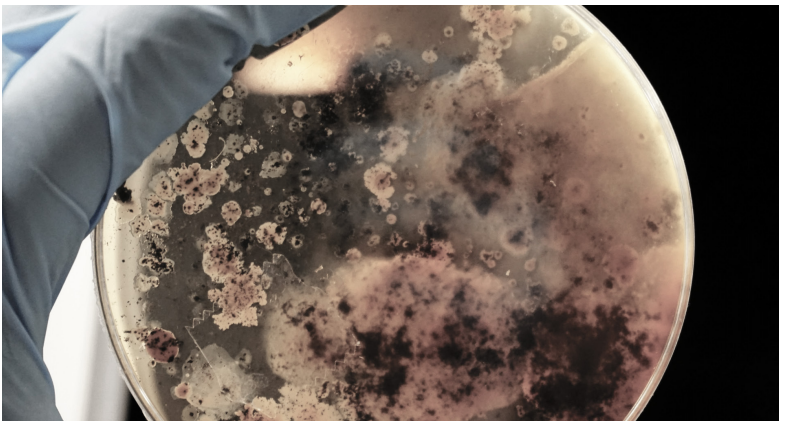

Abstract book

THE ONE HEALTH MICROBIOME SYMPOSIUM

30-31 MAY 2024

THE PENNSYLVANIA STATE UNIVERSITY

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The Pennsylvania State University

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CONFERENCE AGENDA

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May 30, 2024

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Posters & Opening

Life Science Bridge

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8:30 - 9:30 **Poster Session,** *odd numbered*

9:45 - 10:00 **Opening Remarks** *Berg Auditorium*
Seth Bordenstein, PhD
One Health Microbiome Center, Penn State

Section 1: Integrated One Health

Berg Auditorium

Keynote Speaker

10:00 - 10:50 **Steffanie Strathdee, PhD**
University of California San Diego
From Bog to Bedside: The Story Behind the First Dedicated Phage Therapy Program in the U.S.

10:50 - 11:10 **Rupinder Kaur, PhD |** *Penn State*
Pushing Boundaries: Unraveling the Impact of Bacteriophage Proteins on Eukaryotic Host Biology to Aid in Vector Control Strategies

11:10 - 11:30 **Ivan Liachko, PhD |** *Phase Genomics*
Building the World's Largest Virome-Microbe Interaction Atlas Using Proximity Ligation Sequencing Technology

11:30 - 11:50 **Geo Santiago-Martínez, PhD**
The University of Connecticut
Functional Genomics in Methane-producing Archaea from Host-associated Microbiomes

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Dylan Barbera, *QIAGEN Sciences, Inc., Germantown, USA*
Global Product Manager, UNGS Genotyping, GPM Genomics

Day 1. Thursday

May 30, 2024

Section 2: Agricultural Health

Berg Auditorium

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Max Planck Institute for Plant Breeding Research
Reductionist Approaches to Determine Functions of the Plant Root Microbiota

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Variation in Response to Arbuscular Mycorrhizal Fungi across Maize Genotypes and Agroecosystems

2:15 - 2:35 **Fiama Guevara |** *The Ohio State University*
Pathogen-induced Changes in Bacterial and Fungal Communities' Structure in Hydroponically Grown Lettuce

2:35 - 2:55 **Karen Peralta Martínez |** *University of Pittsburg*
Breaking Down the Armor? Effects of Chitin, Found in the Exoskeleton of Insects, on Mammalian Gut Microbiota and Physiology in Wild Mice

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Berg Auditorium

Keynote Speaker

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Windows to the Underground - Live Broadcast from the World of Soil Microbes

4:10 - 4:30 **Marcella Baiz, PhD |** *University at Buffalo, SUNY*
Association Between the Gut Microbiome and Carotenoid Plumage Phenotype in an Avian Hybrid Zone

4:30 - 4:50 **Tim I. Miyashiro, PhD |** *Penn State*
Homoserine Lactone Autoinducer Drives Diversification of the Quorum-sensing Receptor in the Bacterial Symbiont *Vibrio fischeri*

4:50 - 5:10 **Anna Kazarina |** *Kansas State University*
Microbial Metabolites Enhanced Plant-host Growth through Provision of Nutrients and Stress Removal

6:00 - 8:30 **Reception & Networking** *Hintz Alumni Center*

Day 2. Friday

May 31, 2024

Posters

Life Science Bridge

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Rutgers University
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Harnessing Gut Microbes for Glycan Detection and Characterization

10:55 - 11:15 **Sangshan Tian |** *Penn State*
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Metatranscriptomic Activity of the Gut Microbiome Drives Data-driven Precision Nutrition through Food and Supplement Recommendations

1:40- 2:00 **Jennifer Harris |** *Penn State*
Active Microbes in the Rhizosphere are Most Likely to Successfully Colonize Plant Belowground Structures when Probed with Bioorthogonal Non-canonical Amino Acid Tagging (BONCAT)

2:00 - 2:15 **Coffee Break** *Life Science Lobby*

2:15 - 3:00 **Closing remarks** *Berg Auditorium*
Awards, discussion

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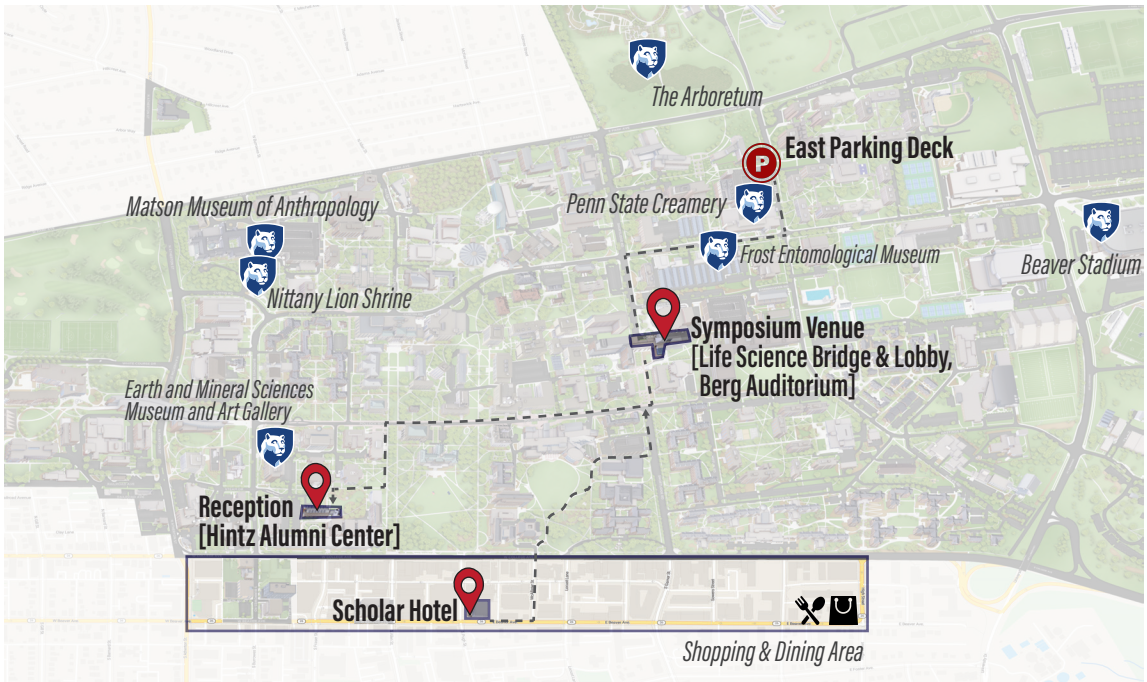
MAP

Symposium Map

Penn State University



Parking Information

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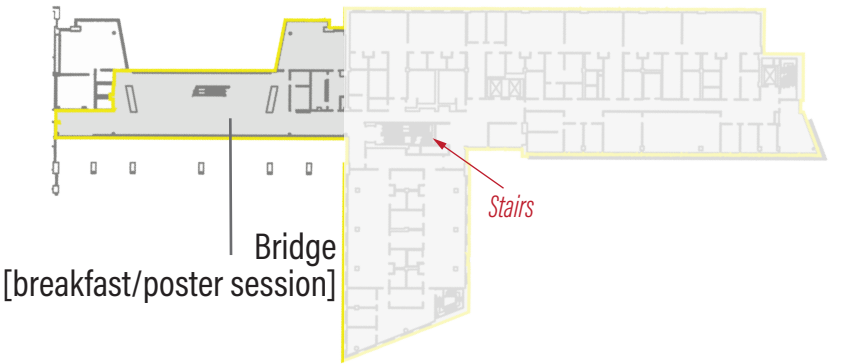
Symposia locations, parking & restaurants in Google Maps

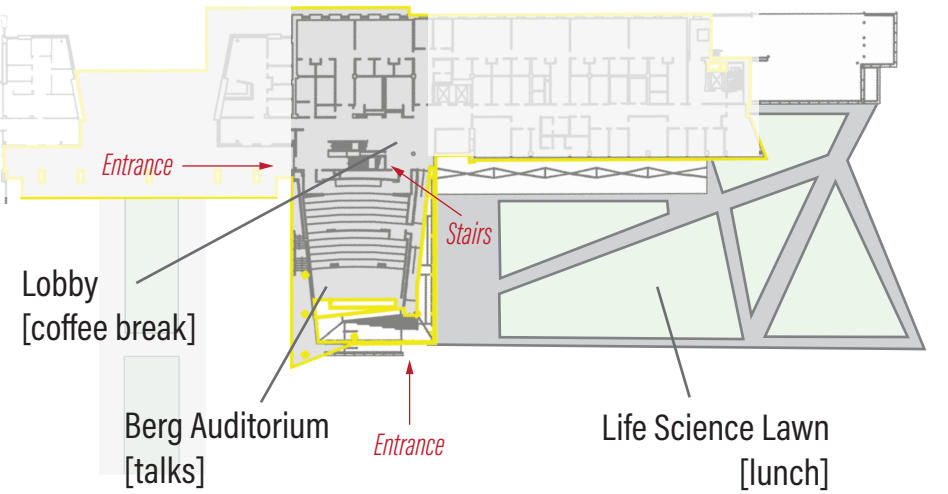
https://bit.ly/ohmc24_map

Symposium Venue Layout

Life Science Building. Floor 3



Life Science Building. Floor 1



TALKS ABSTRACTS

Integrated One Health

Dr. Steffanie Strathdee
Associate Dean of Global Health Sciences
Harold Simon Distinguished Professor
Department of Medicine
California San Diego School of Medicine

Dr. Strathdee is a co-director of the Center for Innovative Phage Applications and Therapeutics (IPATH). In 2016, Strathdee and colleagues were credited with saving her husband's life from a deadly superbug infection using bacteriophages —viruses that attack bacteria. The case, which involved cooperation from three universities, the U.S. Navy and researchers across the globe, shows how phage therapy has potential to treat multi-drug resistant bacterial infections which are expected to kill 10 million people per year by 2050. Strathdee and her husband co-authored their memoir called The Perfect Predator: A Scientist's Race to Save Her Husband from a Deadly Superbug. For her efforts to revitalize phage therapy in the West, she was named one of TIME magazine's Most Influential People in Health Care in 2018.

Agricultural Health

Dr. Paul Schulze-Lefert
Director
Department of Plant Microbe Interactions
Max Planck Institute for Plant Breeding Research, Germany

Previously, Dr. Schulze-Lefert held senior positions at the University of Aachen, Germany, and at the Sainsbury Laboratory of the John Innes Centre, Norwich, UK. His research focuses on the plant innate immune system and the plant microbiota. In recent years, his laboratory has contributed to the development of plant microbiota science as a new field of research. His main goal is to define the molecular principles underlying plant-associated microbial communities and their beneficial services to the host using reductionist approaches. Paul Schulze-Lefert is an elected member of EMBO, the National Academy of Sciences, USA, the German National Academy of Sciences, Leopoldina, and of the American Academy of Microbiology, USA. He is science advisory board member of the Two Blades Foundation and a co-founder and advisor of AgBiome, a for-profit company that explores the crop microbiome to develop biologicals that improve plant health and productivity.

Environmental Health

Dr. Edith Hammer
Senior Lecturer, Associate Professor
Department of Biology
Lund University, Sweden

Dr. Hammer research focuses on microbial processes that drive the nutrient cycles in soils and are the base for healthy soil functions, such as its enormous carbon storage. She has developed so-called soil chips, microfluidic micromodels that mimic soil microstructure to study organisms and processes embedded in their spatial settings. She has a strong interest in processes at the scale of the microbes themselves, and with help of imaging she also wishes to increase awareness of the fragile ecosystem with its intricate biodiversity. She leads the branch for climate and C-cycle science of the strategic research environment BECC, the Section Soil Biology at the European Geosciences Union, and 2023's Microsoil Network.

Human Health & Disease

Dr. Maria Gloria Dominguez-Bello
Professor of Microbiome and Health
Department of Biochemistry and Microbiology,
Department of Anthropology
Rutgers University

Dr. Dominguez-Bello is a Fellow of the American Academy of Microbiology and of the Infectious Disease Society of America (IDSA), as well as a member of the Editorial Board and reviewer at several scientific journals. Her work focuses on understanding human health before urbanization, and the impact of urban practices that impair the microbiome, as well as strategies for restoration. She is a founding member of the Microbiota Vault, a global initiative to preserve the diversity of the microbes relevant to human health, and to educate and to foster collaborative research with the global South to create microbiota collections in hotspots of biodiversity.

Day 1. Thursday

10:00 - 11:50 AM *May 30, 2024*

INTEGRATED ONE HEALTH

SECTION 1

Keynote Speaker:

Dr. Steffanie Strathdee

Associate Dean of Global Health Sciences
Harold Simon Distinguished Professor
Department of Medicine
California San Diego School of Medicine

Thursday, 10:50 - 11:10 am

Dr. Strathdee is a co-director of the Center for Innovative Phage Applications and Therapeutics (IPATH). In 2016, Strathdee and colleagues were credited with saving her husband's life from a deadly superbug infection using bacteriophages —viruses that attack bacteria. The case, which involved cooperation from three universities, the U.S. Navy and researchers across the globe, shows how phage therapy has potential to treat multi-drug resistant bacterial infections which are expected to kill 10 million people per year by 2050. Strathdee and her husband co-authored their memoir called The Perfect Predator: A Scientist's Race to Save Her Husband from a Deadly Superbug. For her efforts to revitalize phage therapy in the West, she was named one of TIME magazine's Most Influential People in Health Care in 2018.

Pushing Boundaries: Unraveling the Impact of Bacteriophage Proteins on Eukaryotic Host Biology to Aid in Vector Control Strategies

Thursday, 10:50 - 11:10 am

Rupinder Kaur[1,2,3], Angelina McGarry[1,2], Dylan J. Shropshire[3,4], Brittany A. Leigh[3], Seth R. Bordenstein[1,2,3]

[1] *Departments of Biology and Entomology, The Pennsylvania State University, University Park, PA, USA.*
[2] *The One Health Microbiome Center, Huck Institutes of the Life Sciences, The Pennsylvania State University, University Park, PA, USA.*
[3] *Department of Biological Sciences, Vanderbilt University, Nashville, TN, USA.*
[4] *Department of Biological Sciences, Lehigh University, Bethlehem, PA, USA.*

The degree to which bacteriophage proteins directly influence animal host biology remains largely unexplored. We show that prophage-encoded proteins from the endosymbiotic bacteria *Wolbachia* alter long noncoding RNA (lncRNA) and DNA during *Drosophila* sperm development to establish a paternal-effect embryonic lethality known as cytoplasmic incompatibility (CI).

