversity of gut microbiota, which is associated with a healthy gut microbiome. The microbes found in fermented foods are believed to have positive impacts on health. Tempe, a popular fermented food in Southeast Asia, lacks a thorough understanding of its bacterial community composition, function, and influencing factors. Through high throughput sequencing, we conducted a comprehensive analysis of the microbial composition, diversity, and functional potential of tempe collected from various locations in Indonesia. Our findings revealed that hygiene practices during tempe production significantly affect the microbial community composition and overall microbial function. Surprisingly, tempe packaging also had a moderate influence on bacterial abundance. Using a genome-centric approach, we discovered that tempe-associated bacteria have potential beneficial functions, such as the production of vitamin B, vitamin K, and short-chain fatty acids. This study serves as a foundation for improving tempe quality by modifying the indigenous microbiota, an aspect that is often overlooked.

No. 10

Using artificial humic acid to encounter the effects of climate change induced droughts on soil microbiome

Daniel Höfle, Sperber Sebastian, Nader Marzban, Barbara Sturm, Thomas Hoffmann, Markus Antonietti, Gabriele Berg, Ahmed Abdelfattah

Leibniz Institut für Agrartechnik und Bioökonomie e.V. (ATB), Potsdam, Deutschland

Increasing temperatures associated with climate change and changing rainfall patterns are impacting the survival of plants. This is particularly challenging in areas like Brandenburg, Germany, where sandy soil is common. The availability of surface water for irrigation is decreasing, leading to more frequent and severe droughts. To address these issues, the use of humic substances has been considered to improve soil fertility and water retention. This study aimed to investigate the effect of artificially produced humic acid on the soil

microbiome. After incorporation of humic acids into soils collected from a field site in Marquardt (Brandenburg) at a concentration of 0.01%, soils were exposed to drought conditions. At regular intervals, the microbiota was monitored in comparison to untreated controls analyzing a strain collection together with an amplicon library of 16S rDNA genes. The bacterial abundance decreased in the absence of humic acid during drought, while conversely, the soil treated with humic acid showed consistent bacterial abundances even in drought conditions. These findings indicate that incorporating artificial humic acid into sandy soil can have a beneficial effect, especially during drought. Additionally, in induced drought and humic acid treatment, we identified several bacterial species that possess potential to enhance drought tolerance in plants, e.g. Bacillus sp., Flavobacterium sp., Pseudomonas sp., Stenotrophomonas rhizophilia and Variovorax sp. Furthermore, the application of humic acid had a significant effect on the community composition as well as species richness. This study highlights the potential of artificial humic substances in mitigating the effects of climate change-induced droughts.

HUMAN HEALTH

No. 11

Associations of age, gender and dietary intervention on serum TMAO concentrations from childhood to early adulthood and the microbiome – The Special Turku Coronary Risk Factor Intervention Project (STRIP)

Gunter Almer, PhD; Andreas Meinitzer, PhD; Noora Kartiosuo; Dietmar Enko, MD; Harri Niinikoski, MD, PhD; Terho Lehtimäki, MD, PhD; Markus Herrmann, MD; Olli T. Raitakari, MD, PhD; and Katja Pahkala, PhD.

KIMCL, Auenbruggerplatz 15, 8036 Graz, Austria

Background and aim: Primary prevention is the cornerstone for cardio-metabolic health. In the randomised, controlled STRIP study, dietary counselling intervention was given to children from infancy to 20 years of age. We investigated the associations of age, sex and dietary intervention on the gut metabolite and cardiac biomarker trimethylamine-N-oxide (TMAO) and the microbiome.

Methods: A subcohort of 592 healthy males (54%) and females (46%) from STRIP was used. Compared to the control group, the intervention group received dietary counselling since age 7 months, focused on low intake of saturated fat and cholesterol, and the promotion of fruit, vegetable, and whole-grain consumption. TMAO serum concentrations were measured at ages 11, 13, 15, 17, 19, and 26 years. Gut microbiome analysis was performed with stool samples from the last age point using 16S rRNA sequencing.

Results: TMAO concentrations increased from age 11 to 26 years in both genders. At all measurement time points, males showed significantly higher serum TMAO concentrations compared to females, but did not differ between the intervention and control groups. Firmicutes to Bacteroidetes ratio on phylum level and Beta diversity on genus level showed differences in the microbiome composition between females and males at age 26 years. Linear regression analysis revealed that the firmicute Roseburia hominis directly correlates with TMAO in males.

Conclusion: TMAO concentrations increased from childhood to early adulthood, but they were not affected by the given dietary intervention. The generally higher TMAO concentrations in males could be associated with higher abundancies of only one taxa, but several sex-based differences in the microbiome composition were found suggesting that sex-related differences need further investigation.

No. 12

Breath of Discovery: Mapping Microbial Constellations in the Human Respiratory Tract through Metagenomic Exploration

Tejus Shinde[1], Christina Kumpitsch[1], Christine Moissl-Eichinger[1,3], Vasile Foris[2]

[1]The Diagnostic & Research Institute for Hygiene, Microbiology, & Environmental Medicine, Medical University of Graz; [2]Division of Pulmonology, Department of Internal Medicine

A dysbiotic airway microbiome is linked to respiratory disorders like chronic obstructive pulmonary disease (COPD) and chronic rhinosinusitis. Understanding the airway microbiome's nature and dynamics is essential for investigating disease causality and mechanisms of microbial dysbiosis-associated disease progression. To address this knowledge gap, we aim to create a pan-genome catalog of microorganisms associated with the human respiratory tract. Whole genome metagenomic sequencing allows us to explore the intricate microbial genomic inventory within this ecosystem. By reconstructing metagenome-assembled genomes (MAGs), we can gain species- and strain-specific insights into the functional and pathogenic potential of the human microbiome.

To achieve this, we retrieved public metagenomic datasets from NCBI-SRA to perform de novo assembly, compiling a representative set of genomes specific to the human respiratory system. Additionally, we will include microbial genomes obtained from sequencing clinical samples and isolates. However, recovering MAGs from human respiratory samples is challenging due to low microbial biomass and high human DNA background. Therefore, we are employing various assembly and binning approaches such as SACB (single-sample assembly with co-binning) and CASB (co-assembly with co-binning) to maximize MAG retrieval from low-diversity, low-abundance samples.

We present summary statistics and preliminary insights into the diversity of the respiratory microbiome based on an initial screening of human respiratory samples. The genome alignment tree for all the high-quality MAGs are from one of the datasets ‘URT_B1’. The final catalog of microbial genomes will facilitate improved taxonomic classification, functional capacity prediction, and metabolic modeling of dysbiosis in the human respiratory system, leveraging the comprehensive gene content information available.

No. 13

Cyanovirin-N binding to N-acetyl-D-glucosamine requires carbohydrate-binding sites on two different protomers

Irene Maier and Christoph Steininger

Department of Internal Medicine I, Medical University of Vienna, Vienna, Austria

Cyanovirin-N (CV-N) binds high-mannose oligosaccharides on enveloped viruses with two carbohydrate-binding sites, one bearing high-, and one low-affinity to Manα(1-2)Man moieties. A tandem repeat of two CV-N molecules (CVN2) was tested for antiviral activity against human immunodeficiency virus type I (HIV-1) by using the domain-swapped dimer. CV-N was shown to bind N-acetylmannosamine (ManNAc) and N-acetyl-D-glucosamine (GlcNAc) when the carbohydrate-binding sites in CV-N were free to interact with these

monosaccharides independently. CVN2 recognized ManNAc at a Kd of 1.5 µM and bound this sugar in solution, regardless of the lectin being expressed with small ubiquition-like modifier (SUMO)-tag and amino acid sidechain contacts on targeted viral glycoproteins. An interdomain cross-contacting residue Glu41, which has been shown to be hydrogen-bonding with dimannose, was substituted in the monomeric CV-N by alanine. Thus, the amide derivative of glucose, GlcNAc, achieved similar binding affinity to the new variant CVN-E41A than high mannose N-glycans, but binding to CVN2 in the nanomolar range with four binding sites involved. Disulfide bond variants were generated by substitution of C58-C73 via Glu – Arg to increase binding affinity to hemagglutinin, and Glu – Arg and Trp – Met to reduce binding when two functional low-affinity binding sites were utilized to target the viral spike proteins near a disulfide bridge. Low-affinity binding was achieved by CVN2 to dimannosylated peptide or GlcNAc with two carbohydrate-binding sites of differing affinities, mimicking biological interactions with the respective N-linked glycans of concern and interest and cross-linking of carbohydrates on human T-cells for lymphocyte activation. The selective binding of GlcNAc by CVN-E41A was measured by isothermal titration calorimetry in the low-micromolar range, making this sugar-lectin interaction a stimulator of immune cell effector functions in response to interleukin-2 release.

