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Session Food

Large Scale Metagenomic analysis of the Fermented Beverage Kefir

Liam Walsh
Teagasc, Agriculture and Food Development Authority, Ireland

Milk kefir, a fermented dairy beverage, has a long history of safe consumption. Particular milk kefir beverages, and the strains therein, have also been associated with specific health benefits. A comprehensive metagenomics-based understanding of the microorganisms present within milk kefir communities can help to define the milk kefir pan-metagenome, which includes details such as how the core milk kefir microbiome and the extent non-core components can vary. Such insights are of considerable scientific and commercial value and can contribute to future efforts to create tailored kefir-based microbial communities for different applications. To facilitate this we undertook a large-scale metagenomic analysis of milk kefir samples, generated by inoculating full fat, pasteurized cow's milk with 64 separate kefir grains sourced from 25 different countries, to study patterns of strain, species, and functional pathway variation. We further apply genome-resolved metagenomic analysis to milk and water kefir metagenomes derived from the same initial microbial population of bacteria, yeast and bacteriophage to study compositional change and microbial evolution at the species and strain level. This was facilitated through the contributions of citizen scientists who used the common source of milk or water kefir grain to carry out fermentations using different substrates and growth conditions for up to 21 weeks.

Challenges and future perspectives on the persistence phenotype of food-associated Listeria monocytogenes

Lauren Alteio
FFoQSI, Austrian Competence Centre for Feed and Food Quality, Safety & Innovation, Austria

Elimination of microbial pathogens in food production is critical for reducing risks to consumers from food products, however organisms such as *Listeria (L.) monocytogenes* are well-adapted to survival in food producing environments (FPEs) for periods of months to years. This so-called "persistence" of

identical isolates in the same facility over time presents an immense challenge for food producers, with far-reaching economic and public health implications. Despite widespread efforts to investigate *L. monocytogenes* prevalence and gene composition in the last decades, this pathogen continues to persist and remains a hazard in food production. This begs the question – what are we overlooking in our investigations of the complex and multifaceted topic of *L. monocytogenes* persistence? In this study, we surveyed the landscape of literature centered on the phenomenon of *L. monocytogenes* persistence. We aim to identify patterns across persistent clones of *Listeria*, investigating potential genes and sets of genes associated with higher probability of persistence, and introduce a unique perspective on the relative importance of ecology and evolutionary contexts for understanding microbial persistence. We argue that the interplay between environmental parameters, inter- and intra-species interactions, and adaptations of *L. monocytogenes* should be considered in future studies of this phenotype. Our ongoing research will be highlighted, which incorporates both facility sampling and laboratory experiments to evaluate the role of these factors in *L. monocytogenes* persistence. Finally, we suggest methodologies and approaches as a path toward disentangling the complex picture of persistence for *L. monocytogenes* and other persistent microorganisms in food systems.

Microbial communities in cheeses and their production environment explored through shotgun metagenomics

V. Valentino, F. De Filippis, M. Yap, J.F. Cobo Díaz, C. Barcenilla, N. Martín Quijada, N. Carlino, C. Sabater, M. Wagner, A. Margolles, A. Álvarez Ordóñez, N. Segata, P.D. Cotter, D. Ercolini
Department of Agricultural Sciences, University of Naples Federico II, Portici, Italy

The food processing environment is a home for a plethora of microbial species that might become residential and specific to the production plant. These microbes might be transferred from surfaces to intermediates of production and, inevitably, to the final product, thus profoundly influencing their quality and safety. Currently, little is known about the impact of the residential microbiome on the quality and safety of cheeses. Therefore, we visited 73 cheesemaking facilities from 4 European countries in order to collect environmental samples (i.e., from sanitized Food Contact/Non-Food Contact surfaces) and ingredients/products. Overall, 1305 samples were collected, then we performed DNA extraction and Whole Metagenome Sequencing. The results show that the residential microbiome might exert several microbial activities potentially protecting the food product from spoilage/pathogenic taxa. Indeed, biosynthesis of bacteriocins was more frequently detected in surfaces than in ingredients/products, highlighting that the Lactic Acid Bacteria (LAB) dominating the production environment might counteract the activities of potential spoilage/pathogens. Furthermore, microbial genes involved in adherence and stress resistance were enhanced in surfaces, suggesting that these communities might survive to the gastrointestinal transit and persist in the gut. Interestingly, we clearly highlighted the presence of environmentally selected facility-specific LAB strains. Pangenome analyses of these strains revealed their potential role in driving the ripening process of cheeses, making their sensory profiles unique. Overall, these results show that the environmental microbiome of cheesemaking facilities is highly complex, and that it might strongly influence the quality of the products, as well as the consumers' health.