The CI phenomenon is of significant interest due to its relevance in strategies targeting pest populations and mosquito-borne viral diseases. At the biochemical level, we report that CI factor A (CifA) is a ribonuclease (RNase) that depletes a spermatocyte lncRNA required for the transformation of histone to protamine during sperm development. CifA and CI factor B (CifB) are deoxyribonucleases (DNases) that nick DNA to compromise insect DNA integrity in late spermatids, which ultimately causes unrepairable embryo damage and sterility.

We conclude that prophage proteins interact with eukaryotic macromolecules during gametogenesis to fashion a worldwide symbiosis, which is crucial to advance human health prospects in the future.

Building the World's Largest Virome-Microbe Interaction Atlas Using Proximity Ligation Sequencing Technology

Thursday, 10:50 - 11:10 am

Ivan Liachko [1]

[1] *Phase Genomics, Seattle, WA, USA*

Phages interact with all life and shape the global ecosystem through their impacts on community composition and horizontal gene transfer. However, phage-host relationships have proven challenging to identify without use of culture-based experiments to generate unambiguous evidence for a phage's presence in a given host. These experiments inherently require that all hosts are culturable, typically restricting the scope and microbial diversity that can be surveyed and limiting our understanding of potentially valuable phage-host relationships.

Metagenomic proximity ligation sequencing is a powerful method for associating viruses and plasmids with their hosts directly in native microbial communities. It captures, in vivo, physical interactions between the host microbial genome, the genetic material of both lytic and lysogenic phage as well as plasmids and AMR genes. Similar to culturing experiments, these linkages offer direct evidence that phage sequences are present within a host cell, thereby establishing a phage-host pair. However, unlike culturing experiments, proximity-ligation methods do not require the propagation of living bacterial cells. The combination of intra-phage and phage-host signal enables us to simultaneously deconvolve viral genome bins (vMAGs) directly from metagenomes and to assign hosts to large numbers of vMAGs without culturing.

Using large-scale application of this technology to samples in agricultural, environmental, and healthcare settings, we have generated the world's largest repository of genomic assemblies of phage, plasmid, and resistance elements connected with their host microbes. We will discuss both published and unpublished work on the application of this technology to combatting AMR and microbial modulation in environmental and agricultural settings.

Functional Genomics in Methane-producing Archaea from Host-associated Microbiomes

Thursday, 11:30 - 11:50 am

Geo Santiago-Martínez [1]

[1] *The University of Connecticut,Storrs, CT , USA*

Each year, around one billion tons of methane are produced by methane-producing microorganisms (methanogens, from Domain Archaea) in free-oxygen environments, playing an important role in nutrient cycles and the Earth's climate. In recent years, methanogens have also been found in diverse host-associated microbiomes, suggesting beneficial roles in microbial interactions and One Health.

Our Microbial Ecophysiology Lab at UConn focuses on understanding the regulation of metabolic pathways in diverse type of methanogens. We use functional genomics and comparative microbial physiology to provide insights into the function and evolution of proteins involved in microbial metabolism. Currently, we are evaluating the physiological role of Carbonic anhydrases and Citrate synthases in methanogens. Our central hypothesis proposes that gene evolution provides unique properties that benefit microbial metabolism. Our main goal is to understand the relevance of these new features in regulating protein function, cellular localization, and microbial metabolism.

Our results suggest that new features of proteins could be predicted by sequence analysis, but the differences are more evident when the proteins are analyzed in a microbial physiology context, since not all genes are expressed under the same condition. For example, in the same microorganism, multiple functions of carbonic anhydrases can be observed depending on the availability of energy sources.

These proteins have greater physiological relevance in environments where nutrient availability is limited, affecting the syntrophic relationships associated with these microenvironments and microbiomes. Finally, we are also testing new tools to find more archaea in soils and aquatic sediments, but also in host-associated microbiomes.

Day 1. Thursday

1:05 - 2:55 PM *May 30, 2024*

AGRICULTURAL HEALTH

SECTION 2

Keynote Speaker:

Dr. Paul Schulze-Lefert

Director
Department of Plant Microbe Interactions
Max Planck Institute for Plant Breeding Research, Germany

Thursday, 1:05 - 1:55 pm

Previously, Dr. Schulze-Lefert held senior positions at the University of Aachen, Germany, and at the Sainsbury Laboratory of the John Innes Centre, Norwich, UK. His research focuses on the plant innate immune system and the plant microbiota. In recent years, his laboratory has contributed to the development of plant microbiota science as a new field of research. His main goal is to define the molecular principles underlying plant-associated microbial communities and their beneficial services to the host using reductionist approaches. Paul Schulze-Lefert is an elected member of EMBO, the National Academy of Sciences, USA, the German National Academy of Sciences, Leopoldina, and of the American Academy of Microbiology, USA. He is science advisory board member of the Two Blades Foundation and a co-founder and advisor of AgBiome, a for-profit company that explores the crop microbiome to develop biologicals that improve plant health and productivity.

Variation in Response to Arbuscular Mycorrhizal fungi across Maize Genotypes and Agroecosystems

Thursday, 1:55 - 2:15 pm

Patrick W. Sydow[1], Sergio Pérez-Limón[1] , Meng Li[1] , Melanie G. Perryman[1] , M. Rosario Ramírez-Flores[2] , Peter Balint-Kurti[3,4], Shannon Sermons[3,4], Jagdeep S. Sidhu[1], and Ruairidh J. H. Sawers[1]

[1] *Department of Plant Science, The Pennsylvania State University, State College, PA, USA.*
[2] *Bioscience Division, Oak Ridge National Laboratory, Oak Ridge, TN, USA.*
[3] *Plant Science Research Unit, USDA-ARS, Raleigh, NC, USA.*
[4] *Department of Entomology and Plant Pathology, NC State University, Raleigh, NC, USA.*

Arbuscular mycorrhizal fungi (AMF) are ubiquitous in cultivated soils, forming symbiotic relationships with the roots of major crop species. Studies in controlled conditions have demonstrated the potential of the symbiosis to enhance the growth of host plants. It is difficult, however, to estimate the actual benefit in the field, not least because of the lack of suitable AMF-free controls.

Here, we report the use of maize genetic mutants to generate AMF incompatible sentinel plants that can be used as a baseline against which to evaluate the impact of the symbiosis. To estimate the overall (main) effect of AMF and characterize the impact of host genotype on symbiotic outcome, we have selectively incorporated AMF-incompatibility into genetic mapping populations. We are using these populations for trait mapping, estimating AMF, host genotype and host genotype x AMF effects by comparison of mycorrhizal and non-mycorrhizal plants.

We present evidence of plant genetic trade-offs between performance with and without AMF, indicating the importance of tailoring crop varieties to the AMF "environment" with respect to different types of agroecosystem. The approaches we present are applicable to other crop species, permit further mechanistic study and are scalable to larger yield trials.

Pathogen-induced Changes in Bacterial and Fungal Communities' Structure in Hydroponically Grown Lettuce

Thursday, 2:15 - 2:35 pm

Fiama E. Guevara[1], Maria Soledad Benitez Ponce[1]

[1] *Department of Plant Pathology, Ohio Agricultural Research and Development Center, The Ohio State University, Wooster, Ohio, USA.*

The plant microbiome plays important roles in host's fitness and functionality including (a)biotic stress tolerance. Besides host genotype, pathogen invasion is one of the main biotic factors driving plant microbiome assembly. Hydroponics is a method of growing plants in a nutrient solution without soil under a climate-controlled environment that allows for precise nutrient delivery and management of pests and diseases.

Nonetheless, hydroponics are susceptible to water-borne pathogens like *Pythium spp*., which cause root rot disease. Despite advancements in understanding soil microbial communities, our knowledge of microbiome function in hydroponics remains limited, thus we aimed to study bacterial and fungal communities shifts in roots of hydroponic lettuce upon inoculation with the plant pathogen *Pythium aphanidermatum*, using metabarcoding of ribosomal markers (16S rRNA and ITS1).

Community composition analysis, based on Bray-Curtis distances showed that bacterial and fungal communities' structure differed in inoculated plants (PERMANOVA; p-value < 0.05) compared with non-inoculated plants. Disease severity was estimated using a root necrosis scale. Root samples with high disease severity presented higher bacterial and fungal ASVs richness compared to low disease severity and non-inoculated controls (Kruskal-Wallis; p-value < 0.001). Differential abundance analysis revealed that potential beneficial bacteria, such as *Pseudomonas* and *Mucilaginibacter*, were enriched in inoculated plants (Log2FoldChange; p-value <0.05) when compared with non-inoculated controls. Similarly, potential opportunistic fungi such as Fusarium were enriched in inoculated plants.

These findings advance our understanding on plant-microbiome interactions in hydroponic systems and could provide important information for safeguarding plants against disease and enhancing agriculture production.

Breaking Down the Armor? Effects of Chitin, Found in the Exoskeleton of Insects, on Mammalian Gut Microbiota and Physiology in Wild Mice

Thursday, 2:35- 2:55 pm

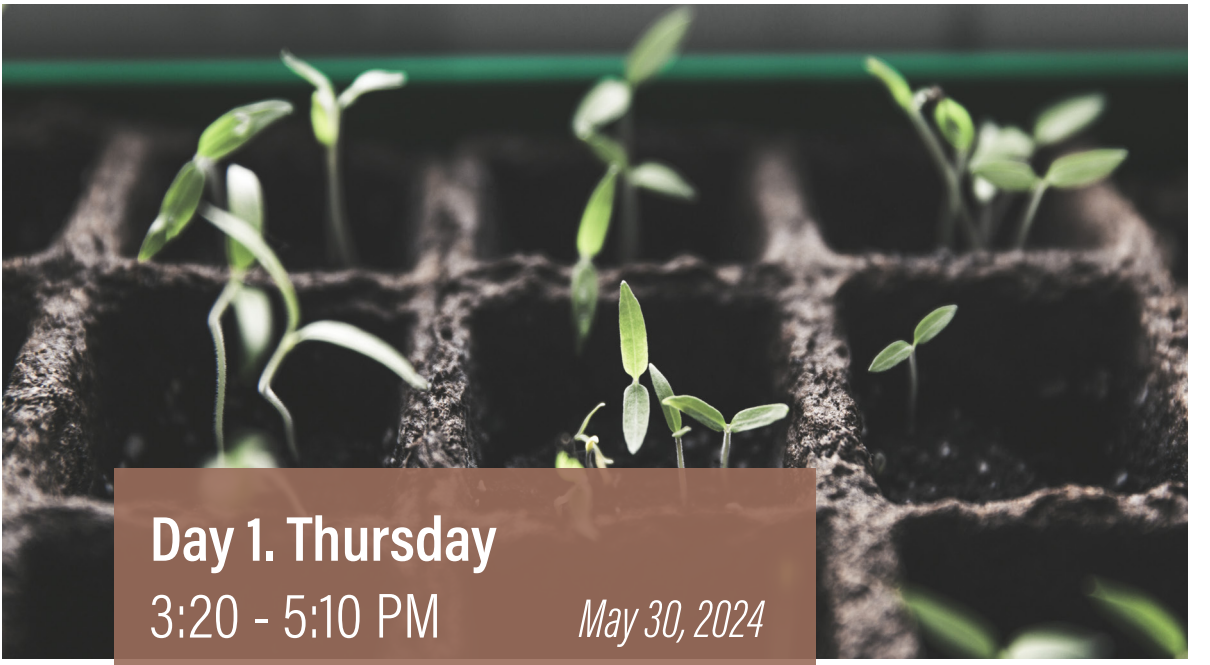
Karen Peralta Martinez[1], Claire Chiang[2], Isabella Bosco[1], Sarah Holleman[1], Nick Barts[3], Jose F. Goyco-Blas[1], and Kevin Kohl[1]

[1] *Department of Biological Sciences, University of Pittsburgh, Pittsburgh, PA, USA.*
[2] *Cornell University College of Veterinary Medicine, Ithaca, NY, USA.*
[3] *Department of Biological and Clinical Sciences, University of Central Missouri, Warrensburg, MO, USA.*

Efforts to incorporate insects, in animal feeds and western diets, are increasing due to the current global food insecurity crisis. Insects serve as a valuable source of protein and animal fibers, particularly chitin, which ranks as the second most abundant biopolymer in nature. Chitin is predominantly found in the exoskeleton of insects and fungal cell walls while chitin-degrading enzymes are conserved across microbes and most vertebrates. However, the loss of functional chitinase genes in mammals begs the question of whether or not chitin is impacting the health of the host and its gut microbiota.

Here, we examine the effects of dietary chitin on the gut microbiota and host enzymatic activity and physiology of wild omnivorous deer mice. Using cecal content samples, we found that bacterial richness and diversity were reduced in mice fed a chitin-containing diet, but the composition of the gut microbiota did not change between the groups. Feces analysis revealed that while in captivity mice lowered their microbial abundance and diversity, yet the chitin-containing diet better resembled the gut microbiota of the wild. In the host, we found that chitinase activity was significantly higher in the stomach of chitin-fed mice compared to the control group, however, we did not observe changes in gut physiology.

Our findings highlight the positive impact that chitin has on gut microbial richness and composition, and host digestion. Future research is necessary to understand the effects of animal fibers on gut health and gut microbiome, which remains one of the "Grand Challenges" in physiology.



Day 1. Thursday

3:20 - 5:10 PM *May 30, 2024*

ENVIRONMENTAL HEALTH

SECTION 3

Keynote Speaker:

Dr. Edith Hammer
Senior Lecturer, Associate Professor
Department of Biology
Lund University, Sweden

Dr. Hammer research focuses on microbial processes that drive the nutrient cycles in soils and are the base for healthy soil functions, such as its enormous carbon storage. She has developed so-called soil chips, microfluidic micromodels that mimic soil microstructure to study organisms and processes embedded in their spatial settings. She has a strong interest in processes at the scale of the microbes themselves, and with help of imaging she also wishes to increase awareness of the fragile ecosystem with its intricate biodiversity. She leads the branch for climate and C-cycle science of the strategic research environment BECC, the Section Soil Biology at the European Geosciences Union, and 2023's Microsoil Network.

Thursday, 3:20 - 4:10 pm

Association between the Gut Microbiome and Carotenoid Plumage Phenotype in an Avian Hybrid Zone

Thursday, 4:10 - 4:30 pm

Marcella Baiz[1], Andrew Wood[2], David Toews[2]

[1] *Department of Biological Sciences, University at Buffalo, SUNY, Buffalo, NY, USA.*
[2] *Department of Biology, Penn State University, State College, PA, USA.*

Birds host complex microbial communities. In the vertebrate gut, microbes play an important role in development and immune function. Previously, we found that in wood-warblers, host evolutionary history plays a role in structuring the gut microbiome. This may suggest that as host populations diverge, so do their gut microbiota, either as a result of tight coevolutionary dynamics with their hosts, or reflecting differential environmental influences in allopatry, or both. Hybridization is common in warblers, but the effects of evolutionary divergence on host-microbiome dynamics during secondary contact are unclear. Further, the fitness consequences of potential host-microbiome mismatches for admixed individuals are not known.

Here, we present an analysis of gut microbiome variation across two geographically disjunct hybrid zones between Blue-winged and Golden-winged Warblers. We performed 16S amplicon sequencing of fecal samples from 123 individuals collected during the breeding season to test the hypothesis that admixture is associated with gut microbiome disruption. We found that hybrids and parental individuals harbored similar microbiome diversities, and microbiomes varied between contact zones. We identified several bacterial taxa associated with host admixture phenotype, suggesting hybrids may carry incompatible combinations of bacteria.

Bacteria in some of these genera encode pathways for carotenoid biosynthesis. Because warblers derive their colorful plumage from carotenoid pigments, this may suggest avian hosts take advantage of bacteria-derived carotenoids. However, the functional significance of species-specific bacteria and whether they impose selection on their hosts remain targets for future study.