No. 14

Disentangling gut microbiota associated with Type-2 Diabetes and Metabolic dysfunction Associated Steatotic Liver Disease

Mohamed S. Sarhan, Paolo Manghi, Giacomo Antonello, Christoph Grander, Herbert Tilg, Cristian Pattaro, Peter Pramstaller, Nicola Segata, Christian Fuchsberger

Institute for Biomedicine, Eurac Reseach, Bolzano Italy (Affiliated institute with Lübeck University, Lübeck, Germany); Department CIBIO, University of Trento, Trento, Italy

Metabolic dysfunction Associated Steatotic Liver Disease (MASLD), formerly known as NAFLD, is a major health concern and is the most common liver disease worldwide. The link between gut microbiota and liver is bidirectional, however, it is not yet fully understood, particularly in relation to the development of liver disease. Existing studies have reported the association between members of the human gut microbiome and MASLD development, however these studies did not dissect the effect of Type-2 diabetes (T2D), which is a major MASLD comorbidity, from the MASLD effect on the microbiome. Therefore, we conducted an observational case-control study on a sub-cohort from the Cooperative Health Research in South Tyrol (CHRIS) cohort, aiming to disentangle the microbiota association with MASLD from T2D. In total, 111 participants of T2D- and MASLD-, 56 participants of T2D+ and MASLD-, 68 participants of T2D- and MASLD+; and 115 participants of T2D+ and MASLD+ were surveyed via shotgun metagenomic sequencing, plasma metabolomics, complete blood counts, and dietary questionnaires. As results, we identified 17 and 10 species-level, and 155 and 240 metabolic pathways-level associations with MASLD and T2D, respectively (FDR 0.2). A machine learning classifier trained on joint taxonomic, functional, and plasma metabolomics profiles was able to stratify the participants according to the disease status at AUC ~ 0.8. The results presented here open a door on the microbiome potential for Type-2 diabetes independent MASLD screening technologies.

No. 15

Efficiency of fecal microbiome data layers in classification of depression

Masa Primozic[1], Leon Deutsch[2], Bostjan Murovec[3], Blaz Stres[2456]

[1]Faculty of Education, University of Ljubljana, Ljubljana, Slovenia; [2]Biotechnical Faculty, University of Ljubljana, Ljubljana, Slovenia; [3]Faculty of Electrical Engineering, University of Ljubljana, Ljubljana, Slovenia; [4]Faculty of Civil and Geodetic Engineering, University of Ljubljana, Ljubljana, Slovenia; [5]Institute Jožef Stefan, Ljubljana, Slovenia; [6]National Institute of Chemistry, Ljubljana, Slovenia

Depression is the most common mental disorder in the world. However, the methods used for its diagnosis are still problematic and inadequate, and can be improved with non-invasive, quantitative tests based on biomarkers. While numerous biomarkers have already been researched, a potentially promising target has remained overlooked. Gut microbiome (GM) is one of the most important parts of the gut-brain axis and represents information rich subsystem, but no single biomarker could be identified. To explore the efficiency of fecal microbiome data layers in classification of depression a metagenomics subset of the Flemish Gut Flora Project was acquired (n=150) including metadata (age, sex, BMI, BSS, RAND), 80 with depression and 70 healthy controls. The sequencing data was pre-processed with our metaBakery implementation of bioBakery (KneadData, HUMAnN3; MetaPhlAn3; mothur; databases) at HPC Vega to generate information layers: taxonomy (Bacteria, Archaea, Fungi, Protozoa, DNA Viruses), diversity, functional genes, enzyme reactions, metabolic pathways, intestinal metabolites. The importance of features within these layers for differentiation between healthy individuals and those with depression were explored and utilized to build, optimize and validate classification models in Orange and JADBio. While results from individual GM layers were promising, the classification model utilizing assembled information from all GM layers yielded >90% classification accuracy establishing the benefits of using gut microbiome information in classification of psychiatric disorders.

No. 16

Environmental enrichment reduces anxiety-like behaviour and changes the microbial community composition of mice

Isabella Faimann, Eva Tatzl, Christine Moissl-Eichinger, Florian Reichmann

Otto Loewi Research Center, Division of Pharmacology, Medical University Graz

Environmental enrichment (EE) is a husbandry procedure known to improve animal welfare. It has beneficial effects on behavior and promotes stress resilience. However, the precise mechanisms underlying the translation of environmental stimulation into changes of brain function are currently unknown. The aim of the current work is therefore to investigate whether the beneficial effects of EE are mediated via gut microbiota and the microbiota-gut-brain axis, a bidirectional communication pathway between visceral organs and the brain.

Four-week-old C57BL6/J mice were co-housed for four weeks in order to homogenize their microbiome. Afterwards the mice were split into EE and standard environment (SE) housing.

Following differential housing, the open field test (OFT) was conducted in both species to assess anxiety-like behaviour. In addition, mouse boli were analyzed with 16S Illumina sequencing in order to determine differences in the intestinal microbial community composition.

In the OFT we found that EE-housed animals entered the central zone of the test arena significantly more often than animals in SE indicating reduced anxiety. The 16S sequencing showed unique microbiome signatures developing over time in SE and EE. Furthermore, we found significant changes in the microbiome of female EE mice compared to SE-housed females after 9 weeks of differential housing.

In conclusion, we found that EE reduces anxiety-like behaviour and changes the microbiome, effects that might be interrelated. Therefore, future experiments should assess causality by using antibiotic models and/or stool transplantation experiments.

No. 17

Infant Microbiome Development: Exploring the Role of Archaea and Anaerobes

Charlotte Neumann, Pei Yee Who, Tanja Kobal, Manuela Pausan, Evelyn Jantscher-Krenn, Christine Moissl-Eichinger

Department of Hygiene, Microbiology and Environmental Medicine, Medical University of Graz, Graz, Austria

Introduction: The establishment and maturation of the infant microbiome play a crucial role in shaping lifelong health outcomes. While extensive research has focused on bacterial communities, the contributions of archaea and anaerobes remain relatively underexplored. This study aims to unravel the dynamics of infant microbiome development by employing a comprehensive analysis of oral and stool samples from a cohort of 30 infants.

Material and Methods: We recruited a diverse cohort of 30 infants and collected both oral and stool samples regularly, starting from the neonatal period and extending monthly throughout the first year of life. Genomic techniques, including amplicon sequencing and metagenomics analysis, were employed to characterize microbial communities. Next to bacteria, special attention was given to archaeal and anaerobic populations, employing targeted approaches to elucidate their roles in the developing infant microbiome. Our methodology aimed to provide a comprehensive understanding of the microbial diversity, abundance, and transition within the upper and lower intestinal tract.

Results: Preliminary results indicate dynamic shifts in the composition of infant oral and stool microbiota over the first year of life. While bacterial communities display expected patterns, the analysis of archaea and anaerobes has uncovered intriguing nuances in their contributions to microbiome development. The monthly sampling strategy has enabled the identification of critical milestones and potential factors influencing the establishment of these microbial populations.