Session Bioinformatics in microbiology

Computational identification and biological interpretation of genome rearrangements in microbes

Olga Bochkareva
University of Vienna

High plasticity of bacterial genomes is provided by numerous mechanisms including horizontal gene transfer and recombination via numerous flanking repeats resulting in genome rearrangements. Such genome rearrangements may alter gene expression, create new genes and shuffle gene domains providing adaptation to new environments or intra-population phenotypic diversity. Whole-genome sequencing of bacterial genomes allows to study evolutionary changes on different levels of genome organization and predict the phenotype-genotype connection. In the first part, I will talk about computational approaches for whole-genome alignment and synteny blocks construction. Then I will present our method, called PaReBrick, for prediction of rearrangements across the phylogenetic tree and demonstrate its efficiency on bacterial data. The last part will be focused on biological applications of our approach such as identification of phase variation mechanisms, genomic islands and host-microbe interactions.

A microbial oasis in extreme cave environments

André Soares
University of Duisburg-Essen

Karstic caves are extreme environments where microbial life persists regardless of constant darkness, oligotrophy and high aqueous Ca2+ loads. Given the low energy available, microbial communities in caves mostly occur in dormant, low metabolic activity states. Secondary speleothems (cave features) such as moonmilk present unique semi-isolated microenvironments that permit microbial life to thrive. In this talk, I will elucidate the taxonomic and metabolic diversity of active and dry moonmilk microbiomes as leveraged from 291 high-quality metagenome-assembled genomes (MAGs). Numerous CRISPR-Cas spacer matches to viral sequences indicate complex virus-host interactions, with potential to shape moonmilk-based microbial contributions towards carbon biogeochemistry in cave systems. This talk will highlight the potentials of moonmilk and karstic caves in elemental cycling as well as the microbial warfare processes ongoing in this as-yet underexplored microenvironment.

Annotating genomes in a custom way

Lovro Trgovec-Greif
University of Vienna

When it comes to bacterial genome annotation, there are many simple to use bioinformatic tools available. However, more or less often we will encounter a case when there is no ready to use tool available to provide annotations needed for a certain question. In that case it is necessary to find a way to obtain the desired annotations using only the general tools for sequence analysis and processing like BLAST. During the analysis of multiple bacterial genomes, the question was to explore the presence of bacterial anti-phage defense systems in certain hotspots which have been described by another paper. The main challenge was to obtain genomic locations of the hotspots in genomes

being analyzed based on the descriptions in the paper. In order to obtain a table appropriate for analysis, I had to combine outputs of multiple tools and derive a simple algorithm based on BLAST searches. In my talk, I will show and describe what kind of files one could encounter when conducting similar analysis and how to add annotations in a systematic and reproducible way. Moreover, I will describe how to use outputs of multiple tools, parse them, extract the desired information and create a resulting file which can be analyzed directly, for example with R software. I will focus on describing how to break a bigger task into smaller subtasks and how to organize the work in a sustainable way

Large-scale meta-analytic approaches for systematic and reproducible associations between the human microbiome and host's conditions

Paolo Manghi
University of Trento

Microbiome studies have produced thousands of microbiome samples which are an unique opportunity for revealing patterns unobservable at the scale of the single dataset, but data harmonization and standardized approaches for their integration are barriers to their utilization. We developed curatedMetagenomicData (cMD) 3, a R/Bioconductor command line tool that gives access to ~22,700 publicly available human shotgun metagenomic samples that allows the integration of massive amounts of metagenomic data with cross-cohort harmonic data and consistent metadata. To analyze these data, we developed machine learning and meta-analysis strategies that were used to unravel microbiome association with sex, age, BMI, 15 disease-types, and to develop a novel oral-to-gut introgression score.