Homoserine Lactone Autoinducer Drives Diversification of the Quorum-sensing Receptor in the Bacterial Symbiont *Vibrio fischeri*

Thursday, 4:30 - 4:50 pm

Edward A. P. Provencher[1,2], Andrew G. Cecere[1,2], Molly R. Ehrig[1], Tim I. Miyashiro[1,2]

[1] *Department of Biochemistry and Molecular Biology, The Pennsylvania State University, University Park, PA, USA.*

[2] *The One Health Microbiome Center, Huck Institutes of the Life Sciences, The Pennsylvania State University, University Park, PA, USA.*

Signaling-based interactions between bacterial cells are critical for microbiome functions. Quorum sensing is one type of signaling mechanism that enables cellular traits to be coordinated across a bacterial population. The marine bacterium *Vibrio fischeri* depends on quorum sensing for controlling bioluminescence production, which is an energetically costly trait that must be expressed during symbiosis with the Hawaiian bobtail squid Euprymna scolopes. In *V. fischeri*, LuxI synthase produces N-3-oxohexanoyl-L-homoserine lactone (autoinducer), which induces bioluminescence production by binding and activating the transcription factor LuxR. While the molecular mechanisms underlying quorum sensing are well understood, how such signaling systems evolve remains less clear.

Here we report findings from a short-term evolution experiment in which cultures of *V. fischeri* were propagated in the presence or absence of autoinducer for over 200 generations. Shortly after 150 generations, lineages constantly exposed to autoinducer produced lower bioluminescence levels under inducing conditions, and the bioluminescence profiles of individuals isolated from terminal populations validated these results. Terminal populations from lineages supplemented with autoinducer also showed enrichment of LuxR alleles predicted to disrupt LuxR function. All lineages yielded multiple individuals with defects in colonizing *E. scolopes* hatchlings, with many defective individuals displaying low or no motility, which is a trait important for *V. fischeri* to colonize the light organ.

Taken together, these results suggest that exogenous autoinducer can be a potent driver of evolution for *V. fischeri,* which has important implications in how such systems evolve in more complex microbiomes.

Microbial Metabolites Enhanced Plant-host Growth through Provision of Nutrients and Stress Removal

Thursday, 4:50 - 5:10 pm

Anna Kazarina[1], Soumyadev Sarkar[2], Bryttan Adams[1], Leslie Rodela[1], Hallie Wiechman[1], Leah Heeren[1], Nicholas Reese[1], Eli Hartung[1], Qinghong Ran[1], Ari Jumpponen[1], Loretta Johnson[1], Sonny T.M. Lee[1]

[1] *Division of Biology, Kansas State University, Manhattan, KS, USA.*
[2] *Biodesign Center for Fundamental and Applied Microbiomics, Arizona State University, Tempe, AZ, USA.*

The plant rhizosphere harbors a diverse range of the microbial species, which play integral roles in plant-host well-being. Although it is known that the plant host selectively recruits microbes from the local soil environment, our knowledge on mechanisms of communication between the plant and its rhizosphere, the functional potential of recruited microbes remain sparse.

Our study on the reciprocal gardens revealed that dry and wet *Andropogon gerardii* (Big Bluestem) ecotypes perform better at recruiting beneficial microbes when grown in their "home" environments. We thus set out to understand functions and mechanisms of these microbial populations by culturing microbial communities from their ecotypic home regions (Dry: Hays, Kansas and Wet: Carbondale, Illinois), and reciprocally inoculating them into dry and wet ecotypes for 12 weeks in the greenhouse experiment. We observed that the dry ecotype had greater biomass when inoculated with Dry inoculum microbes. We further observed that our 287 (Dry: 132 and Wet: 155) high-quality microbial genomes possessed potential functions and metabolites that may enhance the host growth by producing beneficial products and communication signals. We found that Dry inoculum microbes produce Lyso-phosphatidylethanolamine which potentially can have a priming effect and stimulate plant-host immune response. In addition, Dry inoculum microbes exuded several metabolites including Ectoine and Tetraethylene-glycol mitigating host-associated osmotic stress. The Dry inoculum microbes also biosynthesized indole-3-acetic acid hormone, 3-(Methylthio)-1-propano and Pyrocatechol potentially used in microbe communication.

This study improves our understanding of the complex mechanisms of plant host-microbe interactions, and the selective process of the rhizosphere recruitment.

Day 2. Friday

9:45 - 11.35 AM *May 31, 2024*

HUMAN HEALTH & DISEASE

SECTION 4

Keynote Speaker:

Dr. Maria Gloria Dominguez-Bello

Professor of Microbiome and Health
Department of Biochemistry and Microbiology,
Department of Anthropology
Rutgers University

Friday, 9:45 - 10:35 am

Dr. Dominguez-Bello is a Fellow of the American Academy of Microbiology and of the Infectious Disease Society of America (IDSA), as well as a member of the Editorial Board and reviewer at several scientific journals. Her work focuses on understanding human health before urbanization, and the impact of urban practices that impair the microbiome, as well as strategies for restoration. She is a founding member of the Microbiota Vault, a global initiative to preserve the diversity of the microbes relevant to human health, and to educate and to foster collaborative research with the global South to create microbiota collections in hotspots of biodiversity.

Harnessing Gut Microbes for Glycan Detection and Characterization

Friday, 10:35 - 10:55 am

Guy Townsend[1]

[1] *Department of Biochemistry & Molecular Biology, The Pennsylvania State University, University Park, PA, USA.*

Colonizing the mammalian intestinal tract requires gut bacteria to harvest transiently available nutrient pools due to fluctuations in host dietary preferences and co-resident microbial glycan synthesis. The prevalent human intestinal bacterial phylum, *Bacteroidetes*, have evolved unique transmembrane sensors that facilitate nutrient acquisition by specifically recognizing complex polysaccharides, called glycans, derived from dietary, microbial, and mucosal material found in the gut. Following detection of distinct glycans, these sensors mount rapid, dramatic, and predictable gene expression increases that facilitate corresponding glycan utilization. Although thousands of unique glycans are implied to simultaneously exist in the intestine and influence gut microbial activities, current methods are unable to elucidate bacterial-glycan interactions in complex mixtures.

To overcome these challenges, we developed a high-throughput platform that harnesses bacterial sensors to examine biologically derived glycan mixtures by coupling key regulatory elements to an anaerobic transcriptional reporter. We assembled 88 *B. thetaiotaomicron* glycan reporter strains into a high-throughput detection platform and examined dietary, microbial, and mucosal mixtures for microbial accessible nutrients. We determined that a unique signature was present in fungi but absent in other material derived from various intestinal and dietary components. Using co-regulated bacterial glycan binding proteins, we isolated an oligo-mannan from *Saccharomyces* cerevisiae and determined this glycan supports growth of gut microbial subsets.

This indicates that our platform can directly identify previously unknown bacterial substrates to support the expansion of gut microbial populations. We assert that this technology can be implemented in many other species to identify new prebiotic compounds for manipulating the gut microbiome.

Soluble Fiber Mediated Modulation of Colonic Inflammation and Colitis-associated Colon Tumorigenesis in Mice

Friday, 10:55 - 11:15 am

Sangshan Tian[1], Devendra Paudel[1], Fuhua Hao[2], Rita Castro[1], Andrew D. Patterson[2], Vishal Singh[1]

[1] *Department of Nutritional Sciences, The Pennsylvania State University, State College, PA, USA.*

[2] *Department of Veterinary and Biomedical Sciences, The Pennsylvania State University, State College, PA, USA.*

Processed foods are increasingly incorporating partially hydrolyzed guar gum (PHGG), a soluble fiber advocated as prebiotics. Guar gum, the source for PHGG, has been shown to have both beneficial and detrimental effects on colonic inflammation and tumorigenesis. These contrasting findings prompted us to investigate the effects of PHGG on colitis and colitis-associated colon carcinogenesis (CAC) in mouse models.

Mice (both sexes, 4-week-old) were maintained on PHGG (7.5% PHGG, 2.5% cellulose) or control diet (10% cellulose) for 4 weeks. Then basal groups were terminated; colitis groups were given 1.4% dextran sulfate sodium (DSS) for 7 days; CAC groups received azoxymethane (7.5 mg/kg weight, i.p.) followed by three 7-day cycles of DSS/regular water. PHGG-fed mice displayed severe colitis after DSS administration compared to the control, evidenced by loss of body weight, enlarged spleens, and heightened colonic inflammation. These differences were not observed between basal groups. PHGG feeding further resulted in extensive tumorigenesis (average 20.5% colon area) or a high mortality (~50%) but not in control groups (0% colon area and no death). *Clostridia, Bacilli*, and *Bacteroidia* were dramatically altered by PHGG from the control. The PHGG group also exhibited increases in luminal succinate and lactate production. Furthermore, diets consisting of a low dose of PHGG (2.5%; Low-phgg) were employed to mimic human diets. Low-phgg-fed mice displayed moderate inflammation and tumorigenesis between control and PHGG groups.

Our results suggested PHGG promoted colon inflammation and tumorigenesis. The results built on Low-phgg feeding trials incorporated our findings with insights into clinical applications for human nutrition.

Antimalarial Drug Interactions with the Human Gut Microbiome

Friday, 11:15 - 11:35am

Benjamin Anderson[1]

[1] *The Pennsylvania State University, University Park, PA, USA.*

Many common oral drugs elicit antibacterial effects and drug-microbe interactions in the gastrointestinal tract may limit their bioavailability. These interactions can contribute to interindividual variation in drug response due to the highly individualized nature of microbiome compositions. Limiting bioavailability is worrying as it may result in sub-therapeutic serum concentrations of the drugs leading to treatment failure, or, in the case of infectious disease treatment, the development of drug resistant microbes.

We have focused on antimalarial drugs as these compounds have high luminal concentrations, poor bioavailability, high rates of side effects, and drug-resistant malaria-causing *Plasmodium sp.* are on the rise posing a major public health threat. We hypothesize that bidirectional drug-microbe interactions between antimalarials and gut microbes shape microbiome composition and modulate the oral bioavailability of these compounds.

Through sequencing and ex vivo experiments using human fecal material, we determined that there are extensive off-target antibacterial effects of common antimalarial drug families on gut microbial communities which could be replicated in humanized gnotobiotic mice. Notably, these antibacterial effects are donor-specific. To map these interactions, we performed high-throughput screening against a diverse collection of human microbes revealing both clade and strain-specific susceptibility patterns. Through pharmacokinetic analysis performed in gnotobiotic animals, we found that microbiome composition alters drug bioavailability.

Taken together, these results show a bidirectional relationship between antimalarial drugs and the human gut microbiome. The ultimate goal of this project is to leverage understanding of drug-microbe interactions for personalized medicine and microbiota-targeted interventions to support the use of drugs we already have.

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Day 1. Friday

1:20 - 2.00 PM *May 31, 2024*

TOOLS & TECHNOLOGIES

SECTION 5

Metatranscriptomic Activity of the Gut Microbiome Drives Data-driven Precision Nutrition through Food and Supplement Recommendations

Friday, 1:20 - 1:40 pm

Eric Patridge[1], Anmol Gorakshakar[1], Matthew M. Molusky[1], Oyetunji Ogundijo[1], Ryan Toma[1], Cleo Ho[1], Nan Shen[1], Pedro Moura[1], Tiep Le[1], Cristina Julian[1], Lan Hu[1], Janelle Connell[1], Hillary Keiser[1], Grant Antoine[1], Uma Naidoo[1], Damon Tanton[1], Momchilo Vuyisich[1], Robert Wohlman[2], Guruduth Banavar[1]

[1] *Viome Life Sciences, New York, NY USA.*
[2] *Washington Gastroenterology, Bellevue, WA, USA.*

Foods and nutritional ingredients are important to human health and often involve transformation via the microbiome. We have developed an objective, integrated, and automated approach to deliver personalized food and supplement recommendations that are powered by artificial intelligence and depend on individualized molecular data (RNA) from the gut microbiome, the saliva microbiome, the human host (blood), and their interactions.

Our metatranscriptomic analyses of these samples are clinically validated, and the resulting molecular data are converted into personalized nutritional recommendations (foods and supplements) using algorithms derived from clinical research studies and domain knowledge. We describe the application of our platform to human populations with irritable bowel syndrome (IBS), focusing on the ability to use our tools and technologies for pre-diagnostic wellness applications. We present a suite of eight wellness scores which are biologically relevant to IBS and demonstrate their functionality. These wellness scores employ both principal component analysis and the KEGG orthology, and we validate them using a large independent cohort as well as independent Rome IV criteria.

Our approach with IBS has been replicated for other conditions, and we will briefly discuss results from interventional studies which show significant improvements in clinical outcomes for IBS, depression, anxiety, and type 2 diabetes.

Active Microbes in the Rhizosphere are Most Likely to Successfully Colonize Plant Belowground Structures when Probed with Bioorthogonal Non-canonical Amino Acid Tagging (BONCAT)

Friday, 1:40 - 2:00 pm

Jennifer E. Harris[1,2], Regina B. Bledsoe[1], Sohini Guha[1], Haneen Omari[2], Sharifa Crandall[3], Liana T. Burghardt[1], Estelle M. Couradeau[2]

[1] *Department of Plant Science, The Pennsylvania State University, University Park, PA, USA.*
[2] *Department of Ecosystem Science and Management, The Pennsylvania State University, University Park, PA, USA.*
[3] *Department of Plant Pathology and Environmental Microbiology, The Pennsylvania State University, University Park, PA, USA.*

Beneficial microbes have the potential to vastly improve crop growth, yield and overall plant health in agricultural systems. However, microbial inoculum are often ineffective. Major barriers to microbial management include 1) determining which microbes are contributing to beneficial functions in a complex community and 2) developing inoculum that effectively colonize the plant in natural soils.

To address these issues, we leverage a novel activity probing technique, BONCAT, to determine patterns of microbial activity and the composition of the active microbial community across the rhizosphere and plant tissues. For the first time, we successfully labelled active microbes inside plant tissues, allowing for visualization of microbial colonization in a natural microbial population. We find that microbial activity is highest inside plant tissue (~10%). Coupling with FACS (Flow cytometer assisted sorting) and 16S rRNA sequencing we determine the active and total viable cell communities are distinct across the rhizosphere and plant belowground tissues. Finally, we reveal that microbial activity in the rhizosphere was the most effective predictor of successful microbial plant tissue colonization.

We demonstrate that BONCAT implemented in plant systems can reveal key microbial taxa that colonize the plant belowground structures, which could serve to identify candidates for microbial inoculum, instead of screening culture collections or sequencing data dominated by dormant ASVs.