No. 18

Inhibiting Toll-like receptor 2 dampens liver degeneration in aging mice

Haktan Övül Bozkir, Annette Brandt, Patricia De Oliveira Prada, Anja Baumann, Julia Jelleschitz, Annika Höhn, Ina Bergheim

University of Vienna, Vienna, Austria

Background: Even the natural course of healthy aging has been shown to be associated with changes of intestinal microbiota composition and impaired intestinal barrier function subsequently leading to an increased translocation of Toll-like receptor (TLR) ligands like lipoteichoic acid and bacterial endotoxin. Here, we determined if bacterial toxins of gram positive bacteria like lipoteichoic acid and the TLR2 signaling cascade are critical in the onset of the so called ´inflammaging´.

Methods: Seventeen months old male C57BL/6 mice showing an impaired intestinal barrier function as assessed by measuring bacterial endotoxin in peripheral blood when compared to young male C57BL/6 mice were orally treated either with the TLR2 inhibitor ortho-vanillin (60 mg/kg BW) dissolved in drinking water or plain water for four months. Markers of senescence, liver damage, inflammation and fibrosis were determined in blood and liver tissue.

Results: Markers of senescence were significantly lower in aging mice treated with the TLR2 inhibitor compared to age-matched plain water controls. In line with these findings, TLR2 inhibitor-treated mice were also markedly protected from aging associated hepatic inflammation as assessed by scoring liver histology. Moreover, markers of hepatic fibrosis, like hepatic α-smooth muscle actin mRNA expression and sirius red positive areas in liver sections were significantly reduced in TLR2 inhibitor-treated mice compared to age-matched controls. Conclusion: Taken together, our data so far suggest that TLR2 ligands and signaling cascades may be critical in aging-associated liver decline in healthy aging. (funded by FWF and DFG)

No. 19

Investigating the essential role of *Burkholderia pseudomallei* ExoU in lytic cell death and intracellular bacterial loads on epithelial cells and macrophages

Linh K Nguyen, Andrea Zauner, Sabine Saiger, Sabine Lichtenegger*, Ivo Steinmetz*

Diagnostic and Research Institute of Hygiene, Microbiology and Environmental Medicine - Medical University of Graz, Neue Stiftingtalstraße 6, 8010 Graz, Austria

Melioidosis, caused by the Gram-negative pathogen *Burkholderia pseudomallei*, poses a severe threat with an estimated 165,000 cases and 89,000 fatalities per year. Due to intrinsic antibiotic resistance and lacking vaccines, alternative treatments are urgently needed. Gram-negative pathogens, including *B. pseudomallei*, use type III secretion systems (T3SSs) to deliver effectors into host cells. One of these effectors is ExoU, a phospholipase, which is an important virulence factor in e.g. *Pseudomonas aeruginosa* where it induces massive lytic cell death. Despite the importance of ExoU for other pathogens, its function in *B. pseudomallei* remains elusive. Our investigations therefore

aim to elucidate the impact of *B. pseudomallei* ExoU on bacterial survival and lytic cell death. In our study, we identified two ExoU genes in *B. pseudomallei*. Using conjugation, we created a knock-out strain, which lacks both genes. We infected primary murine and human macrophages, and the lung epithelial cell line A549 with the ExoU mutant and the isogenic wild type strain. LDH release and bacterial survival were analyzed at 0, 3, and 24 hours post-infection. The knockout of ExoU in *B. pseudomallei* exhibited no significant impact on both murine and human macrophages. However, when infecting A549 cells a notable reduction in lytic cell death and intracellular bacterial loads was observed for the mutant compared to the wild type. While our data do not predict an essential role for ExoU in macrophages, its impact on epithelial cells suggests cell-type-specific responses. In future investigations, we will explore whether ExoU provides lipids for bacterial metabolism or whether the observed reduction in lytic cell death and intracellular bacterial loads depends on lipid mediators and/or modulation of cell death induction.

No. 20

Microbiota, energy, and macronutrient restrictions as modulators of bile acids metabolism

András Gregor, Joana Séneca, Petra Pjevac, Arturo Auñon-Lopez, Marc Pignitter, Kalina Duszka

Center of Translational Medicine, Warsaw University of Life Sciences, Warsaw, Poland

Our research focuses on nutrition and microbiota-dependent regulation of bile acids (BA) metabolism. We found that in calorie-restricted (CR) mice, BA concentration increases in the liver, intestine, and plasma, whereas it is reduced in the feces, suggesting enhanced synthesis, release, and reuptake. Similar upregulation of liver and ileum BAs concentration was found in animals submitted to intermittent fasting and fasting-mimicking diet, but not upon short-lasting restrictions, like overnight fasting. Moreover, carbohydrate restriction led to an increase in BA levels in the liver, while the increase in carbohydrate and fat amount in the diet of CR mice neutralized BA-related CR phenotype. Also, ketogenic diet tended to increase levels of BAs in the liver but not in the ileum.

Next, we have proven that the deconjugation of taurine following the release of BAs in the intestine depends on the type of cage bedding with which mice are housed and is associated with the intake of cage bedding-derived fiber. Removal of cage bedding neutralized CR phenotype associated with taurine levels, BAs deconjugation, and fecal microbiota composition. Microbiota transplant from CR mice housed with bedding stimulated BAs deconjugation. Application of bile salt hydrolase (BSH) inhibitor prevented the increase in free taurine concentration while increasing taurine-conjugated BA levels in the intestine. Ad libitum consumption of diets high in fiber increased the levels of taurine but did not affect the production and secretion of BAs. Recently, we identified inulin, B-glucan, and psyllium as the types of fiber with the strongest BSH-stimulating properties, and we analyzed bacteria abundance to assign corresponding BSH providers.

We conclude that energy and carbohydrate restriction is required to stimulate BAs production, energy restriction triggers BAs secretion and reuptake, while ingestion of fiber stimulates the capacity of microbiota to deconjugate BAs.

No. 21

Nitrogen recycling by the gut microbiota in sarcopenia

Rosa Haller, Olha Hazia, Angela Horvath, Vanessa Stadlbauer

Gastroenterology and Hepatology, Medical University of Graz, Graz, Austria; CBmed Gmbh, Graz, Austria

Liver cirrhosis is one of the most common causes of death and more than half of the patients suffer from sarcopenia. Due to the lack of therapy, hibernating mammals are studied in this context as several species do not experience muscle loss during hibernation. It was shown that in the gut microbiome of hibernating squirrels, urease producing bacteria have an increased abundance. The nitrogen yield is then incorporated into their proteins. It is unknown if nitrogen recycling is disturbed in sarcopenia. We aim to gain a better understanding of the role nitrogen recycling in sarcopenia.

Therefore, we predicted the functional profiles of microbial communities based on 16S rRNA (Tax4Fun2) of 152 patients with and without sarcopenia and extracted the predicted abundance of urease subunits (alpha, beta and gamma). Additionally, we conducted a systematic literature search to identify urease producing bacteria.

In cirrhotics (n=96), patients without sarcopenia had increased abundance of urease subunit alpha (p=0.045), beta (p=0.042) and gamma (p=0.042). In women (n=49) the subunits alpha (p=0.037), beta (p=0.044) and gamma (p=0.044) were increased in patients without sarcopenia. There was no difference in urease abundance between cirrhotics and non cirrhotics nor between men or women.

The systematic literature search yielded 120 taxa, of which 35 taxa could be extracted from the dataset and tested for their abundance in sarcopenia. Bacteroides fragilis was more abundant in cirrhotic patients with sarcopenia (p=0.026). Micrococcaceae was more abundant in men (n=103) with sarcopenia (p = 0.046). Clostridiacae 1 was increased in non-cirrhotic patients without sarcopenia (p=0.02).