Session Microbe-host interactions

The role of sugar substitutes in *Salmonella typhimurium* virulence

Dr. Bidong Nguyen
Department of Biology, Institute for Microbiology, ETH Zürich, Zürich, Switzerland

Consumed by millions of people to manage blood glucose and body weight, sugar substitutes may have unintended side effects. Here, we found that the ingestion of arabinose, lactulose, maltitol, erythritol, and sorbitol, which can be substituted for table sugar, reduces colonization resistance against the common food borne pathogen, Salmonella Typhimurium and promotes its virulence. In mice, consumption of these sugars upon infection led to gut colonization by the pathogen with high fecal shedding, a disseminated systemic infection and enteropathy. While the gut microbiota can utilize these sugars, it is perturbed by intestinal acidification resulting from accumulating microbiota-derived fermentation byproducts, permitting pathogen colonization. In addition, Salmonella virulence gene expression is induced, which further enhances infection and intestinal disease. Moreover, the gut environment upon ingestion of these sugar substitutes selects for fully virulent, invasive Salmonella, as avirulent mutants are highly attenuated for growth in the gut. Interestingly, the diet can alter the pathogen's tissue tropism along the intestinal tract. These findings highlight that sugar substitutes in diets may pose a risk for enteric infection and offer a new model for studying Salmonella gut infections that is independent of antibiotic pre-treatment.

Biosynthetic flexibility of Pseudomonas aeruginosa leads to hydroxylated 2-Alkylquinolones with proinflammatory host response

Viktoriia Savchenko
Faculty of Chemistry, Institute of Biological Chemistry, University of Vienna, Vienna, Austria

Pseudomonas aeruginosa, a human pathogen, is known to produce a diverse range of 4(1H)-quinolones with various biological functions. Two significant metabolites among these are 2-nonyl-4(1H)-quinolone (NQ) and its N-oxide (NQNO). Our study aimed to explore the biosynthetic pathway of these metabolites, speculating that oxidized fatty acids might play a crucial role in the production of an undiscovered class of quinolones. To achieve this, we devised a divergent synthesis strategy for 2'-hydroxy (2'-OH) and 2'-oxo-substituted quinolones and respective N-oxides. Our findings reveal for the first time the natural production of 2'-OH-NQ and 2'-OH-NQNO, but not their corresponding 2'-oxo compounds, by the PAO1 and PA14 strains of P. aeruginosa. Intriguingly, the main metabolite, 2'-OH-NQ, is produced in concentrations comparable to NQ. Furthermore, the addition of exogenous βhydroxydecanoic acid led to a significant increase in the production of 2'-OH-NQ. In contrast to NQ, we observed that 2'-OH-NQ strongly induces the cytokine IL-8 in a human cell line at 100 nM, suggesting its potential role in host immune modulation. These findings expand our understanding of *P. aeruginosa*'s metabolic capabilities and may open new avenues for therapeutic interventions targeting quinolone-mediated immune responses.

Mathematical framework to elucidate the role of host immune response and its interaction with the microbiome community

Eeman Abbasi
Department of Biology, University of Pennsylvania, Philadelphia, USA

The host microbiome can be considered an ecological community of microbes present inside a complex and dynamic host environment. The host is under selective pressure to ensure that its microbiome remains beneficial. The host can impose a range of ecological filters including the immune response that can influence the assembly and composition of the microbial community. How the host immune response interacts with the within-microbiome community dynamics to affect the assembly of the microbiome has been largely unexplored. We present here a mathematical framework to elucidate the role of host immune response and its interaction with the balance of ecological interactions types within the microbiome community. We find that highly mutualistic microbial communities characteristic of high community density are most susceptible to changes in immune control and become invasion prone as host immune control strength is increased. Whereas highly competitive communities remain relatively stable in resisting invasion to changing host immune control. Our model reveals that the host immune control can interact in unexpected ways with a microbial community depending on the prevalent ecological interactions types for that community. We stress the need to incorporate the role of host-control mechanisms to better understand microbiome community assembly and stability

Session Phages

The impact of storage buffer and storage conditions on fecal samples for bacteriophage infectivity and metavirome analyses