POSTERS

Day 1. Odd-numbered

Life Science Bridge
8:30 - 9:30 AM

1. "Temporal Dynamics of Soil Microbial Communities Following Anaerobic Soil Disinfestation"
Joe Ono-Raphel, *Penn State*

3. "Implications of the Microbiome on Wound Healing and Treatment"
Katrina Bakhl, *Penn State College of Medicine*

5. "The Microbiomes of Raw Commercial Feline Diets Reveal Risks of Seeding Households with Antimicrobial Resistance"
Yewei Tina Yu, *Cornell University*

7. "A CRISPRi System to Elucidate how Microbial Metabolic Pathways Facilitate Mammalian Intestinal Colonization"
Kailyn Winokurr, *Penn State College of Medicine*

9. "Investigating the Role of Bacteriophage on the Gut Microbiome"
Heejung Koo, *Penn State*

11. "Decoding the Polymicrobial Interactions in Bile Acid Metabolism by the Gut Microbiome"
Min Soo Kim, *Penn State*

13. "Using BONCAT to Identify Active Microorganisms During N2O Production"
Jonah Gray, *Penn State*

15. "Microbial Diversity along a Soil Water Saturation Gradient in an Andean high-altitude Peatland, Páramo Chingaza, Colombia"
Sarah Glass, *Penn State*

17. "Understanding and Manipulating Dietary Metal Bioavailability through the Gut Microbiome"
Daniela Betancurt-Anzola, *Penn State*

19. "Exploring Microbiome Modulation in Broilers Through Dietary Interventions: Probiotics and Phytobiotics as Antibiotic Alternatives"
Ana Fonseca, *Penn State*

21. "Identification of Microorganisms and Associated Enzymes from Sourdough Starter Cultures that Enhance Nutritional Attributes of Final Breads"
Ashley Ohstrom, *Penn State*

23. "Impacts of the Host Extracellular Microbiome on an Intracellular Endosymbiont"
Madangchanok Imchen, *Penn State*

Day 1. Odd-numbered

25. "Short-term Caloric Restriction Alters the Microbiome Modulating Resistance to *Clostridioides Difficile* Infection"
Jingcheng Zhao, *Penn State*

27. "*Bifidobacterium bifidum* Strain BB1 Inhibits TNF-α induced Increase in Intestinal Epithelial Tight Junction Permeability via TLR-2/TLR-6 Complex Stimulation of PPAR-g Inhibition of NF-kB p65 Activation"
Raz Abdulqadir, *Penn State College of Medicine*

29. "Oral and Gut Microbiome Profiles in Early Parkinson's Disease"
Keaton Stagaman, *23andMe, Inc.*

31. "Surveying Cattle Microbiomes and Pathobiomes from Smallholder Farms in Meru, Kenya"
Samantha Seibel, *Penn State*

33. "Effect of Processed Fermentable Fibers on Gut Microbiota Function and Colon Tumorigenesis in Mice"
Giang (Tina) Le, *Penn State*

35. "Soft Rot and Black Leg Pathogens: Developing a Metagenomics Pipeline to Identify the Species Diversity in Pennsylvania and Wisconsin"
Sadie Seaman, *Penn State*

37. "A Conserved Peptidase is Required for Glucose-mediated Cur Inhibition in Human Gut Commensal Bacteria"
Kamalesh Verma, *Penn State College of Medicine*

39. "Multidrug-resistant ESBL-producing *Klebsiella pneumoniae* ST147: a High-risk Clonal Lineage in Cat Urinary Tract Infection"
Natália Carrillo Gaeta, *University of São Paulo*

41. "Dietary Sugars Rewire Central Metabolism in Gut *Bacteroides* Species by Inhibiting the Master Regulator of Carbohydrate Utilization"
Seth Kabonick, *Penn State College of Medicine*

43. "A Paleogenomic Approach to Reconstruct Historical Responses of Coral Reefs"
Raúl A. González-Pech, *Penn State*

45. "Phage Therapy for Mitigating Bacterial Blotch Disease in *Agaricus bisporus*"
Marysabel Méndez Acevedo, *Penn State*

47. "Evaluating the Individuality and Stability of Human Gut Bacteriophage *Crassvirales*"
David Cirota, *Penn State*

Day 2. Even-numbered

Life Science Bridge
8:30 - 9:30 AM

2. "Assessing Ethnicity-associated Periodontal Health Disparities Using NHANES Oral Microbiome Data"
Crystal L. Crabb, *Penn State*

4. "Functional Genomics of Gut Microbiome Variation in Golden-winged Warbler x Blue-winged Warbler Hybrids"
Tina Nguyen, *University at Buffalo*

6. "The Living Medicine Inside Us"
K M Salim Andalib, *Khulna University*

8. "A Genomic Exploration of Coral Larvae Settling Inducing Bacteria"
Diego Lera-Lozano, *Penn State*

10. "Crop Management Style Drives the Maize Foliar Fungal Endophyte Microbiome in Central Pennsylvania "
Chelsea L. Newbold, *Penn State*

12. "Interaction of *S. Aureus* and *S. Epidermidis* when Mimicking Atopic Dermatitis Conditions"
Rhodrick Takor, *Binghamton University*

14. "Effect of Manganese on Growth of Methane-Producing Archaea"
Steven Kilmetz, *University of Connecticut*

16. "Effects of a Direct-fed Microbial Inclusion on the Gastrointestinal and Environmental Microbiomes of Turkey Toms"
Sophia Kenney, *Penn State*

18. "Acetate Metabolism by *Methanobrevibacter smithii* Using the Enzyme Acetyl-CoA Synthetase "
Alexander Poulter, *University of Connecticut*

20. "Effects of Root Anatomical Traits on Rhizosphere Microbiota Assembly"
Courtney Tharp, *Penn State*

22. "Validating Candidate Genes for Competitive Colonization of Legume Roots by Nitrogen-fixing Bacteria"
Sohini Guha, *Penn State*

24. "Microbial Seedbank Activation and Substrate-Induced Respiration"
Nina Camillone, *Penn State*

26. "Exploring the Dynamic Interplay of Biotic and Abiotic Factors in Microbial Community Coalescence"
Luana Bresciani, *Penn State*

Day 2. Even-numbered

Life Science Bridge
8:30 - 9:30 AM

28. "A Synthetic Microbiota Designed via Meta-analysis Exhibits *C. difficile* Colonization Resistance"
Susan Tian, *Penn State*

30. "Directed Evolution of Beneficial Microbial Inoculants to Improve Survival in Soil Conditions"
Keya Harshad Thumar, *Penn State*

32. "Exploring the Compositional and Functional Aspects of the Mushroom Devome"
Eoin O'Connor , *Penn State*

34. "Phage against the (machine) Biofilm"
Andres Valdez, *Penn State*

36. "Sex Differences in High-fat Diet Induced Dysbiosis"
Morgan Sotzen, *Penn State*

38. "Symbionts of Symbionts: Disentangling Symbiodiniaceae-microbiome Interactions"
Vivian Yifan Li, *Penn State*

40. "Cell Size Distribution Across a Microbial Activity Gradient in Agricultural Soil"
Luisa Robles Zaragoza, *Penn State*

42. "Coastal Environments Shape Chemical and Microbial Properties of Forest Litters in the Circum-Mediterranean Region"
Borsali Amine Habib, *University of Saida "Dr Moulay Taher" Algérie*

44. "Toward an Integrative Framework for Microbial Community Coalescence"
Gordon F. Custer, *Penn State*

46. "Farm-scale Differentiation of Active Microbial Colonizers"
Sarah Richards, *Penn State*

48. "Multi-omic Analyses Demonstrate the Functioning of a Surface Film-forming Consortium is Altered by Interactions with Resident Soil Community Members"
Ryan Trexler, *Penn State*

50. "Integrating Geospatial and Activity-Based Intelligence to Uncover Microbiome Dynamics"
Camelia Kantor, *Penn State*

52. "Unearthing Phage-Bacteria Interactions in Model Soil Bacteria"
Marissa Gittrich, *The Ohio State University*

Poster Session

INTEGRATED ONE HEALTH

SECTION 1

Acetate Metabolism by *Methanobrevibacter smithii* Using the Enzyme Acetyl-CoA Synthetase

18

Alexander Poulter[1], Michel Geovanni Santiago-Martinez[1], Jack Rodriguez[1], Isis Delmazo Monsalvo[1], Steven Kilmetz[1]

[1] *Department of Molecular and Cellular Biology, University of Connecticut, Storrs, CT, USA.*

In microorganisms, acetyl-CoA synthetase is an enzyme that catalyzes the first step of acetate metabolism and conversion into acetyl-CoA to be used in anabolic pathways. In *Methanobrevibacter smithii*, a host-associated methane-producing anaerobe from the Archaea domain, it is hypothesized that acetate is the sole carbon source for anabolic pathways since it doesn't have genes encoding proteins required for use of metabolic intermediates from carbon dioxide-reducing methanogenesis. This key enzyme hasn't been characterized, so my project involves the isolation and kinetic analysis of this enzyme to better understand its physiological role in the metabolism of *M. smithii.*

Exploring the Dynamic Interplay of Biotic and Abiotic Factors in Microbial Community Coalescence

26

Luana Bresciani[1,2], Gordon F. Custer[1,2], Francisco Dini-Andreote[1,2]

[1] *Department of Plant Science & Huck Institutes of the Life Sciences, The Pennsylvania State University, University Park, PA, USA.*

[2] *The One Health Microbiome Center, Huck Institutes of the Life Sciences, The Pennsylvania State University, University Park, PA, USA.*

Community coalescence is defined as the wholesale mixing of ecological communities. After a coalescence event (e.g., fecal microbiota transplantation, organic amendments in soils), a dynamic interplay of biotic and abiotic factors controls the patterns of community reassembly and stabilization over time. However, we still lack a comprehensive framework to properly parametrize and study compositional and functional outcomes of community coalescence.

Here, we use a soil microcosm experimental system to parametrize the dynamics of microbiota reassembly using time-series analysis. For that, distinct soil types varying in biological and physicochemical properties (i.e., with distinct edaphic and eco-evolutionary histories) were experimentally manipulated using a dilution-to-extinction approach and mixed in pairs. Reassembly of the soil microbial community was tracked over time (i.e., 1, 5, 15, and 30 days) following coalescence. We used a combination of bacterial 16S rRNA high-throughput sequencing, Biolog EcoPlate assays, and a series of enzymatic essays to track temporal shifts in community taxonomy, metabolic profile, and enzymatic activities, respectively.

Overall, our results revealed that microbial diversity affects the relative dominance of donor communities on community reassembly and that donor dominance is strongly associated with the presence of stringent abiotic factors in the donors' home environment. Moreover, we detected an overall decrease in metabolic functions following coalescence, despite a slight increase in enzymatic functioning at high-diversity treatments. Collectively, this study provides a new model that partitions the dominance of donor biotic and abiotic factors that determine the compositional and functional outcomes following community coalescence.

Surveying Cattle Microbiomes and Pathobiomes from Smallholder Farms in Meru, Kenya

31

Samantha Seibel[1], Stephanie Bierly[1], Elizabeth Ransom[1], Frank Onyambu[1], Joan Simam[1], F. Thiakunu[1], G. Kinoru[1], Chege Kariuki[1], Patrick Mutharia[1], James Mutunga[1], Kerry Kaylegian[1], Edward Dudley[1], Nkuchia M'ikanatha[1], Japhet Magambo[1], Erika Ganda[1]

[1] *The Pennsylvania State University, University Park, PA, USA.*

Dairy production and consumption are rapidly increasing in Sub Saharan Africa; there are an estimated five times more dairy cows than people. As dairy is incorporated into the diet, there is increased concern for the spread of zoonotic pathogens. While manufacturers of milk and cheese are improving their food safety protocols for mass distribution, there is not a lot of surveillance occurring at the small farm level, also known as smallholders.

This gap may shroud potential disease in the populations that do not consume pasteurized dairy products. Therefore, the introduction of pathogens via raw dairy may contribute to the spread of diseases like tuberculosis, salmonellosis, brucellosis, etc. Variables measured to examine the effect on the microbiome and pathobiome of cows included herd size, sterilization techniques (pre- and post-dip of iodine), and most recent use of antimicrobials.

The study sampled 24 different farms located outside of Meru County, Kenya. Alpha and beta diversity of taxa remained unchanged for most conditions except for a significant difference in variance of alpha diversity for farms with 6-20 cows and farms with more than 20 cows. Future work entails examining farms extended outside of Meru, specifically the nomadic Maasai cattle, examining the consumption of raw milk to disease prevalence, and monitoring the spread of disease between wildlife, cattle, and humans.

Effect of Processed Fermentable Fibers on Gut Microbiota Function and Colon Tumorigenesis in Mice

33

Sangshan Tian[1], Giang V. Le[1], Fuhua Hao[2], Madangchanok Imchen[3], Seth R. Bordenstein[3], Andrew D. Patterson[2], Vishal Singh[1]

[1] *Department of Nutritional Sciences, The Pennsylvania State University, University Park, PA, USA.*

[2] *Department of Veterinary and Biomedical Sciences, The Pennsylvania State University, University Park, PA, USA.*

[3] *Departments of Biology and Entomology, One Health Microbiome Center, Huck Institutes of the Life Sciences, The Pennsylvania State University, University Park, PA, USA.*

Processed fermentable dietary fibers (p-FDFs), such as partially hydrolyzed guar gum (Phgg), are frequently incorporated into packaged foods. Despite demonstrating benefits in healthy individuals, p-FDFs (inulin and Phgg) are shown to exacerbate intestinal inflammation-associated colon carcinogenesis. This study investigates whether p-FDF Phgg promotes colon tumorigenesis independent of intestinal inflammation.

Four-week-old wild-type (WT, C57BL/6) mice received eight azoxymethane (AOM, a carcinogen) injections over eleven weeks while fed either a diet containing cellulose (control) or Phgg. Notably, the Phgg group exhibited increased colonic tumorigenesis. In contrast, no tumors were observed in the control group receiving non-fermentable fiber cellulose, suggesting that a fiber fermentation-mediated shift in gut microbiota activity promotes colon cancer development.

To test this hypothesis, we assessed alterations in microbiota composition and metabolites following intervention with control or Phgg for 5 weeks. Relative to the control, Phgg significantly reduced alpha diversity and markedly enriched the genus *Bifidobacterium* (>40-fold, phylum: *Actinobacteriota*). Additionally, the Phgg group displayed an increased abundance of specific genera associated with *Firmicutes* phylum. Furthermore, quantitative 1H-NMR metabolome analysis of cecal content revealed luminal accumulation of the intermediate metabolites, succinate and lactate in Phgg group. In agreement, we observed increased abundance of genera encoding genes for succinate and lactate production.

Collectively, our data suggest that Phgg-induced inordinate expansion of Bifidobacterium and luminal accumulation of intermediate metabolites are potentially associated with increased susceptibility to colon tumorigenesis. However, the causal relationship between Phgg-mediated microbiome alterations and colon cancer development warrants further investigation.

Phage Against the (machine) Biofilm

34

Andres Valdez[1], Bruce Levin[2], Igor Aranson[3]

[1] *Department of Biomedical Engineering, The Pennsylvania State University, University Park, PA, USA.*
[2] *Department of Biology, Emory University, Atlanta, GA, USA.*
[3] *Departments of Biomedical Engineering, Chemistry, and Mathematics, The Pennsylvania State University, University Park, PA, USA.*

Bacteria form self-organized communities on physical structures known as biofilms. While the colony gains optimal nutrient consumption and waste removal the spatial heterogeneity provides resistance to external changes. This resistance motivates our study. We use phages to annihilate bacteria biofilms. We show our numerical simulation results willing to discuss a (the most) effective way to kill a microcolony.

Multidrug-resistant ESBL-producing *Klebsiella pneumoniae* ST147: a High-risk Clonal Lineage in Cat Urinary Tract Infection

39

Victoria Tiemi Sorbello Sakauchi[1], Amanda Haisi[2], João Pessoa Araújo Júnior[2], Marcos Bryan Heinemann[1], Natália Carrillo Gaeta[1,3]

[1] *Department of Veterinary Medicine and Animal Health, University of São Paulo, São Paulo, SP, Brazil.*
[2] *State University of São Paulo, Botucatu, SP, Brazil.*
[3] *Veterinary Medicine, Faculdades Integradas Campos Salles, São Paulo, SP, Brazil.*

Urinary tract infections are pervasive in human and veterinary medicine, notably affecting companion animals. These infections frequently lead to the prescription of antibiotics, contributing to the rise of antimicrobial-resistant bacteria. This escalating concern is underscored by the emergence of a previously undocumented case: a broad-spectrum cephalosporin-resistant *K. pneumoniae ST147* strain denoted USP-275675, was isolated from a cat with urinary tract infection in Brazil.