We could show that there are changes in the nitrogen recycling between patients with sarcopenia and without sarcopenia. The effects observed depend on the underlying disease and on gender. Further research is required to understand the effect of the gut microbiota on muscle health.

No. 22

Probiotics via the gut microbiota-lung axis reduce the duration of respiratory tract infections in older people

Maja Strauss[1], Dušanka Mičetić-Turk[2], Mateja Lorber[1], Maja Šikić Pogačar[1,2], Anton Koželj[1], Sabina Fijan[1]

University of Maribor, Faculty of Health Sciences, Maribor, Slovenia

Introduction: Respiratory infections are more common in the autumn and winter seasons. Various agents can cause respiratory infections. Respiratory infections include infections of the upper and lower respiratory tract. Colds and flu are the most common, whilst

pneumonia causes the greatest mortality, especially in the older population. Other common respiratory diseases are nose, throat, trachea, and lung infections. In adults they are associated with absence from work and high treatment costs. It is well-known that respiratory tract infections are associated with an intestinal microbiota dysbiosis along with the upper respiratory tract microbiota in older people. The aim of our double-blind randomised clinical study was to determine if OMNi-BiOTiC® Active reduces the duration of upper respiratory tract infections.

Methods: The study included 95 older people randomised into the intervention and control group. The test group received a probiotic drink containing 11 probiotic strains twice a day and the control group received placebo for 3 months.

Results and discussion: The duration of upper respiratory tract infections was statistically significantly different between the two groups (p=0.011). Participants taking the probiotics had a mean duration of illness of 3.1±1.6 days, while participants receiving placebo had symptoms for a mean of 6.0±3.8 days (p=0.011). Current evidence shows that probiotics can influence the gut microbiota-lung axis by lowering the duration of acute upper respiratory tract infections in older people. More high quality large-scale properly controlled clinical studies are warranted.

No. 23

Skin microbiome dynamics as predictor and pathogenesis mechanism for severe radiodermatitis in breast cancer patients

Avidan U. Neumann, Claudia Hülpüsch, Matthias Reiger, Julius Fischer, Amedeo de Tomassi, Gertrud Hammel, Carina Gülzow, Megan Fleming, Hendrik Dapper, Michael Mayinger, Marco Vogel, Christina Ertl, Stephanie E. Combs, Claudia Traidl-Hoffmann, Kai Borm

Institute of Environmental Medicine, Helmholtz Munich, Augsburg, Germany; Environmental Medicine, Faculty of Medicine, University of Augsburg, Augsburg, Germany.

Radiodermatitis is commonly observed during radiotherapy in post-surgery breast cancer patients. However, the factors associated with severe radiodermatitis, which poses important clinical complications, are not currently understood. Here we conducted a longitudinal pilot study with 20 women undergoing radiotherapy to elucidate the role of skin microbiome and skin physiology in the development of radiodermatitis. Strikingly, 16S sequencing revealed that low (<5%) colonization with commensal skin bacteria (Staphylococcus epidermidis, Staphylococcus hominis, Cutibacterium acnes) at baseline, in both the affected and unaffected breasts, was predictive of the development of severe radiodermatitis with 100% accuracy. Commensal bacteria relative abundance on the skin was significantly inversely correlated with the skin pH, but the latter was not significantly predictive of severity. Interestingly, only in the same patients with severe radiodermatitis, qPCR bacterial quantification revealed a general non-species-specific overgrowth of skin bacterial load, only in the affected breast, after initiation of radiotherapy but prior to the onset of severe symptoms. Subsequently, again in same severe patients, the abundance of commensal bacteria was increased, coinciding with a decline in total bacterial load. These findings indicate a potential skin microbiota related mechanism for the pathogenesis of

severe radiodermatitis and can be used as a predictive biomarker for personalized treatment of radiodermatitis.

No. 24

Spatially resolved transcriptomic profiling of severe covid-19

Martin Zacharias, Moritz Schütte, Marija Durdevic, Bernd Timmermann, Gregor Gorkiewicz

Institute of Pathology, Medical University of Graz, Graz, Austria

The pathophysiology of severe covid-19 is still poorly understood. Autopsy studies revealed remarkable morphological heterogeneity within affected lung tissue, however, less is known about the corresponding molecular heterogeneity and how it affects pathogenesis. For that purpose, we used the 10x Genomics Visium Spatial Gene Expression for FFPE workflow in a well-characterized autopsy cohort of severe covid-19 cases and correlated the spatial transcriptomic landscape with previous outputs, including bulk RNA sequencing. Through this approach, we delineated spatially distinct molecular signatures associated with host-pathogen interaction, immunity, as well as tissue damage and repair. Our study provides a comprehensive framework for elucidating the spatial heterogeneity of host responses to SARS-CoV-2 infection within the lung microenvironment, offering novel insights on the pathophysiology of severe covid-19.

No. 25

The outer membrane vesicle (OMV)-associated protein ObfA facilitates biofilm formation of Vibrio cholerae

Stephan P. Ebenberger, Fatih Cakar, Yi-Chi Chen, Adina Schulze, Leo Eberl, Stefan Schild

Institute of Molecular Biosciences, University of Graz, Graz, Austria

The facultative human pathogen Vibrio cholerae is a potent colonizer of the human intestinal tract and the causative agent of the severe diarrheal disease cholera. Between epidemic outbreaks, V. cholerae persists in the aquatic reservoirs in the biofilm mode, which enhances environmental fitness and host transition. Similar to other Gram-negative bacterial pathogens, V. cholerae releases outer membrane vesicles (OMVs) that have so far been characterized for their role during in vivo colonization. In this study, we analyzed the impact of V. cholerae OMVs on biofilm formation being a pivotal feature for ex vivo survival. In contrast to OMVs from planktonic cultures, our results show that biofilm-derived OMVs facilitate V. cholerae biofilm formation, which depends on a proteinaceous factor. Subsequent comparative proteomic analyses revealed distinct differences between planktonic- and biofilm-derived OMVs. We identified a previously uncharacterized outer membrane protein as an abundant component of biofilm-derived OMVs, which was shown to enhance OMV-dependent biofilm maturation. The protein was consequently named as OMV-associated biofilm facilitating protein A, i.e. ObfA. Comprehensive molecular studies identify ObfA as a negative modulator of HapR activity. HapR is a key transcriptional regulator of the V. cholerae quorum-sensing cascade and a potent repressor of biofilm formation. Surprisingly, our results suggest that ObfA does not affect HapR via the quorum-

sensing system, but through the Csr-cascade by deregulation of small regulatory RNAs. In summary, this study elucidates an intraspecies OMV-based communication in V. cholerae that influence biofilm formation via a novel pathway involving HapR, Csr-cascade and the OMV-associated protein ObfA.

No. 26

Toll-like receptor 2 is a trigger of `inflammaging´ and aging-associated liver decline in healthy aging mice

Annette Brandt, Haktan Övül Bozkir, Patricia De Oliveira Prada, Anja Baumann, Julia Jelleschitz, Annika Höhn, Ina Bergheim

University of Vienna, Vienna, Austria

Background: Studies suggest that even healthy aging in mice and humans is associated with alterations of intestinal microbiota composition in both small and large intestine, a decline in intestinal barrier integrity and increased translocation of bacterial toxins including ligands of Toll-like receptor 2 and TLR4. The role of this increased translocation of bacterial toxins in the development of aging-associated decline has not yet been understood. Here, we assessed the effects of a genetic depletion of TLR2 on the natural course of aging and especially the development of aging-associated liver degeneration.

Methods: Male C57BL/6 and TLR2 Knockout (TLR2 KO, B6.129-Tlr2tm1Kir/J) mice were housed in groups with free access to standard diet and tap water ad libitum. At the age of 4 and 20 months blood and liver were collected and snap-frozen or fixed in neutral-buffered formalin.