Xichuan Zhai[1], Josué L. Castro-Mejía[1], Alex Gobbi[2], Antonios Aslampaloglou[1], Witold Kot[2], Dennis S. Nielsen[1], Ling Deng[1]
[1]Section of Microbiology and Fermentation, Department of Food Science, University of Copenhagen, Rolighedsvej 26, 1958 Frederiksberg C, Denmark
[2]Section of Microbial Ecology and Biotechnology, Department of Plant and Environmental Sciences, University of Copenhagen, Thorvaldsensvej 40, 1871 Frederiksberg C, Denmark

Background: There is an increasing interest in investigating the human gut virome for its influence on the gut bacterial community and its putative influence on the trajectory towards health or disease. Most gut virome studies are based on sequencing of stored fecal samples. However, relatively little is known about how conventional storage buffers and storage conditions affect the infectivity of bacteriophages and influence the downstream metavirome sequencing.
Results: We demonstrate that the infectivity and genome recovery rate of different spiked bacteriophages (T4, c2 and Phi X174) are variable and highly dependent on storage buffers. Regardless of the storage temperature and timespan, all tested phages immediately lost 100% (DNA/RNA Shield) or more than 90% (StayRNA and RNAlater) of their infectivity. Generally, in SM buffer at 4°C phage infectivity was preserved for up to 30 days and phage DNA integrity was maintained for up to 100 days. While in CANVAX, the most effective buffer, all spiked phage genomes were preserved for at least 100 days. Prolonged storage time (500 days) at –80°C impacted viral diversity differently in the different buffers. Samples stored in CANVAX or DNA/RNA Shield buffer had the least shifts in metavirome composition, after prolonged storage, but they yielded more contigs classified as “uncharacterised”. Moreover, in contrast to the SM buffer, these storage buffers yielded a higher fraction of bacterial DNA in metavirome-sequencing libraries. We demonstrated that the latter was due to inactivation of the DNases employed to remove extra-cellular DNA during virome extraction. The latter could be partly avoided by employing additional washing steps prior to virome extraction.
Conclusion: Fecal sample storage conditions (buffer, time, temperature) strongly influence bacteriophage infectivity and viral composition as determined by plaque assay and metavirome sequencing. The choice of buffer had a larger effect than storage temperature and storage time on the quality of the viral sequences and analyses. Based on these results, we recommend storage of fecal virome samples in SM buffer at 4°C for the isolation of viruses and at –80°C for metagenomic applications if practically feasible (i.e. access to cold storage). For fecal samples stored in other buffers, samples should be cleared of these buffers before viral extraction and sequencing.

Phage-host interaction regulating skin microbiota

Aryan Rahimi-Midani[1], Maria Sequeira Lopes[2], Parul Chandorkar[1], Janina Marissen[1,3], Lilith Reichert[1,3], Sara Amigo Guillen[1], Jessica Bohlender[1], Louisa Channon[1], Anna Wenning[5], Wilma Ziebuhr[6], David Cameron[7], Dorothee Viemann[3,4], Christoph Härtel[3], Luis Rodrigues Melo[2], Mercedes Gomez de Agüero[1]
[1]Host-microbial interactions group, Institute of Systems Immunology, Max Planck research group, University of Würzburg, Germany
[2]Laboratory of Research in Biofilms Rosário Oliveira, Centre of Biological Engineering, University of Minho, Portugal

[3]University Children's Hospital, University of Würzburg, Würzburg, Germany
[4]Translational Pediatric Department, University Children's Hospital, University of Würzburg, Würzburg, Germany
[5]Mucosal Immunology Group, Department for Biomedical Research, University of Bern, Switzerland
[6] Institute for Molecular Infection Biology, Julius-Maximilians-University of Würzburg, JosefSchneider-Strasse 2, 97080, Würzburg, Germany
[7]Department of Intensive Care Medicine, Inselspital, Bern University Hospital, Switzerland