Characterized by a multidrug-resistant profile, whole genome sequencing exposed several antimicrobial-resistance genes within this strain, notably blaCTX-M-15, blaTEM-1B, blaSHV-11, and blaOXA-1. Recognized as a high-risk clone, the ST147 has historically disseminated globally and is frequently associated with carbapenemases and extended-spectrum ^{2}-lactamases. Notably, the core-genome phylogeny of *K. pneumoniae ST147* strains isolated from urine samples revealed a unique aspect of the USP-276575 strain. Unlike its counterparts, it did not cluster with other isolates. However, a broader examination incorporating strains from both human and animal sources unveiled a connection between USP-276575 and a Portuguese strain from chicken meat. Both were part of a larger cluster of ST147 strains spanning various geographic locations and sample types, sharing commonalities such as IncFIB or IncR plasmids.

This elucidates the multidrug resistance signature inherent in widespread *K. pneumoniae ST147* strains carrying these plasmids, highlighting their pivotal role in disseminating antimicrobial resistance. Finally, the discovery of the high-risk clone *K. pneumoniae ST147* in a domestic feline with a urinary tract infection in Brazil highlights the urgent necessity for thorough antimicrobial resistance surveillance through a One Health approach.

Dietary Sugars Rewire Central Metabolism in Gut *Bacteroides* Species by Inhibiting the Master Regulator of Carbohydrate Utilization

41

Seth G. Kabonick[1], Jennifer L. Modesto[1], Kamalesh Verma[1], Victoria H. Pearce[1], Eduardo A. Groisman[2], Guy E. Townsend II[1]

[1] *Department of Biochemistry and Molecular Biology, Penn State College of Medicine, Hershey, PA, USA.*
[2] *Department of Microbial Pathogenesis, Yale School of Medicine, New Haven, CT, USA.*

Various human diseases are associated with altered gut microbiota compositions, which potentiate important roles for microbial products in health and development. Identifying microbial pathways that can be pharmacologically targeted offers a promising avenue to manipulate the intestinal microbiota and its products to improve human health. Members of the dominant gut bacterial phylum, *Bacteroidetes*, encode a conserved transcription factor called Cur that promotes intestinal occupation by coordinating expression of colonization factors and nutrient utilization genes. Host consumption of excessive dietary glucose and fructose, abundant additives in industrialized diets, rapidly and dramatically silence Cur-dependent products in the gut.

We elucidated how dietary sugars inhibit Cur activity in *Bacteroides thetaiotaomicron* (Bt) by introducing a cur-dependent transcriptional reporter into mutants defective for discreet steps in glucose and/or fructose metabolism. Here, we determined that a distinct phosphofructokinase responsible for ATP-dependent fructose-1,6-bisphosphate (FBP) synthesis produces excess FBP during growth in the presence glucose and fructose, which facilitates Cur inhibition. Additionally, we determined that this enzyme is completely dispensable for in vitro growth, which alternatively requires pyrophosphate-dependent FBP synthesis mediated by a distinct enzyme. Strikingly, mutants defective for ATP-dependent FBP synthesis exhibit no intra-intestinal fitness defects when Cur is absent, suggesting that FBP largely serves a regulatory role rather than an energetic one.

Our data establish the molecular basis by which glucose and fructose hinder intestinal colonization by gut commensal bacteria illustrating the consequences of dietary choices gut microbial composition and metabolism.

Poster Session

AGRICULTURAL HEALTH

SECTION 2

Temporal Dynamics of Soil Microbial Communities Following Anaerobic Soil Disinfestation

1

Joe Ono-Raphel[1,4], Francisco Dini-Andreote[1,2,4], Gordon F. Custer[1,4], Kathleen Arrington[3], Raymond Balaguer[1], Jason Kaye[3], Erin Rosskopf[5], Francesco Di Gioia[1]

[1] *Department of Plant Science, The Pennsylvania State University, University Park, PA, USA.*
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Anaerobic Soil Disinfestation (ASD) is an agricultural management practice used to control soilborne pathogens. This practice is used in specialty cropping systems, and it is based on the principle of carbon-rich amendments and temporal water saturation in the topsoil layer.

In this study, we implemented a field trial to evaluate the efficiency of distinct carbon sources – namely, crimson clover, triticale, and their mix as sole carbon sources or in combination with wheat middlings – on ASD efficacy and soil microbial community dynamics. Soil samples were collected at distinct time points during ASD (i.e., a total period of 21 days), and after ASD (i.e., initial stages of tomato planting). At each time-point, soil samples were subjected to physicochemical measurements and bacterial community profiling via 16S rRNA gene amplicon sequencing. Our results show that ASD treatments induced variations in soil redox potential, electrical conductivity, and pH. Pairwise treatment comparisons revealed that the amendment with wheat middlings had a direct influence on the bacterial composition up to 21 days post-treatment. We also found such variation to be mostly associated with shifts in soil pH, potentially mediated by the production of organic acids during the anaerobic phase of ASD. Most interestingly, after 21 days post-treatment, soil bacterial communities showed a convergent pattern across treatments, becoming not statistically different after tomato planting (ca. 55 days after ASD).

Taken together, our study provides new insights into the dynamics of soil biotic and abiotic factors during ASD and highlights the absence of legacy impacts of ASD in soils.

Crop Management Style Drives the Maize Foliar Fungal Endophyte Microbiome in Central Pennsylvania

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Foliar fungal endophytes are fungi that live asymptomatically within leaves and are major players in crop health as beneficials that can ameliorate plant stress or cause disease. It is important to empirically test how organic crop management practices shape foliar fungal endophytic communities, specifically potential pathogens, in maize production systems where disease pressure is high because synthetic fungicides and transgenic seed are not used.

Here we investigate how organic production of maize, with the application of cover crops, shapes the foliar fungal endophyte community, with a focus on the important pathogenic genus, *Fusarium spp.* The fungal microbiome and *Fusarium spp.* specific community members were characterized from leaves of field-grown maize under conventional and organic production and cover crop monocultures: crimson clover (*Trifolium incarnatum*), triticale (*Triticosecale*), and radish (*Raphanus sativus*). We sampled asymptomatic tissues across host development from vegetative to senescence. Results indicate that management practice and host development are drivers of maize foliar fungal endophyte communities; taxonomic composition was unique between production styles (PERMANOVA, r2= 0.09, p-value 0.05) and developmental stages (PERMANOVA, r2= 0. 0.04, p-value 0.05).

Preliminary analyses suggest, however, that *Fusarium spp.* subcommunities are not driven by host development, nor management practices. This may indicate that *Fusarium spp.* serve diverse ecological functions beyond disease-causing agents driven by different host and agronomic conditions. Future research is needed to control for the variable of host genotype, a known driver of endophytes, which may be a contributing factor toward the maize leaf fungal endophytic microbiome.

Using BONCAT to Identify Active Microorganisms During N_2O Production

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Nitrous oxide (N_2O) is a potent greenhouse gas with a warming potential ~300 times higher than CO_2 and is produced by incomplete denitrification and as a byproduct of nitrification. Denitrification is mediated by fungi, archaea and bacteria that each utilize different nitrogen species along the denitrification pathway to produce energy when oxygen is unavailable. Incomplete denitrification occurs because nitric oxide (NO), a higher yielding electron acceptor, inhibits the use of the lower yielding N_2O. Although we know many of the species that denitrify and the enzymes associated, there is little known about which taxa and what percentage of microorganisms are active under optimal conditions for N2O production.

We will be conducting a microcosm experiment where soil is incubated in anaerobic vials with added carbon and nitrogen for 12-16 hours while tracking N_2O production. Using Bioorthogonal Non-canonical Amino Acid Tagging (BONCAT), we will probe the active microorganisms and correlate the active taxa with N_2O production. Before peak N_2O production, a methionine analog will be added to the vials, and active organisms will incorporate it into proteins. Flow cytometry activated cell sorting will isolate active cells and 16S rDNA library will be built to correlate active microbial community structure to N_2O production.

This experiment will provide a basis for making future agricultural management decisions to reduce the activity of N_2O producing microorganisms.

Effects of a Direct-fed Microbial Inclusion on the Gastrointestinal and Environmental Microbiomes of Turkey Toms

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Probiotic supplementation in poultry production has been increasingly explored for its potential health and growth promotion. Specifically, *Bacillus subtilis*-based direct-fed microbial (DFM) inclusions have been shown to promote weight gain, pathogen control, and beneficial changes to intestinal morphology. Given this, we sought to determine 1) the effects of a *B. subtilis*-based DFM on the ileal, cecal, and cloacal microbiomes of turkeys and 2) the impact of the DFM on their environmental microbiomes. Additionally, we evaluated if boot sock environmental sampling was representative of gastrointestinal microbiome changes.

To this end, 720 Nicholas Select turkey toms (N=360/treatment) were reared on a conventional diet or a *Bacillus subtilis*-supplemented diet over a 19-week period. Samples were collected using cloacal, cecal, and ileal swabs on the last day of study as well as from the pens in which the birds were housed using boot sock covers. Following targeted sequencing of the V4 region of the 16s rRNA gene, microbial taxonomic assignment was performed.

Between control and probiotic-supplemented groups, no significant differences in microbiome composition or diversity were observed. Significant differences in alpha diversity (p-value 0.01) and beta diversity (PERMANOVA, p.adj = 0.006) were observed between sample types. Across all samples, *Firmicutes*, *Bacteriodota*, and *Actinobacteriota* were the most abundant phyla. In this study, the use of DFM supplementation did not significantly impact the host- or environmental-associated microbiomes. Observed microbiome composition is, however, impacted by the choice of sampling method, with boot sock environmental sampling being non-representative of microbiome changes within the gastrointestinal tract.

Exploring Microbiome Modulation in Broilers Through Dietary Interventions: Probiotics and Phytobiotics as Antibiotic Alternatives

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The poultry industry is evolving towards antibiotic-free production to meet market demands and decelerate the increasing spread of the antimicrobial resistance. Successful approaches for microbiome manipulation including the use of feed supplements (e.g., probiotics and phytobiotics) in broilers diet are promising alternatives to enhance poultry health and growth performance.

The goal of our study was to explore the potential of chickens' microbiome modulation through dietary interventions focus on the effects of a probiotic and an essential oils blend across two feeding phases (starter and grower). A total of 320 Cobb 500 (1-day-old) chicks were raised for 21 d in 32 randomly allocated cages. Treatments consisted of 4 experimental diets: a basal diet, and a basal diet mixed with an antibiotic (bacitracin methylene disalicylate), an essential oils blend (oregano oil, rosemary, and red pepper), or a probiotic (Bacillus subtilis). Droppings were collected daily (1–21 days) to characterize broilers' microbiota by targeted sequencing of the bacterial 16S rRNA gene. Feed supplements did not affect alpha diversity but did impact microbial beta diversity. Feeding phases impacted microbiota turnover as differences in alpha and beta diversity were detected. Furthermore, when looking into the specific taxa, *Enterococcus* and *Escherichia-Shigella* were relatively more abundant in the starter phase while *Lactobacillus* and *Faecalibacterium* relatively more abundant in the grower phase.

Overall, probiotic supplementation but not essential oils supplementation directly caused directional shifts in broilers' microbiota structure. The findings suggest that strategic manipulation of the diet through probiotics are promising approach to optimize chicken health.

Effects of Root Anatomical Traits on Rhizosphere Microbiota Assembly

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The micro-scale physicochemical gradients in the plant rhizosphere are directly determined by plant-root traits and the exudation of root-derived metabolites. These gradients affect the assembly and functioning of root-associated microbiota. A large body of the literature has been focusing on studying the effects of distinct plant genotypes on the rhizosphere microbiota. However, relatively less attention has been given to elucidating the fine-scale mechanisms determining the perception and metabolisms of rhizosphere-competent microbes. Also, the extent to which root traits (individual units of the phenotype) impact the chemistry and biology of the rhizosphere remains largely elusive.

This project investigates the role of root anatomical traits (cortical cell size, density), altered by polyploidization and domestication, in shifting root exudate metabolites and the assembly of the rhizosphere microbiota. Here, we used a collection of wheat lines varying in ploidy levels (diploid, tetraploid, and hexaploid) and domestication statuses (domesticated vs. wild). These genotypes display a gradient in root anatomical trait parameters. The initial experiment consisted of growing 320 plants encompassing 16 genotypes for a period of 21 days (the point of phene expression). Sample collection consisted of root and rhizosphere samples to profile the microbiota inside and outside the root, in addition to root metabolites and anatomical analysis. Samples are currently being analyzed with laser ablation tomography (root anatomy), mass spectrometry (metabolomics), and DNA sequencing (microbial community).

Taken together, this project will elucidate how distinct root anatomical traits affect root-exudate metabolites that act as resources for the recruitment and functioning of root-associated microbiota.

Identification of Microorganisms and Associated Enzymes from Sourdough Starter Cultures that Enhance Nutritional Attributes of Final Breads

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Sourdough bread is made using flour and water that is leavened with wild bacteria and yeast. Commercial bread is leavened with a single yeast strain, Saccharomyces cerevisiae. In a sourdough starter microbiome, the diversity and abundance of bacteria and fungi can shape the structure of communities and subsequent function.

Previously, we demonstrated that 20 distinct sourdough starter microbiomes significantly influence the physical and chemical properties of final bread. One limitation to the previous study is how sourdough starter microbiomes affect the nutritional attributes of final bread. Preliminary results suggest that sourdough microbiomes can alter free amino acid levels in dough, and distribution of low-, medium-, and high molecular weight gliadin peptides in final breads.

Here, we seek to identify microorganisms and associated enzymes that enhance nutritional attributes of final breads. Of particular interest are microorganisms that can hydrolyze gluten. Enzymes for hydrolyzing gluten have been characterized in sourdough bacterial populations, yet the role of fungal enzymes in this process remains less explored and understood. We hypothesize that fungal communities could be strong drivers of biochemical properties of final breads compared to bacteria.

Using the predictive functional program PICRUSt, we identified enzymes across 20 sourdough microbiomes with potential to hydrolyze gluten. Metagenomic sequencing of 20 sourdough microbiomes further support functional genes and pathways present in microbial communities with the ability to hydrolyze gluten. Outcomes from this work could help with developing fermentation technologies that will address the next generation of consumer demands for high-quality “clean label” products with reduced gluten immunogenicity.

Microbial Seedbank Activation and Substrate-Induced Respiration

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Soil microorganisms carry out many processes that are fundamental to soil functions. Among the millions of bacterial cells present in a gram of soil, however, less than 2% are commonly estimated to be active at any point in time. Because the respiratory response of a bulk soil to carbon substrate addition would be expected to reflect the number of active cells, we hypothesized a positive correlation between active cells and soil respiration rates during substrate-induced respiration (SIR) assays.

To test this, we monitored respiration and active bacterial cell counts during 24-h incubations of agricultural soil subsamples after treating with two carbon substrates or a water-only control. We enumerated active bacterial cells with the Bioorthogonal Non-canonical Amino Acid Tagging (BONCAT) method. BONCAT provides a labeled amino acid for active cells to incorporate into newly synthesized proteins, which can then be tagged with a fluorescent dye to enable enumeration by flow cytometry. Both respiration rates and active cell counts increased over time and were positively correlated with each other after 6 h of incubation. After 24 h, however, increases in active cells were proportionally greater than increases in respiration. Additionally, carbon-amended soils had higher respiration rates than water-only soils with similar active cell counts, suggesting differences in carbon use efficiency.