Results: Markers of senescence were markedly higher in 20 months old C57BL/6 mice compared to young animals while similar differences were not found after comparing young and old TLR2 KO mice. In line with these findings, 20 months old TLR2 KO mice were significantly protected from the development of aging-associated hepatic inflammation and fibrosis as assessed by liver histology. Moreover, markers of inflammation, like interleukin 1ß mRNA expression and number of neutrophil granulocytes were significantly lower in livers of 20 months old TLR2 KO mice compared to age-matched C57BL/6 mice.

Conclusion: So far, our data suggest that the loss of TLR2 is related to diminished `inflammaging´ and liver decline. (funded by FWF and DFG)

No. 27

Vibrio cholerae's ToxRS Bile Sensing System

Nina Gubensäk, T. Sagmeister, C. Buhlheller, B. D. Geronimo, G. E. Wagner, L. Petrowitsch, M. Gräwert, M. Rotzinger, T. M. I. Berger, J. Schäfer, I. Usón, J. Reidl, P. A. Sánchez-Murcia, K. Zangger, T. Pavkov-Keller

Institute of Molecular Biosciences, University of Graz, Austria

The since 1960 ongoing seventh pandemic of the diarrheal cholera disease is caused by the Gram-negative bacterium Vibrio cholerae. Its environmental persistence provoking recurring sudden outbreaks is enabled by V. cholerae’s rapid adaption to changing

environments involving sensory proteins like ToxR and ToxS. Located at the inner membrane, ToxR and ToxS react to environmental stimuli like bile acid, thereby inducing survival strategies e.g. bile resistance and virulence regulation. The presented crystal structure of the sensory domains of ToxR and ToxS in combination with multiple bile acid interaction studies, reveals that a bile binding pocket of ToxS is only properly folded upon binding to ToxR. Our data proposes an interdependent functionality between ToxR transcriptional activity and ToxS sensory function. These findings support the previously suggested link between ToxRS and VtrAC-like co-component systems. Besides VtrAC, ToxRS is now the only experimentally determined structure within this recently defined superfamily, further emphasizing its significance.

In-depth analysis of the ToxRS complex reveals its remarkable conservation across various Vibrio species, underlining the significance of conserved residues in the ToxS barrel and the more diverse ToxR sensory domain. Unravelling the intricate mechanisms governing ToxRS's environmental sensing capabilities, provides a promising tool for disruption of this vital interaction, ultimately inhibiting Vibrio's survival and virulence. Our findings hold far-reaching implications for all Vibrio strains that rely on the ToxRS system as a shared sensory cornerstone for adapting to their surroundings.

No. 28

Impact of Oral Human Trefoil Factor 3 in DSS-Induced Colitis, a Mouse Model of Ulcerative Colitis

Lara Valentina Ofner, Laura Schmidt, Gene Hopping, Markus Muttenthaler, Aitak Farzi

Lehrstuhl für Pharmakologie; Medizinische Universität Graz; Neue Stiftingtalstraße 6; 8010 Graz, Austria

Abstract not available.

MECHANISTIC STUDIES

No. 29

Adaptive evolution of gut microbiomes and host specialization in insects

Polpass Arul Jose and Cassandra G. Extavour

Department of Molecular and Cellular Biology, Harvard University, Cambridge, MA, USA

Host specialization is an important process in the organismal diversification. Microorganisms associated with insects are often critical for the host insect's survival and reproductive fitness, and encounter the same environmental changes as the host insect during the course of specialization. However, the microbial molecular mechanisms associated in turn with host insect's specialization on different substrates, remain unclear. Our project is aimed to characterize host specialization processes through comparative hologenome and developmental studies on selected Drosophila species that show different levels of specialization on distinct substrates in the wild. To study the potential microbial impacts on the process of animal host specialization, we plan to experimentally evolve laboratory populations of D. melanogaster harboring defined microbial communities with different adaptive potential to noni fruit. We expect that the results of these experiments will help us to identify significant causal links between the microbiome and adaptive mechanisms in these insects. In my talk, I will describe unpublished results from this study and few former studies on Tephritidae which shed light on adaptive evolution of gut microbiomes and host specialization.

No. 30

Caenorhabditis elegans as a non vertebrate colonization model for bacterial pathogens

Cornelia List, Andreas Grutsch, Claudia Radler, Fatih Cakar, Franz G. Zingl,..., Stefan Schild, Kristina Schild-Prüfert

Institute of Molecular Biosciences, Department of Microbiology, University of Graz, Austria

The water-borne diarrheal disease cholera is caused by the bacterium Vibrio cholerae. The facultative human pathogen persists as a natural inhabitant in the aquatic ecosystem between the outbreaks. In contrast to the human host, V. cholerae requires a different set of genes to survive in an environment with constant threat by predatory protozoa and nematodes. We successfully adapted a genetic reporter technology and identified more than 100 genes activated by V. cholerae upon exposure to the bacteria-grazing nematode Caenorhabditis elegans. This allowed us a first glimps into the adaptational strategies of V. cholerae against such a natural predator.

Phenotypic characterization of several genes revealed, that the mannose-sensitive hemagglutinin IV pilus (MSHA) was significantly induced upon exposure to the nematode. MSHA could be shown to be critical for the attachment of V. cholerae in the pharynx of the C. elegans and initiate colonization, which resulted in growth retardation and developmental delay of the worm. This implicates a strategy of V. cholerae to induce its

fitness upon contact with this bacteria-grazing nematodes. The herein established assays also highlight the potential of C. elegans as a non-vertrebrate colonization and virulence model for bacterial pathogens.

No. 31

Elucidating diversity and roles of endohyphal bacteria in a filamentous fungus model

Wallner Adrian, Kiss Tomáš , Antonielli Livio, Pastar Milica, Compant Stéphane

AIT Austrian Institute of Technology GmbH

Fungi, unlike other multicellular eukaryotes, have long been considered “terminal” organisms, colonizers of larger life but not able to host independent biological units themselves. This paradigm has been refuted since, and several examples of endohyphal associations with bacteria are described. Still, data regarding the diversity of fungi-hosted bacteria and their functions in fungal biology is scarce. The recently started HyphoBiome project aims at shining light on these questions through the study of the fungal grapevine pathogen, Fomitiporia mediterranea. Microscopy observations of this Basidiomycete reveal complex communities of endosymbiotic bacteria, further confirmed by amplicon sequencing and paving the way for promising subsequent analyses on the mechanisms and roles of these associations.

No. 32

Single-cell mass distributions as a tool to understand bacterial growth

Benjamin R. K. Roller, Cathrine Hellerschmied, Yanqi Wu, Teemu P. Miettinen, Annika L. Gomez, Scott R. Manalis, Martin F. Polz

Division of Microbial Ecology, Centre for Microbiology and Environmental Systems Science, University of Vienna, Vienna, Austria

Optical density is a proxy of total biomass concentration and is commonly used for measuring the growth of bacterial cultures. However, there is a misconception that exponential optical density growth is equivalent to steady-state population growth. Many cells comprise a culture and individuals can differ from one another. Hallmarks of steady-state population growth are stable frequency distributions of cellular properties over time, something total biomass growth alone cannot quantify. Using single-cell mass sensors paired with optical density measurements, we explore when steady-state population growth prevails in typical batch cultures. We find the average cell mass of Escherichia coli and Vibrio cyclitrophicus growing in several media increases by 0.5–1 orders of magnitude within a few hours of inoculation, and that time-invariant mass distributions are only achieved for short periods when cultures are inoculated with low initial biomass concentrations from overnight cultures. These species achieve an effective steady-state after approximately 2.5–4 total biomass doublings in rich media, which can be decomposed to 1 doubling of cell number and 1.5–3 doublings of average cell mass. We also show that typical batch cultures in rich media depart steady-state early in their growth curves at low

cell and biomass concentrations. Achieving steadystate population growth in batch culture is a delicate balancing act, so we provide general guidance for commonly used rich media. Quantifying single-cell mass outside of steady-state population growth is an important first step toward understanding how microbes grow in their natural context, where fluctuations pervade at the scale of individuals. I will outline how we can take the next step to estimate growth rates in complex microbiomes using single-cell mass measurements coupled with quantitative growth experiments.