Commensal microorganisms, called microbiota, colonize mammalian body surfaces at birth and contribute to the barrier function. *Staphylococcus epidermidis*, a major constituent of the skin microbiota, strengthens the neonatal skin barrier by enhancing the immunity and inducing pathogen exclusion. Nevertheless, pathogenic strains of *S. epidermidis* have emerged as major agents causing neonatal sepsis, especially in preterm. Bacteriophages are efficient and natural modulators of the composition of the microbiota. However, their role as the modulators of skin microbiota has not been fully studied. In this study, we aimed to investigate infection patterns of bacteriophages and their potential to shape skin microbiota. Using 11 lytic bacteriophages isolated from human skin, we showed a preferential and strong lytic capacity against *S. epidermidis* strains isolated from the same niche. In addition, they showed a low inter-species target spectrum. Commensal -non-biofilm forming- *S. epidermidis* strains showed a larger phage-spectrum susceptibility than pathogenic-biofilm forming-strains. On the other hand, using a gnotobiotic murine model and epidermal 3D-organoid model, we observed that bacteriophage and *S. epidermidis* strain can interact with each other and also keratinocytes. Overall, our study indicates a close relationship between the bacteria and bacteriophages in the skin. Further studies are planned to address how, where, and when the bacteria and bacteriophage interaction happen in the skin and its contribution to the skin microbiota composition.

Z-DNA containing phages and Z-DNA biosynthetic pathways using the concept of metaGPA

Weiwei Yang, Shuangyong Xu, Laurence Ettwiller
Research Development, New England Biolabs, Ipswich, MA, USA

In the evolutionary arm race between phage and host, phages develop a variety of strategies to rebel to the defensive mechanisms of host bacteria. One classical strategy is that phage genomes can be fully modified to prevent cleavage by host restriction endonucleases. Understanding the different “flavors” of DNA modification carried by phage microbiomes and how these modifications are synthesized and maintained in the phage genomes inspire innovation of nucleic acid products and techniques. Here, we applied our previously established metagenomics genome-phenome association (metaGPA) pipeline to study phage metagenomes containing diaminoadenine (dZ) from environmental microbiome samples. We established a next generation sequencing method to selectively enrich dZ genome sequences and through this study, thousands of unculturable, novel dZ containing phage genomes were discovered. We then applied MetaGPA association analysis to scrutinize protein domains with significant higher frequencies in dZ genomes. These protein domains are potentially involved in production and maintenance of dZ genome. We are able to further build the dZ biosynthetic pathway with the co-occurrence network. Our result suggests the involvement of a novel component in the dZ pathway. In addition, we performed metaGPA differential residue association analysis on two conserved genes in related phage genomes: PurZ (homolog of adenylosuccinate synthetase) and DpoZ (DNA replicative protein in dZ genome) sequences from de novo and public databases. Our findings implicate key residues which may play roles on the dZ vs dA specificity.

Rapid hydrogel-based phage susceptibility test for pathogenic bacteria

Sheetal Patpatia[1], Eric Schaedig[1], Anna Dirks [1], Lauri Paasonen[2], Mikael Skurnik[1,3] and Saija Kiljunen[1,3]
[1]Human Microbiome Research Program, Faculty of Medicine, University of Helsinki, Helsinki, Finland,
[2]UPM Biomedicals, Helsinki, Finland,
[3]Division of Clinical Microbiology, HUSLAB, Helsinki University Hospital, Helsinki, Finland

Phage therapy is one alternative to cure infections caused by antibiotic resistant bacteria. Due to the narrow host range of phages, hundreds to thousands of phages are required to cover the diversity of bacterial pathogens. In personalized phage therapy, fast selection of the phages for individual patients is essential for successful therapy. The aims of this study were to set up a rapid hydrogel-based liquid phage susceptibility assay (PST) for the selection of phages for therapeutic use and to establish a "ready-to-screen" plate concept, where phages are readily stored in hydrogel as small droplets in microtiter plate wells. We first tested four commercially available hydrogels (GrowDex, Askina, Purilon, and Intrasite) for their suitability as phage matrices in PSTs with four phages, two of which infecting Escherichia coli and two Staphylococcus aureus. Of these four hydrogels, GrowDex was the best matrix for PST, as it did not inhibit bacterial growth, released phages quickly when mixed with bacterial culture, and maintained phage viability well. We then optimized the assay for both optical density and microscopy readers using GrowDex as a matrix with 23 bacterial strains representing 10 different species and 23 phages possessing different morphologies and genome sizes. When the bacterial growth was monitored by microscopy reader, the PST was executed in just 3 hours, and there was no need for overnight culturing bacterial cells prior to the assay, whereas using optical density reader, bacteria had to be pre-cultured overnight, and the assay time was five hours. Finally, we evaluated the effect of three different chemical stabilizers (trehalose, hyaluronic acid, and gelatin) in a six-month stability assay with six model phages. These phages assay behaved very differently in respect to the chemical stabilizers, and there was not a single stabilizer suitable for all phages. However, when gelatin (0.01%) or hyaluronic acid (0.2 mg/ml) was used as stabilizer, all tested phages were still considered as positives in PST after a six-month storage in 1 ml volume. In "ready-to-screen" plates, the differences in phage stabilities were even more profound, varying from two to six months for the most and least stable phages, respectively.