Our results indicate that the number of translationally active bacteria is an important but not sole contributor to observed bulk soil respiration.

Exploring the Compositional and Functional Aspects of the Mushroom Devome

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The impacts of microorganisms as partners in host developmental crop phenotypes is an emerging field of study. The term devome (developmental microbiome) describes microbiomes that are necessary or contribute to the development of a host organism. Cultivation of *Agaricus bisporus* involves changes in the compost and casing microbiome which are crucial for productive mushroom development. The casing layer is host to beneficial bacteria that are crucial for this development.

We compositionally and functionally characterized the casing devome using metagenomics and metaproteomics. Casing material was manipulated by collecting the substrate at the point of pinning (mushroom primordia initiation) and adding it to a fresh standard casing substrate (ratio 1:10). The passaged casing triggered early pinning of mushrooms and was also suppressive to a common commercial disease, bacterial blotch. Metaproteomics revealed among the most highly represented phyla were the *Pseudomonadota*, *Bacteroidota*, and *Bacillota*. Biological processes related to monoatomic ion transport, transmembrane transport and cell adhesion were highly represented among taxa. Differential protein abundance analysis revealed that nearly 10% of protein abundance was related to TonB receptors. Their role in casing is hitherto unknown.

Taken together, manipulating the casing devome offers advantages in shorter cropping cycles and suppression of blotch. The overall aim of this work is to identify the compositional and functional role of a microbial cohort from passaged casing that may have benefits commercially. While our understanding of devomes is still in its infancy, their intersection in microbiome sciences and developmental biology offers new prospects into the field of crop production.

Soft Rot and Black Leg Pathogens: Developing a metagenomics pipeline to identify the species diversity in Pennsylvania and Wisconsin

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Potatoes (*Solanum tuberosum*) are the top tuber vegetable produced globally and the United States stands as the fourth-largest producer. In the US, Wisconsin is one of the top potato producers and a leader in seed certification; while Pennsylvania focuses on chip manufacturing. Soft rot and blackleg, caused by the seed-borne pathogenic bacteria in the *Pectobacteriaceae*, continue to threaten the US potato industry. However, current molecular diagnostic tools are limited to only detect known species. Understanding the diversity of pathogens present can be a crucial factor in improving disease diagnosis and surveillance.

We used metagenomics to evaluate the potential impact of these pathogens in symptomatic soft rot tissue from Pennsylvania and Wisconsin. Plant metagenomic methods can capture DNA of all bacterial species present in a sample. We analyzed potato microbiomes using short-read sequencing collected from both states. We used two approaches to recover all reads associated with soft rot pathogens, using Kraken2 and BWA. We created a tailored database with whole genomes of 20 species of Pectobacterium and 11 species of *Dickeya* to describe soft rot pathogen diversity in Pennsylvania and Wisconsin. Understanding the differences in diversity is crucial for developing state-specific strategies for disease management.

The findings may contribute to the broader field of agricultural microbiology by developing microbiome analysis for disease diagnostics and surveillance.

Cell Size Distribution Across a Microbial Activity Gradient in Agricultural Soil

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Cell size is a fundamental trait that is key to determine a microbe's energy maintenance requirements, nutrient acquisition, growth rate, and waste release. Studies in aquatic systems have shown a reduced cell size for nutrient deprived microbes and the opposite in resource-rich environments.

However, there is a gap of knowledge for agricultural systems that have undergone decades of soil disturbance and degradation affecting biological activity. It is established that the majority of soil bacteria are less than 0.5µm in diameter; however, it is unknown if an increase of activity can change cell size.

This study aims to explore the relationship between soil microbial activity and cell size distribution to answer if we can use cell size as a novel biological indicator for soil health. We hypothesized that agricultural soils with higher induced microbial activity will harbor a higher abundance of bacteria and archaea with larger cell sizes. To capture microbial activity, we measured substrate induced soil microbial respiration with the addition of glucose and nitrate. To measure bacterial abundance and cell size, we used flow cytometry. We used 0.5, 1, and 2µm size beads as a proxy for cell size distributions calibrated against a sequential filtering of cells with 0.4, 0.6, 0.8, 1.2, 2, and 5µm filters.

We found that contrary to our hypothesis, more active soils did not have larger cell sizes. We instead found constant cell size fractions across treatments which have implications for soil microbial ecology and carbon use efficiency.

Phage Therapy for Mitigating Bacterial Blotch Disease in *Agaricus bisporus*

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Agaricus bisporus, a highly sought-after edible mushroom, faces quality challenges due to bacterial infections, notably blotch disease caused by *Pseudomonas* species like *P. tolaasii*. This research explores environmentally acquired phages as a potential biological solution to counteract the impact of bacterial blotch.

To assess this effect 72 *A. bisporus* mushrooms were treated with eight phage-bacteria combinations. The bacterial load was examined on day 0 and day 3 by extracting the infected section of the mushrooms and inoculating them in King medium agar with novobiocin, penicillin, and cycloheximide. The progression of the disease was assessed from day 1 to day 3 through visual inspections of the mushrooms, employing a disease scale. Both tests were statistically analyzed by using analysis variance (ANOVA) and post hoc Tukey test. In the initial trial, the BP1230+1210 phage and strain combination exhibited significant efficacy against the BP1230 strain ($p<0.05$). However, this effect was not replicated in the second trial. In addition, in both trials, there was no significant difference between the disease progression per day and the treatment.

These results suggest the BP1230+1210 phage and strain combination hold biocontrol potential against bacterial blotch in white cap mushrooms.

Farm-scale Differentiation of Active Microbial Colonizers

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Microbial movement is important for replenishing lost soil microbial biodiversity and driving plant root colonization, particularly in managed agricultural soils, where microbial diversity and composition can be disrupted. Despite abundant survey-type microbiome data in soils, which are obscured by legacy DNA and microbial dormancy, it is not well understood how active microbial pools are shaped by local soil properties and agricultural management at differing spatial scales.

To determine how active microbial colonizers are shaped by spatial scale and environmental conditions, we buried microbial traps (sterile soil enclosed by permeable mesh membranes) under 11 cover crop treatments in a randomized block design within an organically managed agricultural research farm. Bulk soil and deployed traps were collected for 16S rRNA gene and fungal ITS amplicon sequencing at two time-points to identify early re-colonization and community development.

We hypothesized that 1) different cover crop mixtures, either single- or multi-species, would stimulate distinct pools of active microbial colonizers, and 2) assessing recolonized pools would be a more sensitive method for detecting plant influence on microbial composition compared to bulk. Briefly, we found that the presence or absence crop cover was the most significant driver of compositional differences in our bacterial active colonizers across treatments, demonstrating the potential for cover crops to substantially influence soil microbiome trajectories, even within the span of a single growing season.

Understanding how environmental constraints and spatial scales impact microbial recolonization dynamics and community assembly are essential for identifying how farm management can be used to intentionally shape agricultural microbiomes.

Unearthing Phage-Bacteria Interactions in Model Soil Bacteria

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Bacteriophages (phages) play a pivotal role in driving nutrient and energy cycling across various ecosystems, yet comprehensive model systems for studying phage-bacteria interactions, particularly in soil environments, are scarce.

In this study, we isolated 48 double-stranded DNA phages infecting three soil-derived, plant-growth promoting rhizobacteria *Klebsiella sp. M5a1* (n=24), *Pseudomonas simiae* (n=13), and *Paraburkholderia phytofirmans* (n=11). These phages were used to challenge three genome-wide loss-of-function transposon mutant libraries (RB-TnSeq). Our analysis identified 133 bacterial genes necessary for at least one phage. These genes encompass diverse functionalities, including carbon cycling, amino acid biosynthesis, global regulators, and surface proteins. Furthermore, we investigated whether these bacterial gene requirements were based on phylogenetic relatedness. Our results indicate that phages belonging to the same species and genus (>95% and >70% Average Nucleotide Identity (ANI), respectively) generally exhibited similar requirements for bacterial genes.

However, there was a divergence in genetic requirements at the family level. Discrepancies in bacterial gene requirements at the family level were further scrutinized through comparative analysis of phage genomes, revealing unique genes that potentially account for these differences.

Collectively, our findings lay the groundwork for developing predictive models to elucidate similarities and differences in phage infections, with implications for both environmental and therapeutic contexts.

Poster Session

ENVIRONMENTAL HEALTH

SECTION 3

Functional Genomics of Gut Microbiome Variation in Golden-winged Warbler x Blue-winged Warbler Hybrids

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Birds, like other vertebrates, harbor essential gut microorganisms crucial for development and well-being. Previous 16S amplicon sequencing revealed that colorful warbler species exhibit variable gut microbial community compositions. Despite this, frequent natural hybridization among warblers, including Blue-winged and Golden-winged warblers, occurs.

Hybridization may offer advantages, for example by potentially exposing individuals to more beneficial symbiotic partners. Notably, we found that certain bacteria abundances, such as *Sphingomonas sp.*, correlate with host ancestry and feather coloration. We hypothesize that birds utilize carotenoid (yellow) pigments produced by gut bacteria, potentially mediated by the host gene, BCO2.

Here, we use metagenomic sequencing to delineate gut microbiome taxa unique to each host species and to unravel the potential functions carried out by the gut microbiome. Additionally, we ask whether the distinct microbiomes of parental species exhibit similar or divergent functions. Lastly, we explore the role of host genetic factors in mediating host-microbiome interactions. Altogether, our data yield important insights on functional aspects influenced by hybridization in the context of host-microbiome interactions.

The Microbiomes of Raw Commercial Feline Diets Reveal Risks of Seeding Households with Antimicrobial Resistance

5

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Bone and raw food diets for pets are now widely commercially available. Previous studies on the risks and benefits have focused on frozen products for dogs despite the growing popularity of freeze-dried products, especially amongst cat owners.

To profile the microbial communities present in these products, a collection of commercial frozen, refrigerated, and freeze-dried raw meat foods were compared to conventional foods using both aerobic culture and direct molecular testing. A total of 112 products were purchased and tested, yielding bacterial isolates almost exclusively from raw and freeze-dried foods. Genera cultured included *Salmonella, Escherichia, Klebsiella, Enterobacter,* and *Cronobacter. Carbapenem*-resistant *Pseudomonas aeruginosa* and *Stenotrophomonas maltophilia* were isolated from frozen raw products. Metabarcoding approaches were carried out on the food homogenates to assess prokaryotic and eukaryotic diversity, including confirmation of protein source labeling.

By 16S rRNA, *Clostridium sensu stricto 1* and *Pseudomonas* were correlated with raw products, while *Bacillus* was correlated with conventional processing. Deep multiplex amplicon sequencing yielded significantly higher presence of antimicrobial resistance genes in raw compared to conventional foods.

The presence of pathogenic species and high load of resistance genes in raw commercial food products suggests a considerable risk to cats and the families who care for them.

A Genomic Exploration of Coral Larvae Settling Inducing Bacteria

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Three bacterial strains were extracted from crustose coralline algae known to promote the settling of coral larvae. In this project we explored using nanopore technology to sequence the entire bacterial genome, then assemble and annotate it with the goal of finding genes that relate to the generation of known chemical cues for larval settlement.

Effect of Manganese on Growth of Methane-Producing Archaea

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Understanding the adaptability of microorganisms in harsh environments is essential to study the effects of metals on biological processes. Here, we use a marine methanogen from the domain Archaea, *Methanosarcina acetivorans*, to study cellular responses to manganese. We investigate the tolerance of this microbe to high manganese concentrations, a mining pollutant that is also found naturally in coastal sediment.

We tested the effects of a water-soluble form of manganese, manganese (II) chloride, on cell growth using three concentrations: 0.25 mg/mL, 0.5 mg/mL, and 1.0 mg/mL. The cells were cultivated in oxygen-free conditions using synthetic seawater to simulate physiological conditions. We measured absorbance over the course of five weeks to quantify cell growth alongside other microbial parameters such as methane production and quantification of remaining manganese from the spent media.

Our results suggest that *Methanosarcina acetivorans* can grow in presence of manganese. Cells grew in all concentrations of manganese, although they grew faster in 0.25 mg/mL and 0.5 mg/mL manganese compared to the cultures without any manganese. Meanwhile, the 1.0 mg/mL cultures gave mixed results; in two samples, cells grew faster than in control conditions, but there was virtually no growth in the other 1.0 mg/mL culture. This variability underscores the complex relationship between manganese and cell growth, emphasizing the need to further investigate mechanisms behind *M. acetivorans'* tolerance to high amounts of manganese.

Our findings demonstrate the resilience of *M. acetivoran*s in these environments, highlighting its potential as a model organism for studies aimed at studying bioremediation of metals.

Microbial Diversity Along a Soil Water Saturation Gradient in an Andean High-altitude Peatland, Páramo Chingaza, Colombia

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Peatlands are globally distributed ecosystems that store up to a third of all soil organic carbon while accounting for only about 3% of the earth's land area. Páramos are peatland ecosystems in the Andes Mountains which serve various and vital ecosystem functions that are threatened by both land use and climate change. Biogeochemical cycles in the páramos are primarily mediated by microbes and so an understanding of the fate of páramo soil organic carbon hinges on our ability to characterize and predict soil microbial communities and their ecological functions.

We sampled soil from six sites in the Páramo Chingaza, Colombia, representing a natural soil water saturation gradient along the landscape terminating in a seasonal lagoon. We used amplicon sequencing to evaluate bacterial, archaeal, and fungal communities at these sites. We evaluated shifts in community structure and predicted functional potential along the landscape. We found significant shifts in community composition largely attributable to soil saturation levels. Contrary to our predictions, soils from saturated sites harbored greater microbial diversity than unsaturated soils.

Our findings will be used to generate hypotheses regarding how these microbial communities will respond to varying hydrological regimes in relationship with the rapid decline of precipitation that is predicted for the region. This study provides an essential baseline to monitor how these ecosystems will respond to climate change and to predict the future of the organic carbon stored in páramo soils.

Validating Candidate Genes for Competitive Colonization of Legume Roots by Nitrogen-fixing Bacteria

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Understanding the genetic basis of microbial competitiveness in plant roots is vital for strain establishment, a key trait for effective bioinoculant formulation. Here, we investigated the influence of candidate *Sinorhizobium* genes, identified as relevant for competition through genome-wide association studies, on competition outcomes during root nodule symbiosis.

We constructed deletion mutants of the focal candidate genes - B, Heat-shock protein 90 and a conserved exported protein of unknown function within *Sinorhizobium* strains of contrasting and used them in 1:1 competition assay with their corresponding wild type strains . Intriguingly, we observed that the mutations transformed the poor competitor into a strong one, and diminished the competitiveness of the previously dominant strain. Furthermore, the impact of these genes on competitiveness was found to be genotype and strain dependent . Moreover, we explored the broader implications of these candidate genes on host investments and on host and rhizobial benefits. While none of the genes affected host investments (nodulation) there were strain and host genotype specific cases of significant reduction in benefits as indicated by the altered plant biomass and rhizobial population sizes.

Our findings underscore the utility of GWAS for identifying novel candidate genes altering microbial competitiveness and provide a promising avenue for increasing microbial competitiveness through targeted manipulation of these novel candidates during root nodule symbiosis.

Impacts of the Host Extracellular Microbiome on an Intracellular Endosymbiont

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Animal holobionts contain diverse extra- and intracellular microorganisms, yet the impacts of the extracellular microbiome on an intracellular endosymbiont have not been studied. Central research questions include do endosymbionts function in the absence of an extracellular microbiome? If so, do endosymbionts take advantage of the newfound host state? What changes in host biology occur in a strict state of one host, one endosymbiont, and no extracellular microbiome?