METHODS

No. 33

De-biasing microbiome sequencing data: bacterial morphology-based correction of extraction bias and correlates of chimera formation

Luise Rauer, Amedeo De Tomassi, Christian L. Müller, Claudia Hülpüsch, Claudia Traidl-Hoffmann, Matthias Reiger, Avidan U. Neumann

Environmental Medicine, Faculty of Medicine, University of Augsburg, Augsburg, Germany

Introduction: Microbiome amplicon sequencing data are distorted by multiple protocol-dependent biases from bacterial DNA extraction, contamination, sequence errors, and chimeras, hindering clinical microbiome applications. In particular, extraction bias is a major confounder in sequencing-based microbiome analyses, with no correction method available to date. Here, we suggest using mock community controls to computationally correct extraction bias based on bacterial morphological properties.

Methods: We compared dilution series of 3 cell mock communities with an even or staggered composition. DNA was extracted with 8 different extraction protocols (2 buffers, 2 extraction kits, 2 lysis conditions). Extracted DNA was sequenced (V1-V3 16S rRNA gene) together with corresponding DNA mocks.

Results: Microbiome composition was significantly different between extraction kits and lysis conditions, but not between buffers. Independent of the extraction protocol, chimera formation increased with high input cell number. Contaminants originated mostly from buffers, and considerable cross-contamination was observed in low-input samples. Comparing the microbiome composition of the cell mocks to corresponding DNA mocks revealed taxon-specific protocol-dependent extraction bias. Strikingly, this extraction bias per species was predictable by bacterial cell morphology. Morphology-based computational correction of extraction bias significantly improved resulting compositions when applied to different samples, even with different taxa.

Conclusions: Our results indicate that higher DNA density increases chimera formation during PCR amplification. Furthermore, we show that computational correction of extraction bias is feasible based on bacterial cell morphology, thus constituting an important step towards overcoming protocol biases in microbiome analysis.

No. 34

Genotyping scheme for monophyletic Klebsiella oxytoca Species Complex

Johanna Dabernig-Heinz[1], Gabriel E. Wagner[1], Sabine Kienesberger[2,3,4], Ivo Steinmetz[1], Eva Leitner[1]

Diagnostic & Research Institute of Hygiene, Microbiology and Environmental Medicine, Medical University of Graz, Graz, Austria

The environmental bacterium Klebsiella oxytoca displays an alarming increase of antibiotic resistant strains, that recently caused outbreaks in intensive care units. Due to its prevalence in the environment and opportunistic presence in the human microbiome, molecular surveillance (including resistance marker screening) and high-resolution cluster analysis is of high relevance. Furthermore, K. oxytoca previously described in studies is rather a species complex (KoSC) than a single species comprising at least 6 closely related species that are not easily differentiated by standard typing methods. To reach a discriminatory power high enough to identify clusters within these species whole genome sequencing is necessary. The resolution is achievable with core genome MLST extending typing of a few housekeeping genes to thousands of core genome genes. Opposed to SNP calling approaches cgMLST is highly standardized and provides a nomenclature enabling cross laboratory reproducibility and data exchange. We established a cgMLST scheme not only capable of resolving the KoSC species but also producing reliable and consistent results for published outbreaks. We also validated resistance markers against known resistance gene patterns and successfully linked genetic results to phenotypically confirmed toxic strains carrying the til gene cluster. In conclusion, our novel cgMLST enables highly reproducible typing of 4 different species of the KoSC by introducing a common database for allele calls, and thus facilitates molecular surveillance and cluster investigations.

No. 35

Gut Microbiome Response Chip (GMRC): differences between the fecal surface and core microbiome activities in NCDs

Maša Ošlak[1], Boštjan Murovec[2], Blaž Stres[1,3,4,5]

University of Ljubljana, Biotechnical Faculty, Department of Animal Science, Jamnikarjeva ulica 101, SI-1000 Ljubljana, Slovenia

Human gut microbiome is inherently linked to the vast amount of chemical and physical signatures that change in response to variations in the thermodynamic conditions of the environment. Mechanistic understanding of the complex interplay between starting conditions, microbiome physiological responses and the resulting chemical space (metabolomic signatures) in real time is lacking. To fill this gap, we present the first version of our newly developed Gut Microbiome Response Chip (GMRC). Fecal surface and core samples were exposed to chemical challenges (e.g. organic acids, sugars, fats, mucus) and data were recorded at a rate of 1 readout per minute for up to 48 h, including the analysis of the chemical stability of the activity reporters. Basal metabolic activity and ten chemical compounds were utilized per challenge in four replicates per fecal specimen. In-house routines were utilized to reorganize and deconvolute the data into biologically relevant descriptive summaries such as lag phases, rates of change, attained levels of signals, signal

to noise ratios and others, next to curve fits, data restructuring and other derivatives, making them highly amenable to machine learning (numerous algorithms, hyperparameter search, reporting statistics, biomarker constellation search). The results show clear, reproducible and significant differences in microbiome responses to chemical challenges characteristic of individual subjects. This testifies as a proof of concept that the tool provides sufficient resolution and reproducibility to serve as a starting point for precision medicine of the Non-Communicable Diseases (NCDs) at the level of the individual microbiome responses to chemical challenges corresponding to environmental chemical changes.

No. 36

HLA-II immunopeptidomics and deep learning reveal features of antigenicity to inform antigen discovery

Martin Stražar, Jihye Park, Jennifer G. Abelin, Hannah B. Taylor, Thomas K. Pedersen, Damian R. Plichta, Yuan-Mao Hung, Eric M. Brown, Basak Eraslan, Kayla Ortiz, Karl R. Clauser Steve A Carr, Daniel B. Graham, Ramnik J. Xavier

Broad Institute of MIT and Harvard, Cambridge, MA, USA

Commensal microbes at mucosal sites promote education and maturation of the adaptive immune systems. Here, CD4+ T cell responses directed against specific peptide antigens displayed by human leukocyte antigen class-II (HLA-II) on antigen presenting cells. Rules of antigen presentation that underpin recognition of self- and non-self are key to understanding immune responses.

Here, we employ monoallelic immunopeptidomics to retrieve unique peptides presented by HLA-II heterodimers in vivo, profiling major alleles across diverse ancestries. This expansive dataset revealed novel binding rules and properties of naturally presented peptides. Using neural networks and ensemble learning, we develop Context-Aware Predictor of T cell Antigens (CAPTAn). Improving on existing models, we predict peptide ligands from their whole protein sequence, increasing the accuracy of retrieval from large proteomes. Our method leverages both amino acid binding preferences and contextual features related to antigen structure, trafficking, and localization, which are learned in annotation-agnostic manner.

Integration of machine learning, microbial genomics and metatranscriptomics predicts prevalent antigens from the human microbiome and SARS-CoV-2 which are experimentally validated through cytokine assays and clonally expanded TCRs from CD4+ T cells. Exposing features of antigenicity through CAPTAn enabled discovery of bacterial and viral antigens that drive functionally heterogeneous T cell responses in humans.