Session Plant Soil Microbiome

Functional resilience in the soil microbiome under long term anthropogenic disturbance: Insights from the seven-decade-old Centralia coal mine fire

Samuel E. Barnett[1] and Ashley Shade[2]
[1]Department of Microbiology and Molecular Genetics, Michigan State University, East Lansing, MI 48824, USA
[2]Universite Claude Bernard Lyon 1; CNRS, UMR 5557 Écologie Microbienne, 16 rue Dubois 69622 Villeurbanne Cedex, France

Soil microbiomes are essential mediators of ecosystem services, and their resistance and resilience to anthropogenic disturbances can have profound effects on soil function. However, we know little about soil microbiome resilience in the context of long-term (press) disturbances, especially as it comes to dynamics of community functional potential during recovery. We utilized the unique seven-decade-old underground coal seam fire in Centralia, Pennsylvania, USA, to examine surface

soil microbiome resilience to long-term heat disturbance. As the fire slowly advances along coal seams, formerly fire-affected soils cool to ambient temperature enabling geographically localized comparisons of impacted soils across a gradient of fire intensities as well as reference soils. We aimed to assess dynamics in potential functions of soil microbiomes during recovery. We performed deep metagenomic sequencing on soils repeatedly sampled over seven years from sites across the disturbance intensity gradient. We examined the genomic investment in genes and gene families of interest, including nitrogen cycling genes, antibiotic resistance genes, and biosynthetic gene clusters, among others. Across these gene families we see varying responses to soil cooling. For example, some nitrogen cycling gene families (e.g., genes involved in denitrification) were enriched in warmer soils, indicating differing nitrogen cycling functionality across disturbance intensity. Overall, our findings indicate that long term soil heating alters the functional potential of soil microbiomes which may broadly affect ecosystem function. We further suggest that while the overall bacterial community is largely resilient to long term heat disturbance, some functional potentials of these communities may remain disturbed for extended periods.

Composition and functional profiles of soil bacterial communities in lemon groves of a dedicated production area

Alexandros Mosca[1], G. Dimaria[2], F. Modica[3], D. Nicotra[2], M. E. Massimino[2], V Catara[2]
[1]Department of Physics and Astronomy, University of Catania, 95123 Catania, Italy
[2]Department of Agriculture, Food, and Environment, University of Catania, 95123 Catania, Italy
[3] Department of Life Sciences, University of Modena and Reggio Emilia, 42122 Reggio Emilia, Italy

The Mediterranean Basin accounts for approximately half of lemon (*Citrus limon*) production. In this study, five lemon groves located in Syracuse (Sicily, Italy), where lemons with Protected Geographical Indication ‘Siracusa’ are cultivated, were selected for the soil bacterial communities analyses. Groves differed for plant age, farming and rootstock (*Citrus aurantiumor*, *Citrus volkameriana*). Moreover, Citrus Mal secco disease, caused by the xylematic fungus *Plenodomus tracheiphilus*, was recorded in the groves. Soil is a reservoir for fungal phytopathogens, and the antagonistic activity of the bacterial communities could be determining for disease management. Total genomic DNA was extracted from soil samples using the DNeasy PowerSoil Pro Kit (Qiagen). High-throughput amplicon sequencing was then performed for microbial community profiling using an Illumina NovaSeq 6000 instrument. Beta diversity analysis revealed that each grove harbours different bacterial communities. Relative abundances of the most abundant phyla, namely *Proteobacteria*, *Actinobacteriota*, *Acidobacteriota* and *Planctomycetota* change in the groves. The bacterial core community, present in at least 75% of the samples, was represented by genera with putative beneficial activity for plants such as *Pseudomonas*, *Streptomyces* and *Nitrospira*. Differential abundance analysis showed enriched plant growth promoting rhizobacteria (PGPRs), such as *Bacillus*, *Stenotrophomonas* and *Lysobacter*, across the groves. Furthermore, predictive functional analysis of the bacterial communities through PICRUSt2 indicated that the most abundant Kegg Orthology modules mapped in biosynthesis of amino acids, quorum sensing and pathways involved in antibiotics biosynthesis. Further studies on microbial communities may dissect the ecological functions of soil microbiome under Mal secco disease infections to increase citrus health.