Here we cultivate *Drosophila melanogaster* with and without its extracellular microbiome and reproductive endosymbiont *Wolbachia* that adaptively cause a paternal-effect lethality of aposymbiotic embryos. We report three main findings: (i) The endosymbiont's adaptation is not only self-sufficient but significantly stronger in a host lacking an extracellular microbiome. (ii) the expression of host gene candidates related to the endosymbiont's adaptation is augmented in a microbiome-free host, even when the endosymbiont densities are reduced. (iii) The intact holobiont contains markedly higher densities of the extracellular microbiome and endosymbiont than a host containing only one of these microbial constituents.

Altogether, this study demonstrates, for the first time, the self-sufficiency of an intracellular endosymbiont in manipulating host reproduction and a previously unknown influence of the extracellular microbiome on the penetrance of an endosymbiotic adaptation.

Symbionts of Symbionts: Disentangling *Symbiodiniaceae*-microbiome Interactions.

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Cnidarians, including corals and jellyfish, are holobionts made up of the animal host and a myriad of other microorganisms. Microalgae in the family *Symbiodiniaceae* live within cnidarian host cells as photosynthetic endosymbionts that provide the host with resources that support growth, development, reproduction, and metabolism. *Symbiodiniaceae* physiology has significant influences on the health, fitness and adaptability of the cnidarian host, which is critical today as corals face mass bleaching and mass disease events in the face of rapid global environmental change.

This study aims to dissect the interactions between *Symbiodiniaceae* and their microbiomes to explain how both parties influence each others' ecology and evolution. We characterized diverse *Symbiodiniaceae* microbiomes using bacterial full-length 16S sequencing employing Oxford Nanopore Technology. We first did this for laboratory cultures across the *Symbiodiniaceae* phylogeny but also plan to repeat this for freshly isolated symbionts from cnidarian hosts in nature.

We hypothesize that diverse *Symbiodiniaceae* microbiomes will differ in structure and composition but share a small number of conserved bacteria that likely play essential roles in algal-bacterial nutrient exchange. Additionally, we expect microbiome structure and composition to be more similar among *Symbiodiniaceae* strains that are more evolutionarily related.

Coastal Environments Shape Chemical and Microbial Properties of Forest Litters in the Circum-Mediterranean Region

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This study explores how chemical and microbial properties of litters can be affected by coastal environments across the Mediterranean basin. A litterbag experiment including *Pinus halepensis Mill.* and *Pistacia lentiscus L.*, collected from both inland and coastal areas, was set up in France, Greece and Algeria. Control litterbags were left in their sampling sites and a transfer of litterbags from inland to coastal areas was performed to test whether the effect of the specific constraints of coastal environments varies according to the country and the litter type. After 10 months, litter chemical composition (CP/MAS 13C-NMR) and microbial activities (cellulase activity, basal respiration, catabolic diversity using Biolog) and community structure (TRFLP) were analysed.

Coastal conditions led to various responses: (i) litter aromaticity differed in the coastal zones depending on the country (high in the Greek coastal area, low in the Algerian coastal zone), (ii) fewer functionally diversified microbial communities were found in the Greek coastal area compared to the French and Algerian coasts, (iii) genetic diversity and richness were strongly impacted after transfer to the coastal zone whatever the country.

The type of litter shaped microbial communities: (i) at a local scale (i.e., in either coastal or inland areas) catabolic profiles and cellulase activities varied with the plant species, (ii) at a regional scale, the effect of coastal conditions differed with the plant species (basal respiration, Shannon-Weaver index, catabolic diversity H′, cellulases and catabolic profiles).

Thus, litter microbial properties differed in coastal environments across the Mediterranean basin and plant litter type plays a major role in microbial properties at a large spatial scale.

A Paleogenomic Approach to Reconstruct Historical Responses of Coral Reefs to Anthropogenic Change

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Coral reefs are declining worldwide due to anthropogenic-driven environmental change. The foundation and health of these ecosystems rely on the harmonic functioning of all members of coral holobionts, i.e., cnidarian host, symbiotic microalgae (families Symbiodiniaceae and Ostreobiaceae), and associated microbiome. Coral stress responses often involve shifts in the taxonomic identity of their symbionts and microbiomes. Tracing back changes in coral holobiont composition over prolonged time periods can help us reconstruct health history of reefs and gain a better understanding of coral response to current stressors.

Here, we focused on a major Caribbean reef-builder coral, *Orbicella faveolata*, from the Varadero Reef, Colombia. This reef has undergone extensive freshwater sediment discharge for decades as result of urbanization. We show, for the first time, that a paleogenomic approach can be used to reconstruct historical, microbial shifts of coral holobionts, potentially associated with stress.

Toward an Integrative Framework for Microbial Community Coalescence

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Community coalescence is defined as the mixing of intact ecological communities. From river confluences to fecal microbiota transplantation, community coalescence constitutes a common occurrence affecting natural and engineered microbial systems.

Here, we propose an integrative framework for community coalescence to guide advances in our understanding of this important – yet underexplored – ecological phenomenon. We begin by aligning community coalescence with the unified framework of biological invasion and enumerate commonalities and idiosyncrasies between these two analogous processes. Specifically, we emphasize the importance of the dynamic interplay of abiotic and biotic filtering during coalescence events. In doing so, we extend key literature from the field of invasion biology to describe the process of community coalescence and multi-species invasion in microbial systems more appropriately. We then discuss how organismal interactions and cohesive establishment may affect coalescence outcomes with direct implications for community functioning and emergent properties. Last, we propose the use of ecological null modeling to study the interplay of ecological processes structuring community reassembly following coalescence.

Together, our framework guides prospective experimental designs and promotes the study of community coalescence across distinct microbial systems.

Multi-omic Analyses Demonstrate the Functioning of a Surface Film-forming Consortium is Altered by Interactions with Resident Soil Community Members

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Cyanobacteria-based soil surface consortia (SSC) are currently being developed as potential agricultural inoculants for adding fixed carbon and nitrogen to soils and to improve soil structure. Similar to other microbial inoculants, little is known about how already established microbes, and the resulting interactions, influence inoculant establishment, persistence, and function after addition. *Cyanobacteria*- based SSCs provide a tractable model for examining these interactions as their abundant surface growth allows for direct observation and sampling.

Here we added a *Cyanobacteria*-based SSC (DG1) to soil microcosms differing in the presence or absence of a diverse, established microbial community (low / high diversity) and in nitrogen status (added urea / no added urea). The presence of a diverse soil microbiome did not affect *Cyanobacterial* relative abundance, though non-*cyanobacterial* members of the consortium decreased in relative abundance. Addition of urea reduced the relative abundance of *Cyanobacteria*. The abundance of transcripts assigned to *Cyanobacteria* was lower in microcosms with a diverse soil community and in microcosms with added urea, indicating both interactions with native soil microorganisms and abundant nutrient levels can lead to lower inoculant activity.

Results demonstrate the utility of this system to examine interactions between SSC inoculants and established soil microbiomes. Future work will specifically focus on determining SSC gene expression as a factor of underlying microbiome diversity and soil nitrogen status, and whether these factors affect functions related to inter-microbial competition.

Poster Session

HUMAN HEALTH & DISEASE

SECTION 4

Assessing Ethnicity-associated Periodontal Health Disparities Using NHANES Oral Microbiome Data

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Racial and ethnic oral health disparities represent a critical US healthcare challenge. Among these, periodontal disease is a polymicrobial oral health condition that results in gum inflammation and ultimately tooth and bone loss. Periodontal disease is more prevalent among Mexican American and non-Hispanic Black Americans than other US residents, yet little is known of how oral microbiome contributes to these disparities. Therefore, this study aims to disentangle these racial disparities by analyzing the relationship between the diversity and composition of oral microbes involved in periodontal disease severity.

This study used microbial composition, microbial diversity, race/ethnicity, gender, and periodontal status data among 7874 participants from the NHANES between 2009-2012. This project used descriptive statistics to analyze the prevalence of periodontal disease among different ethnicities, and then examined how microbial composition and diversity related to these observations using a multivariate ANOVA for beta diversity and a Kruskal-Wallace test for alpha diversity.

Within the samples, the highest prevalence of total periodontal disease and severe periodontal disease was found in Mexican Americans and NH Blacks, while the lowest prevalence was found within NH Whites. Males had a higher prevalence than females within the sample. ADONIS tests showed significant intersectional variation in microbial composition associated with periodontal disease between racial/ethnic categories and gender.

This analysis demonstrates that microbial composition varies between gender, race/ethnicity, and periodontal severity. Differences in the oral microbiome may be linked to the oral health disparities among Mexican American and non-Hispanic Blacks and require further investigation.

Implications of the Microbiome on Wound Healing and Treatment

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The skin and gut microbiome are harbored by diverse microorganisms, varied by the human host and environmental factors, and have been an evolving research topic given the significant impact it has shown to have on various disease processes and prevention. One of these processes is wound healing. This project takes a deeper dive into the impact of the skin and gut microbiome on wound healing and whether these factors impact the effectiveness of various wound care treatments and etiologies.

Research articles published after 2019 were identified using a PubMed search. Articles published prior to 2019 were excluded and systemic reviews, meta-analyses, qualitative studies, and randomized-control studies were included in this analysis. The following MeSH terms were utilized in PubMed: "hyperbaric oxygen therapy," "negative pressure wound therapy," "human microbiome," "microbiome," "human microbiomes," "research, outcomes," and "pressure ulcers."

While wound healing has been evidenced to show a complex, structured mechanism, the human microbiome can interrupt it depending on the organisms present on the patient's skin, therefore impacting the immune cell types involved in wound healing promotion. Moreover, in cases of tissue damage, microbial diversity is lost, with bacteria such as *Staphylococcus, Streptococcus, Pseudomonas,* and *Corynebacterium*, leading to prolonged inflammation and delaying wound healing.

Evidence has shown the impact of the skin and gut microbiome on wound healing and the efficacy of wound treatments. Further directions can assess microbial effects on wound etiologies and examine possible therapies that target the microbiome, therefore bypassing antibiotics.

The Living Medicine Inside Us

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Large body of empirical evidence have confirmed the intrinsic relationship between human gut microbial metabolism and its host health. These findings not only establish the foundation for pioneering bio-therapeutic strategies utilizing gut microbiota but also propel the ongoing pursuit of identifying gut bacteria with potential health benifits.

To further contribute into the knowledge pool, this study isolated 6 gut bacteria focusing on their health-promoting attributes, from a fecal consortium of 30 healthy donors. Further, 16S rRNA gene sequencing identified the bacteria and subsequently, metabolites derived from the bacteria were meticulously scrutinized for their anti-microbial, anti-oxidant and anti-thrombotic activities.

Series of in vitro experiments reveal significant therapeutic potentials of the gut bacteria. Crude metabolites derived all 6 bacteria demonstrated robust anti-oxidant properties (IC50 values ranging from 150 to 350 µ g/mL). Furthermore, metabolites from isolates B1, B2, and BHI2 exhibited bacteriostatic effects on various clinically pathogenic strains, including *E. coli* ATCC-8739, *S. typhi* ATCC-1408, and *S. aureus* ATCC-6538. Notably, M2-derived metabolites showcased superior efficacy in lysing blood clots. Additionally, extracts from all the isolates displayed a substantial capacity to prolong blood coagulation time, thereby affirming their anti-thrombotic potential.

The outcomes of this preliminary research offer fresh insights into the metabolic functions of gut bacteria, extending beyond the confines of the gastrointestinal tract. This study underscores the paramount importance of exploring these active metabolites for prospective therapeutic and clinical applications.

A CRISPRi System to Elucidate how Microbial Metabolic Pathways Facilitate Mammalian Intestinal Colonization

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Human gut commensal bacteria employ the transcription factor Cur to colonize the mammalian gut by optimizing gene expression in response to cellular cues. Unidentified metabolic signals differentially control Cur activity by altering its binding to target promoters and reprogramming the global transcriptome.

We hypothesize that discreet steps in central metabolic pathways control Cur activity by stimulating production of an unknown signaling molecule. However, using loss-of-function mutations to investigate the role of the processes controlling Cur activity is hindered by the essentiality and redundancy of proteins that mediate cellular metabolism.

To overcome these limitations, we have developed an inducible CRISPRi platform that can silence the expression of genes required for essential metabolic steps. We used this system to target a ribose-5-phosphate isomerase, encoded by rpiA, in the human gut commensal, *Bacteroides thetaiotaomicron*. We now demonstrate dose-dependent growth inhibition of Bt when sgRNAs targeting rpiA are expressed and this effect is enhanced by simultaneous expression of multiple sgRNAs.

We determined that CRISPRi-mediated silencing is specific because the inhibitory effect of rpiA-targeting sgRNAs no longer function when a distinct rpi gene is present. We plan to employ this CRISPRi system to genetically dissect the contributions of distinct metabolic steps to controlling Cur activity.

Investigating the Role of Bacteriophage on the Gut Microbiome

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Bacteriophage, viruses that infect bacteria, are important modulators of gut microbial communities driving ecological changes through predation and gene transfer. Altered viral communities have been seen in inflammatory bowel disease and metabolic syndrome. The mechanism by which bacteriophage impacts microbial communities, and consequently host health, remains to be fully characterized.

We hypothesize that phages alter the structure and metabolism of gut microbial communities, particularly by determining competition within bacterial species. We are using a 38-strain microbial community with phages obtained from wastewater. We isolated HKP09, a *Bacteroides uniformis*-targeting bacteriophage, which exhibits high-specificity against *B. uniformis* DSM 6597, but not other members within the community. Based on electron microscopy and genome sequencing, HKP09 is a phage in the Myoviridae family with a 32.8 Kb genome. Co-cultures of *B. uniformis* DSM 6597 and HKP09 demonstrate reduced growth rates but not complete inhibition of growth due to the generation of spontaneous resistance.

To understand how these interactions impact the community, we compared the effect of HKP09 on communities with *B. uniformis* DSM 6597 to communities where B. uniformis DSM 6597 is absent. Quantitative PCR analysis shows the phage lowers the absolute abundance of *B. uniformis* DSM 6597 by 7-fold allowing other microbes to outcompete it. We are assaying the impact of HKP09 through metabolomic analysis of community metabolism, both in vitro and in gnotobiotic animals.

The ultimate goal is to understand the role of phage in modulating community composition and function and how this is essential in targeting the microbiome for health.

Decoding the Polymicrobial Interactions in Bile Acid Metabolism by the Gut Microbiome

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The complex interplay between the gut microbiome and the host is mediated by the constant exchange of macromolecules that are generated and modified by both microbes and mammalian cells, among which bile acids (BAs) hold significant importance. Gut microbes convert host-derived primary bile acids into secondary bile acids, such as 7α-dehydroxylated deoxycholic acid (DCA) and lithocholic acid (LCA), which are implicated in various diseases and pathogen infections. While the 7α-dehydroxylation of bile acids is attributed to bacteria encoding the bile acid-inducible (bai) operon, our preliminary findings suggest an alternative mechanism in a microbial community devoid of the bai operon.

Our preliminary results indicate that a small microbial cohort of 37 bacterial strains is capable of producing 7α-dehydroxylated products in gnotobiotic mice and in culture, despite the absence of the bai operon. We hypothesize that a subset of this community orchestrates the 7α-dehydroxylation reaction through an uncharacterized pathway, possibly involving polymicrobial cross-feeding of intermediates. Through combinatorial testing, quantitative LC/MS methods, and comparative genomics approaches, we aim to elucidate the chemical substrates and the microbial and genetic determinants necessary to complete this novel pathway.

A deeper mechanistic understanding of this novel bile acid metabolism by the microbial community could significantly contribute to our knowledge of the origin of the abundant bioactive and physiologically relevant metabolites in the gut. Moreover, it could pave the way for the development of targeted therapies for gastrointestinal disorders and innovative strategies for pathogen management.