No. 37

Isolation of arabinogalactan-stimulated cells in the human gut microbiome using Raman-activated cell sorting

Sanaz Khadem[1], Hamid Rasoulimehrabani[1], Georgi Nikolov[1], Rebecca Gallo[1], Miriam Philipp and David Berry[1,2]

[1]Center for Microbiology and Environmental Systems Science, Department of Microbiology and Ecosystem Science, Division of Microbial Ecology, University of Vienna, Austria
[2] Joint Microbiome Facility of the Medical University of Vienna and the University of Vienna, Austria

Prebiotics as non-digestible dietary components that are selectively fermented by the gut microbiota. They thereby influence its composition and activity, ultimately contributing to human health. In this study, we evaluated stimulation of the gut microbiota by the candidate prebiotic arabinogalactan (AG). Thus far, standard microbiological isolation and cultivation approaches have primarily identified *Bacteroides* and *Bifidobacterium* species as AG degraders. However, these approaches may bias against isolation of certain AG-degrading species as well as cross-feeding organisms.

Here, we employed heavy water activity labeling followed by Raman microspectroscopy and Raman-activated cell sorting (RACS) to directly isolate and culture cells from the gut microbiota that are stimulated by AG addition. Specifically, we performed short (6 h) incubations of fresh human fecal samples from 10 donors with AG and heavy water as a cellular activity marker. Subsequently, we confirmed cellular metabolic activity through the detection of deuterium incorporation with Raman microspectroscopy and sorted active cells with high-throughput automated RACS.

With this approach, we isolated 98 strains from 10 genera. Almost half of the isolates belonged to the genus *Bifidobacterium* (46 / 98 isolates), but also included members of the genera *Alistipes, Bacteroides, Collinsella, Eggerthella, Faecalibacterium, Parabacteroides, Phascolarctobacterium Phocaeicola*, and *Ruminococcus*, some of which may be novel AG utilizers. We are currently evaluating the ability of isolates to degrade AG or to engage in cross-feeding or indirect stimulation during AG degradation.

With this approach, we were able to successfully overcome some of the previous methodological challenges in exploring prebiotic-stimulated gut microbes. This work deepens our insights into the role of AG in shaping the composition and activity of the gut microbiota.

No. 38

Mass-spectrometry based lipidomics to uncover membrane structural features, mediating host-pathogen interactions

Jacqueline Wolf, Ina Schmidt, Björn Krenz, Viktoria Weinberger, Christine Moissl-Eichinger and Meina Neumann-Schaal

Leibniz Institute DSMZ-German Collection of Microorganisms and Cell Cultures GmbH, Inhoffenstraße 7 B, 38124 Braunschweig, Germany

The cell envelope of pathogens is a key player at various stages of host-pathogen interactions, including pathogen docking, intracellular trafficking and phagocytosis. Structurally fatty acids, glycerolipids or mycolic acids play an important role in pathogen recognition and uptake.

We established different mass-spectrometry based methods to analyse the composition of different lipid classes in bacteria and archaea as well as in virus-infected plant tissue. We developed a laser dissection-based method to successfully isolate nuclei from plants infected with different begomoviruses and analysed their fatty acid profile to identify virus induced changes upon infection. We observed a tendency towards decreased abundance of unsaturated fatty acids in infected nuclei compared to non-infected nuclei. The successful analysis of fatty acids from isolated nuclei provides an essential basis for future investigations of cell compartments.

Methanogenic archaea are an important part of the human gut microbiome and involved in shaping the hosts immune system. As the general architecture of archaeal glycerolipids largely differ from their bacterial counterparts, consisting mainly of glycerol dibiphytanyl glycerol tetraethers, detailed knowledge of their role in the recognition through immune cells is limited. We developed methods to elucidate the structural cell wall components of human associated archaea, in order to identify structures which influences recognition and uptake by immune cells.

The emerging role of lipids not only as structural component of the cell envelope but also as recognition pattern in host-pathogen interactions highlights the need for continuous development of novel approaches for lipid analysis. Our mass-spectrometry based methods for the identification of fatty acids, polar lipids and mycolic acids will provide a lipidomics platform not only for future investigations of microbial membrane structures but also for their role in different host interactions.

No. 39

ML models for colorectal cancer and colorectal adenoma diagnostics from publicly available cohorts based on taxonomical and functional gut microbial metagenomics

Leon Deutsch, Boštjan Murovec, Blaž Stres

Biotechnical Faculty, Department of Animal Sciences, Ljubljana, Slovenia; The NU B.V.m J.H. Oortweg 21, 2333CH Leiden, The Netherlands

In this study, a comparative analysis was conducted on paired read sequences from publicly available datasets, encompassing CRC patients (n=380), colorectal adenoma (CRA) patients (n=110), and a healthy cohort (n=2461). The objective was to develop a robust machine learning model applicable to the general population. Employing our Metabakery pipeline (in preparation), encapsulated in a Singularity image container and executed on the Slovenian supercomputer Vega, each dataset underwent comprehensive processing with tools such as Kneaddata, MetaPhlAn3, Mothur, HUMAnN3, and MelonnPan. For data modeling, the JADBIO autoML method was employed, utilizing a 70:30 split ratio for the datasets, where the first served as a test dataset and the second for model validation. From a pool of 2000 model configurations based on five metrics encompassing taxonomic units, functional genes, enzymatic reactions, and metabolic pathways, models with the highest AUC were selected. No significant differences were observed based on the Shannon index and other diversity metrics. Linear discriminant analysis (LDA) revealed distinctions between groups. Subsequently, an autoML approach was applied to craft models suitable for diagnostic purposes. Most models required only the top ten features from a list of 25 to achieve 90% predictive performance. Our models demonstrated an AUC of approximately 0.8 for all data metrics, excluding metabolites predicted with MelonnPan (AUC=0.621). This outcome implies the potential for daily diagnostics of CRC and CRA based on microbial characteristics, especially when considering diverse healthy cohorts. While these findings underscore the feasibility of constructing robust models from publicly available datasets, it is crucial to underscore that the next pivotal phase involves clinical validation to affirm the accuracy and efficacy of these models.

No. 40

Optimizing in vitro modeling of the human gut: exploring microbial dynamics in intestinal health and disease

Kristina Žukauskaitė, Christian Pacher, Selina Tripolt, Irina Balazs, Angela Horvath, Vanessa Stadlbauer

1. Department of Gastroenterology and Hepatology, Medical University of Graz, Graz, Austria
2. Institute of Biosciences, Life Science Center, Vilnius University, Vilnius, Lithuania

The gut microbiome plays a critical role in our health and disruptions of this ecosystem can contribute to various diseases. This study introduces a novel approach, employing the DASbox® mini bioreactor system and stool samples, to mimic the human gut environment, with the aim of adapting it for studying diseases and various clinical applications.

The stool sample collection procedure was optimized to preserve composition of the donor's microbiome. Stool samples were collected using an anaerobic microbiome collection kit GutAlive® and stored for up to 48 hours. Fecal slurries were cultured in a DASbox® mini bioreactor system for 5 days. 16S rRNA gene sequencing data analysis showed that there was a decline in alpha and beta diversity similarity metrics, which was found to be dependent on culture time ($p<0.050$), but storage time in GutAlive® containers did not significantly alter the microbial community structure.

Next, it was tested how different modes of nutrient supplementation to the bioreactors influence bacterial composition. Alpha diversity analysis showed that culture time had a significant impact on alpha diversity indices (all $p<0.003$). Beta diversity analysis showed that there were significant differences based on nutrient supplementation modes and culture time (both $p<0.050$), with the least difference in the "fasted" culture, which received feeding only once daily.

Simulating various disease scenarios using stool samples from healthy donors and sarcopenic individuals, beta diversity analysis indicated stable differences in microbiome compositions ($p<0.050$). The system's modulability was demonstrated by treating microbiomes with glutamine, which induced consistent and reproducible pH drops in both groups.

In summary, this study shows a successful optimization of the conditions for in vitro modeling of the human gut microbiome. This model enables the study of disease-specific microbiomes, showcasing potential for the integration into clinical practice.