Overlap between stigma and seed bacterial communities of commercial watermelon and their effects on seedling vigor

Gillian Bergmann
Department of Plant Pathology, University of California-Davis, Davis California, USA

Seeds, like all plant tissues, contain microbial communities that can have a myriad of effects on plant health, including increased seed germination and seedling growth. As such, it is important to determine from where seeds acquire microbes in order to predict community assembly outcomes and plant health impacts. One known pathway for microbial transmission to seeds is from floral stigmas, which potentially serve as a first source of inoculum during fertilization and early seed development. However, the overlap between stigma and seed microbial communities is largely unknown. In this study, I compared stigma and seed bacterial communities in fields of commercial watermelon (*Citrullus lanatus*) using culture-dependent and sequence-based approaches. Additionally, I conducted a seedling viability assay to determine the effects of stigma- and seed-sourced bacteria on seedling health. Whereas bacterial communities were significantly different between the two tissue types, I found that 20% of culturable bacteria and 1% of sequenced bacterial amplicon sequence variants (including *Acinetobacter*, an unclassified *Lachnospiraceae* and *Microbacterium*) were shared between stigmas and seeds. Additionally, culturable bacteria had diverse effects on seedling viability, with seed-sourced *Acidovorax* and *Bacillus* improving seed germination and seedling growth whereas stigma-sourced bacteria reduced germination and growth. These data demonstrate a major bottleneck between stigma and seed bacterial communities of watermelon, and that seed-borne bacteria contribute to seedling vigor.

Exploring the Microbiome of Cannabis Seeds through Domestication and Breeding Stages

Carolina Lobato[1], João Machado de Freitas[2], Daniel Habich[1], Gabriele Berg[1,3,4], Tomislav Cernava[1,5]
[1]Institute of Environmental Biotechnology, Graz University of Technology, Petersgasse 12, 8010 Graz, Austria
[2]Know-Center GmbH, Graz, Austria
[3]Leibniz Institute for Agricultural Engineering and Bioeconomy, Max-Eyth-Allee 100, 1446 Potsdam, Germany
[4]Institute for Biochemistry and Biology, University of Potsdam, Karl-Liebknecht-Str. 24-25, 14476 Potsdam OT Golm, Germany
[5]School of Biological Sciences, Faculty of Environmental and Life Sciences, Highfield Campus, SO17 1BJ Southampton, UK.

Seeds are interesting organs from the ecological point of view as they represent the start and the endpoint of a plant's life cycle, allowing for sexual reproduction and dispersal, which ultimately contributes to plant diversification. Seed-associated microorganisms have been shown to contribute to seed preservation and release from dormancy, as well as to improve the germination rate. Furthermore, seed endophytes can be transmitted from one generation to the next, assisting in plant establishment during early development. This study aimed to identify genotype-specific differences in seed microbiomes of Cannabis plants originating from different breeding approaches. This was achieved by (i) characterizing seed endophyte diversity, structure, and distribution across 46 Cannabis varieties, (ii) identifying shared and flexible endophytic taxa, and (iii) determining variety-associated biomarkers at the ASV level that could facilitate further breeding efforts. Bacterial richness was found to be generally low in Cannabis seeds with an uneven distribution of species

prevalence, but significantly higher in less inbred varieties. The fraction of shared endophytes (core microbiome) accounts for a high proportion of the total amplicon reads. Moreover, Cannabis variety was the main factor explaining variations in bacterial composition. Seed communities of plant varieties from the same chemotype, domestication status or lineage are also clustered together, which can be an indicator of co-divergence between plants and their seed microbiome during breeding. Based on the large-scale dataset that was generated in this study, potential bacterial markers as indicators of domestication state and cannabinoid content were identified. As seeds are emerging as biotechnological sources and carriers of beneficial microorganisms, addressing the impact of breeding on their microbiomes can contribute for the development of targeted engineering approaches and promote sustainable agriculture.