Interaction of *S. aureus* and *S. epidermidis* when Mimicking Atopic Dermatitis Conditions

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Atopic dermatitis (AD), which is also referred to as eczema is a chronic disease affecting the skin of about 20% of the world's population, mostly children. Two main bacterial species are involved in the establishment of AD: *Staphylococcus* aureus and *Staphylococcus epidermidis*. Usually, *S. aureus* is the predominant bacteria in AD while *S. epidermidis* is typically found on the skin of healthier individuals as part of the natural microbiota.

The pathology of AD usually involves dysbiosis which involves switching of bacterial population from a predominant *S. epidermidis* to predominantly *S. aureu*s. In addition, AD involves skin ceramide deficiency which aids the establishment of the condition in patients, with sphingosine being one of the important components.

In this work, we have been mimicking AD conditions in vitro, by growing biofilms with different inocula ratios of *S. aureus* and *S. epidermidis* both on plastic and on keratinocytes. We quantified the bacterial adhesion, biofilm development using COMSTAT and quantified gene expression using qPCR. We found that *S. aureus* disrupts the keratinocyte barrier earlier than *S. epidermidis* and that once a balance between the 2 bacterial species is reached, the gene expression relating to virulence is decreased.

Results from this study are expected to lead a better understanding of the pathogenesis of AD and aid in the development of novel therapies to prevent or cure the disease.

Understanding and Manipulating Dietary Metal Bioavailability through the Gut Microbiome

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Gut microbes shape the bioavailability of nutrients and xenobiotics consumed through the diet. Metals are ubiquitous in the diet and play important roles in host and microbial physiology; however, some metals like mercury are toxic to both. Microbial metal resistance often occurs through biotransformation reducing toxicity for both host and microbe. This is true of organomercurial lyase (MerB), an enzyme which demethylates highly bioavailable methylmercury (MeHg) to poorly-bioavailable forms.

To better understand how the gut microbiome could be manipulated to modulate metal bioavailability, we analyzed the metal resistome of 398 lab isolates. While metal-resistance determinants are widespread, the genes for mercury biotransformation are rare and sporadically distributed. Based on these observations, we experimentally determined the important species and pathways involved in mercury biotransformation by exposing fecal samples from human donors to MeHg ex vivo and studying community growth and composition. We observed interindividual variation in susceptibility to MeHg and changes in community composition.

Finally, to supplement the rarity of MerB in the gut microbiome, we have engineered strains of *Lacticaseibacillus* through chromosomal insertion of a constitutively expressed codon-optimized MerB derived from a highly MeHg-resistant *Bacillus megaterium* strain. We have confirmed the function of this construct hypothesizing that MerB results in intracellular accumulation of inorganic mercury which may then be carried out of the body by the non-colonizing engineered strain.

Our work is seeking to uncover how microbial biotransformation of metals affects their oral bioavailability and offers promise for microbiome-targeted interventions to improve human nutrition and health.

Short-term Caloric Restriction Alters the Microbiome Modulating Resistance to *Clostridioides difficile* Infection

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Diet is a major factor modifying the gut microbiota and our previous work has shown that a 2-month 800 kcal/day liquid diet disrupted the microbiome and modified susceptibility to *C. difficile*.

To contrast the effects of diet processing and duration on gut microbiota and colonization resistance to *C. difficile*, we conducted a randomized, cross-over study of 10 healthy individuals consuming either a liquid very-low-calorie diet or a solid-food nutritionally matched diet over 5-day intervals. Both diets had significant effects on microbiota composition with an increased impact of highly bioavailable liquid diets. Fecal microbiota from the participants was transplanted to germ-free mice which were challenged with *C. difficile*. Contrasting our previous results, both short-term diets increased resistance to *C. difficile*. We next confirmed that short, but not long-term CR, protected against *C. difficile* infection in a conventional mice model.

To demonstrate that these effects were determined by microbe-diet and not immune-diet interactions, humanized mice underwent CR for 1 week, and then their microbiota was transplanted into diet-naive germ-free mice. Short-term CR increased *C. difficile* resistance in both donor and recipient animals which was associated with an increase in *Bacteroides caccae*. We isolated the representative strain of B. caccae and confirmed its protection against *C. difficile* infection in vivo. Furthermore, in vitro assays demonstrated that B. caccae could utilize mucin to survive during caloric restriction producing short-chain fatty acids.

These findings reinforce the interplay between diet and microbiome in affecting host health while pointing towards new strategies for infectious disease management.

Bifidobacterium bifidum Strain BB1 Inhibits TNF-α induced Increase in Intestinal Epithelial Tight Junction Permeability via TLR-2/TLR-6 Complex Stimulation of PPAR-g Inhibition of NF-kB p65 Activation

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Bifidobacterium bifidum (BB) strain BB1 causes a strain-specific enhancement in the intestinal epithelial tight junction (TJ) barrier. TNF-a induces an increase in intestinal epithelial TJ permeability and promotes intestinal inflammation. The major purpose of this study was to delineate the protective effect of BB1 against the TNF-a induced increase in intestinal TJ permeability and to unravel the intracellular mechanisms involved.

Filter-grown Caco-2 monolayers and animal models of in-vivo recycling perfusion of mouse intestine were used to assess intestinal epithelial TJ permeability.

TNF-a produced an increase in intestinal epithelial TJ permeability in Caco-2 monolayers and in mice; BB1 inhibited the TNF-a increase in intestinal TJ permeability in a strain-specific manner. BB1 inhibited the TNF-a induced increase in intestinal TJ permeability by interfering with enterocyte NF-kB p50/p65 and MLCK gene activation. The BB1 protective effect on TNF-a induced increase in intestinal permeability was mediated by TLR-2/TLR-6 heterodimer complex activation of PPAR-g and PPAR-g inhibition of IKK-α which in turn resulted in inhibition of NF-kB p50/p65, MLCK gene, and MLCK kinase activity.

These studies unravel novel intracellular mechanisms of BB1 protection against the TNF-a induced increase in intestinal TJ permeability. Our data show that BB1 protects against the TNF-a induced increase in intestinal TJ permeability via a PPAR-g dependent inhibition of NF-kB p50/p65 and MLCK gene activation.

A Synthetic Microbiota Designed via Meta-analysis Exhibits *C. difficile* Colonization Resistance

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Clostridioides difficile (Cd) is a common cause of recurrent antibiotic-associated diarrhea and colitis for which long-term efficacious and safe treatments are needed. Antibiotics are initially effective in treating CDI; however, their persistent disruption to the microbiome frequently leads to recurrent infection. Fecal microbiota transplant (FMT) is an effective treatment but the reliance on human donors makes FMT composition intrinsically irreproducible and may carry adverse effects on host physiology and/or transmit undesirable microbes such as multidrug-resistant pathogens.

We created a synthetic FMT (sFMT) using a meta-analysis from 12 studies (N=899), predicting Cd colonization status and identifying anti-Cd bacteria using classifiers. sFMT1, consisting of 37 lab-derived strains, exhibits stable colonization while inhibiting Cd growth in vitro and reducing infection symptoms in mice whereas the community positively correlates with Cd had no effect, which further demonstrates the specificity of sFMT's composition to maintain its efficacy. To determine sFMT's mechanism of action, each member of the sFMT1 was screened for plausible mechanisms including bile acid metabolism and Stickland fermentation through which sFMT may inhibit *C. difficile* colonization. Variants of sFMT1 by functional strain reduction showed one bacterial member capable of Stickland fermentation is sufficient and necessary to offer protection against infection outcomes while bile acid metabolism is dispensable for the efficacy of sFMT1.

Taken together, meta-analysis-based sFMTs offer a reproducible, effective alternative to FMT and its tractability allows for detailed research into microbiome mechanisms. This study provides insights into Cd treatment and highlights the potential of synthetic microbiota for translational research.

Oral and Gut Microbiome Profiles in Early Parkinson's Disease

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Early detection of Parkinson's disease (PD), a neurodegenerative disease with central and peripheral nerve involvement, ensures timely treatment access. Microbes influence nervous system health and are altered in PD.

We examined gut and mouth microbiomes from recently diagnosed patients in a geographically diverse, matched case-control, metagenomics study. We demonstrated greater alpha-diversity in 464 PD patients versus 249 controls. The microbial signature of PD included overabundance of 16 OTUs, including Streptococcus mutans and Bifidobacterium dentium, and depletion of 28 OTUs. Machine learning models indicated sub-species level oral microbiome abundances best distinguished PD with relatively high accuracy (area under the curve: 0.758). Microbial networks were disrupted in cases, with reduced connectivity of short-chain fatty acid-producing bacteria from stool. Microbiome diversity metrics associated with non-motor autonomic symptom severity.

Our results provide evidence for predictive oral taxonomic PD microbiome signatures that may be useful for early detection, particularly with peripheral nervous system involvement.

Directed Evolution of Beneficial Microbial Inoculants to Improve Survival in Soil Conditions

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Microbial inoculants promise benefits for sustainable agriculture by enhancing soil ecosystem services, yet their efficacy across diverse soil types remains a challenge due to limited survival under environmental change.

Addressing this, our project applies directed evolution to enhance persistence of the phosphate solubilizing bacterium *Priestia megaterium* (Pmeg) across various soil conditions. Over approximately 400 generations, Pmeg was exposed to liquid cultures with a gradient of carbon substrates, from low to high diversity, to simulate the resource heterogeneity found in natural soils. This regimen aimed to select for adaptive traits without compromising beneficial functions like phosphate solubilization. Growth curves were used to gauge fitness changes between the ancestral and adapted Pmeg lines, while a solid media halo zone assay was used to evaluate changes in phosphate solubilization efficiency. Fitness increases were observed in all adapted strains when grown in their respective media compared to the ancestor, and no loss of the focal function was detected in adapted strains. Upon reaching ~600 generations, strain-specific qPCR primers will be employed to track the in-soil viability of both ancestral and adapted Pmeg strains in resource-amended soils.

Our expectation is that Pmeg strains evolved under high resource diversity will have enhanced survival and persistence compared to those evolved in low resource diversity, due to their ability to utilize a wider spectrum of resources. Insights from this research are expected to enhance the conditioning of microbial inoculants for agricultural use, while simultaneously enriching our understanding of microbial evolution within complex soil ecosystems.

Sex Differences in High-fat Diet Induced Dysbiosis

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Chronic consumption of high-fat diet (HFD) is a potent modulator of gut microbiota. While this interaction is established in male animal models, less is known about the impact of HFD on female microbiota. Considering that sex differences exist in the microbiota of lean males and females and the pathophysiology of obesity in males and females, it is likely that gut microbiota response will differ between sexes.

Here we aim to investigate sex differences in HFD induced gut dysbiosis. Adult male and female rats were fed HFD or standard chow for 6 weeks and found significantly increased food intake and body weight and white adipose weights. 16S rRNA analysis was utilized to examine differences in fecal microbiota. We found that alpha-diversity metrics were decreased in HFD-fed females. However, in males, HFD produced a trend to increased evenness and only marginal changes in richness. Beta diversity was altered by HFD to a similarly in both sexes, where HFD-fed males and females displayed a distinct separation from their chow-fed counterparts. Abundance of over 60 taxa was reduced and another 40 increased in females by the HFD. While the numbers of altered taxa were similar in males, the specific taxa affected were largely sex divergent. Further analysis of the cytokines and metabolites in plasma and brain was performed to determine the primary microbial drivers of each parameter per sex.

Our data indicate drastic differences in the specific effects of HFD on the gut microbial community, with links to systemic and central metabolism and inflammation.

A Conserved Peptidase is Required for Glucose-mediated Cur Inhibition in Human Gut Commensal Bacteria

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The mammalian microbiota plays critical roles in host health and development. *Bacteroides* are an abundant bacterial collection comprising the human gut microbiome that establish persistent relationships with lean, healthy hosts. *Bacteroides* species dominate in the mammalian gut by expressing various gene products that facilitate processes crucial for intra-intestinal survival.

The conserved transcription factor, Cur, is required for *Bacteroides* species to utilize distinct carbohydrates, facilitate GTP-independent translation, and produce factors that mediate immunotolerance. Intriguingly, host consumption of the abundant human dietary additives, glucose and fructose, inhibit Cur activity in the gut, reducing Bacteroides thetaiotaomicron (Bt) abundance even though this bacterium can utilize either monosaccharide as a carbon source. Thus, host dietary sugar consumption reduces *Bacteroides* intestinal colonization by inhibiting this critical transcription factor.

We employed a genetic screen to identify determinants governing glucose-mediated Cur inhibition and identified a conserved M16_C peptidase, encoded by BT3803, differentially necessary for Cur inhibition by glucose. This indicates that BT3803 proteolytically acts on an unknown target involved in glucose signaling but not fructose. Intriguingly, BT3803 putatively resembles insulinase, the enzyme that degrades insulin, the mammalian peptide hormone that controls glucose homeostasis in mammals. We hypothesize that gut Bacteroides encode a unique signaling pathway whereby the BT3803 peptidase alters global gene transcription in response to changes in glucose availability by inhibiting Cur activity.

Evaluating the individuality and stability of human gut bacteriophage Crassvirales

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Human gut virome diversity dominates the bacterial microbiome, yet the types, tempos, and applications of evolutionary and ecological change in the gut virome remain largely enigmatic. Crassvirales (a.k.a. CrAssphage) is a highly abundant bacteriophage found in the human gut.

Here, I investigate its longitudinal, evolutionary diversity as a forensic identification tool through levels of individualization and stability. One person's community of CrAssphage must be reliably and identifiably different from another's to make any associations between a suspect and a scene. Additionally, the detection of CrAssphage must remain stable in the face of perturbations in the gut, such as sudden diet changes. Using assembled shotgun metagenomic data from 36 participants sampled over an eight-day period and a shift to and from a non-habitual vegetarian diet, I assessed longitudinal stability and individuality of CrAssphage. This dataset specifically allows for comparison of viral sequence shifts before, during, and after the diet within (stability) and between (individuality) individuals.

Employing sequence searches, amino acid alignments, and phylogenetic clade typing of evolutionarily conserved genes, my results indicate that CrAssphage communities in human gut microbiomes are individualized and longitudinally stable in detection. With these qualities, the potential applications of CrAssphage may expand to scene-suspect, forensic associations when human DNA is insufficient in amount or quality.

Integrating Geospatial and Activity-Based Intelligence to Uncover Microbiome Dynamics

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Integrating Geospatial Intelligence (GEOINT) and Activity-Based Intelligence (ABI) with microbiome research presents a groundbreaking approach to understanding the intricate relationships between environmental factors, human behavior, and microbial communities.

The microbiome, a complex community of microorganisms living in and on all living organisms, plays a crucial role in health and disease. By applying the principles of GEOINT and ABI to study patterns of life, we can gain unprecedented insights into how the microbiome interacts with its environment, influencing public health and ecosystems at large.

I propose an innovative framework that harnesses the power of GEOINT and ABI to explore the multifaceted relationships within microbiome dynamics. By leveraging high-resolution satellite imagery, remote sensing technologies, and comprehensive data analytics, this tapestry aims to elucidate how alterations in human patterns of life and environmental changes impact microbial communities across various scales. I explore the potential of GEOINT and ABI to monitor environmental modifications affecting soil and water microbiomes, track human-microbe interactions influencing health outcomes, and provide epidemiological insights into disease transmission and antibiotic resistance.

This integration has the potential to offer a novel perspective on the microbiome, highlighting its role as a critical nexus in the interaction between humans and their environments. I present a holistic approach to microbiome research, emphasizing the need for innovative methodologies to navigate the complexities of life sciences in the age of big data and advanced geospatial technologies.

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