No. 41

Overcoming Database Dependent Errors in Microbiome Analysis: Examples from INSTAND Round Robin 2023

Driscoll M., Jackson E., Fasulo D., Denslow P., Prazuch A., Gencik M., Schöller Alfred

Praxis für Humangenetik, medgene microbiome, Vienna, Austria

Incomplete, incorrect, or arbitrary taxonomic classification of bacteria is common. Furthermore, databases containing only tens of thousands of bacterial genomes are unlikely to be a good description of the millions or billions of novel strains encountered in real microbiome datasets. Database independent analysis is needed.

Taxonomic errors and database naming convention variability can confound even a very simple mock microbiome analysis with the straightforward goal of identifying known species. We show how multiple different errors in taxonomic assignment can lead to faulty analysis, even with 100% accurate sequence data.

DNA from INSTAND 580 microbiome ring trial 2023 samples was purified (DNA Miniprep Kit; ZymoBiomics), 16S-ITS-23S amplicons (Shoreline Wave™ NanoID™ Kit Version 2; Intus Biosciences) were prepared and sequenced (Ligation Sequencing Kit V14, MinION R10.4.1 flow cell, Mk1C sequencer; Oxford Nanopore Technologies). Barcoded fastq files were error corrected with the Titan-1TM bioinformatic pipeline (Intus Biosciences) to enable strain-level sequence analysis.

Taxonomic misclassifications of high accuracy, strain-level sequence data with 99.93-100% identity to genomes in the NCBI BLASTN database were identified; the supplier species name was incorrect, the assemblies in NCBI were incomplete and therefore absent from BLASTN, or the best matching strain used naming conventions that returned a different species name, despite being the same species. Dependence on the resulting taxonomic mis-assignments resulted in misclassification of accurate, strain level sequences of the selected mock microbiome strains.

We have shown that taxonomic errors and naming convention variability can confound the simplest mock community analysis. As a result, any microbiome data analysis reliant upon taxonomic rather than direct sequence data may miss important findings or draw incorrect conclusions.

No. 42

Patient-Donor signature in predicting clinical response of Faecal Microbiota Transplantation (FMT)

Marija Durdevic, Daniel Podlesny, Patrizia Kump, Christoph Högenauer, Marinka Zitnik, Gregor Gorkiewicz

Core Facility Computational Bioanalytics, Center for Medical Research, Medical University of Graz, Austria; Institute of Pathology, Medical University of Graz, Graz, Austria

In recent years, the incorporation of Fecal Microbiota Transplantation (FMT) into clinical practice has brought a novel therapeutic option for patients suffering from severe active ulcerative colitis (UC). Our latest findings suggest that microbial biomarkers detected just after the FMT could serve as a helpful predictor of the FMT treatment response.

The aim of this study is to construct a robust machine learning (ML) model capable of predicting the FMT response at early stage. Several techniques for classification and regression will be employed to predict post-FMT bacterial populations and abundance. Furthermore, by utilizing MICOM [1], we will be able to evaluate important prognostic metabolites and model the metabolic capacity of the predicted community.

Using this strategy, we can computationally combine patients with several FMT donors in order to find patient-donor pairs with the best possible metabolically competent microbial community. These computational modeling techniques have the potential to improve FMT strategies for UC patients by expanding our knowledge and prognostication abilities.

[1] MICOM: Metagenome-Scale Modeling To Infer Metabolic Interactions in the Gut Microbiota, Christian Diener, Sean M. Gibbons, Osbaldo Resendis-Antonio, mSystems 5:e00606-19, https://doi.org/10.1128/mSystems.00606-19

No. 43

Unmasking nature's collaborators with PyCoMo: Computing all feasible compositions of microbial communities

Michael Predl, Marianne Mießkes, Thomas Rattei, and Jürgen Zanghellini

Department of Analytical Chemistry, University of Vienna, Vienna, Austria

Communities of microbial organisms and their metabolic capabilities are vital for human health, agriculture, and ecology. However, studying naturally occurring microbial communities poses significant computational challenges due to the complex interactions among the species involved.

While genome-scale metabolic modeling has proven effective for individual species, adapting it for communities demands specialized attention. Here we have developed an automated, SBML-compliant workflow, PyCoMo, http://github.com/univieCUBE/PyCoMo, which merges multiple genome-scale metabolic models to overcome these challenges. PyCoMo facilitates the analysis of (microbial) communities using conventional constraint-based analysis tools.

To illustrate the feasibility of our approach, we applied it to a biogas-producing microbial community. This allowed us to explore the full spectrum of potential metabolic behaviors. Our results affirm the critical nature of considering interactions between species to comprehend and predict the collective metabolic capacity. Notably, the cumulative impact of interactions between community members explains up to 96% of the observed community behavior. This highlights the fundamental role of these inter-species relationships in shaping overall metabolic behavior.

PROJECTS

No. 44

canSERV – providing cutting edge cancer research services across Europe

canSERV Consortium, Manuela Pausan

Biobanking Development, BBMRI-ERIC, Graz, Austria

canSERV is a collaborative 14 Mil. Euro project funded by the European Commission with a core mission of making cutting-edge and customised research services available to the cancer research community in the EU, enabling innovative R&D projects and fostering precision medicine for patients benefit across Europe. canSERV involves a consortium of 19 partners from across Europe, consisting of leading Life Science Research Infrastructures and ERICs, key organisations and experts in the field of oncology.

canSERV four main objectives are: (i) offer at least 400 different unique relevant and valuable cutting-edge services for life science research in Europe and beyond over the next three years; (ii) establish a single, unified, transnational access platform to request services and trainings; (iii) ensure oncology-related data provided will be fully compliant with the FAIR principles and complement and synergise with other relevant EU initiatives such as EOSC4Cancer and UNCAN.eu and (iv) ensure the long-term sustainability of the network and unified resources of oncology-related service provision beyond the duration of the project.

canSERV offers a series of open and challenge-driven calls throughout its duration in which it provides researchers with free of charge transnational access (TNA) to over 400 services covering the entire pipeline of cancer research. These calls are designed to support researchers in developing innovative research projects that explore cutting-edge methodologies and target critical gaps in cancer research and patient care. By encouraging the submission of collaborative proposals, canSERV aims to foster transnational cooperation and support a vibrant scientific community. Researchers can access services to help them understand the connection between the human microbiome and cancer development, such as access to human samples, access to microbiome sequencing facilities, access to biomarker discovery services and many more.

No. 45

Let us pave the way for microbiome biobanking. MICROBE - the Microbiome Biobanking (RI) Enabler

Tanja Kostic, Sara Pipponzi, Perrine Portier, Andreas Moser, MICROBE Consortium

AIT Austrian Institute of Technology

Microbiomes are complex communities of microorganisms (bacteria, archaea, protists, fungi, microalgae) that are characteristic of a specific habitat and play a key role in maintaining life on Earth by providing a range of essential ecosystem services and are indispensable for the health of plants, animals and humans. By harnessing microbiome

functions, society would be better placed to tackle global challenges such as food security, health and wellbeing, food waste management, and climate change mitigation. To facilitate the science necessary to achieve key advances in microbiome research, methodologies and technologies to capture or create, ensure stable long-term maintenance, and experimentally perturb microbiomes are required. Research infrastructures currently lack optimized methodologies and technologies to preserve and provide access to microbiome samples and associated data. The MICROBE project (received funding from the EU´s Horizon Europe programme, Grant No. 101094353; 2023-2027) brings together key research actors, biological resource centers and European infrastructures in order to address these issues by building upon and connecting: (1) technical solutions for microbiome preservation, propagation and functionality assessment, (2) novel ecological concepts (i.e., “core microbiome” and “microbial keystone taxa”), and (3) data infrastructures. In addition, MICROBE will address essential framework issues such as standardization, ethical and legal requirements and new business opportunities. The long-term ambition is to ensure widespread uptake in microbiome research communities and thus support the development of novel microbiome-based applications.