Mid-Atlantic Microbiome Meet-up Symposium on Engineering and Analytics in Microbiome Research

Abstract Booklet

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Opening Keynote

Ophelia Venturelli, associate professor of biomedical engineering, Duke University

Talk: The Programmable Microbiome: Engineering Diet and Probiotics to Enhance Gut Resilience

Bio: Ophelia Venturelli is an associate professor of biomedical engineering at Duke University. The Venturelli Lab aims to understand and engineer microbiomes using systems and synthetic biology for applications spanning human health, agriculture and bioprocessing. Venturelli began as an assistant professor of biochemistry at UW–Madison after completing a Life Sciences Research Foundation Fellowship at UC Berkeley.

Her postdoctoral research focused on developing data-driven methods to decipher microbial interactions shaping assembly of synthetic human gut microbiomes and strategies to manipulate intracellular resource allocation by exploiting tools from synthetic biology. She received her Ph.D. in biochemistry and biophysics from Caltech in 2013, where she studied single-cell dynamics and the role of feedback loops in a metabolic gene regulatory network.

Venturelli received numerous awards including Shaw Scientist Award (2017), Army Research Office Young Investigator Award (2017), the Wisconsin Alumni Research Foundation Innovation Award (2019), OVCRGE Early Career Innovator Award (2023), ACS Synthetic Biology Young Investigator Award (2023) and the Thomas Langford Lectureship Award (2024). Her lab has published >30 high impact publications and she holds 6 patent applications. Venturelli has served on the Modeling and Analysis of Biological Systems (MABS) NIH study section since 2020 and has organized 11 international conferences and workshops.

Oral Presentations I: Computational Tools Innovation

Somatem: An Open Source and Modular Analysis Tool for Ensemble Long-Read Metagenomics

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Over a decade ago, MetAMOS was one of the first computational pipelines to provide reproducible analysis of short-read metagenomic data. Since its initial release in 2012, long-read sequencing technologies have come of age; long-read metagenomics enable enhanced taxonomic profiling with species-level resolution and less fragmented metagenome assemblies than short-read platforms, in addition to facilitating strain level analyses. However, analyzing long-read data remains challenging due to inherent error rates and rapidly evolving bioinformatic tools with varying trade-offs and learning curves.

To address this gap, we have developed somatem, an ensemble metagenomic analysis tool that incorporates a litany of metagenomic analysis tools optimized for long-read data analyses. To accommodate diverse computational resources and analytical needs, we partition our workflow into distinct operational modes. Somatem “laptop” mode enables taxonomic profiling from 16S amplicon sequencing data or low coverage metagenomic datasets. This mode runs efficiently within resource-constrained environments without compromising analytical accuracy, and within a reasonable timescale. The “workstation” mode supports compute-intensive tasks such as metagenomic assembly and variant detection from time-series samples. To facilitate usability and enhance user experiences, we developed a companion web app featuring an LLM copilot (Omi) which facilitates the configuration and launch of somatem. Omi also generates an interactive results page to facilitate exploration of the generated results, promoting interactive analysis of complex datasets without requiring command-line experience.

In summary, somatem combines best-practice long-read metagenomic analysis with a reproducible and user-friendly solution that we envision will help bridge the gap between data generation and microbiome insight and discovery.

Pracken Dramatically Reduces False Positive Species Identifications in Metagenomic Samples

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The k-mer-based metagenomic profilers KrakenUniq and Kraken2 are indispensable components of many genomic sample analysis pipelines. Although they are remarkably sensitive for taxonomic classification of sequences, both systems sometimes produce false positive species identifications.

Pracken (precise kracken) uses distinct k-mer statistics to provide a maximum-likelihood estimate of average nucleotide identity (ANI) for a species. We show that ANI is substantially more informative than both raw read count and unique k-mer count for determining which organisms are present in a sample. Pracken can dramatically reduce false positives in metagenomic reports while maintaining high sensitivity, strengthening overall confidence in metagenomic analysis.

TIPP-SD: A New Method for Species Detection

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We present TIPP-SD, a new method for species detection in microbiome samples. Like its predecessor TIPP3 (Plos Computational Biology 2025), TIPP-SD uses maximum likelihood phylogenetic placement into taxonomies based on marker genes. However, to provide high accuracy for species identification, TIPP-SD uses a novel way of combining statistical support obtained from these placements. Our experiments on a wide range of datasets show that TIPP-SD provides improved accuracy compared to well-established methods, such as Kraken2, Bracken, and Metapresence, with the greatest improvements when there are species in low abundance or the sequencing technology introduces high error rates.

Lightning Talks

Internal Standards Improve Metagenomic Sequencing Data

Sam Forry[1], Stephanie Servetas[1], Jason Kralj[1], Monique E. Hunter[1], Jennifer N. Dootz[1]

[1] National Institute of Standards & Technology (NIST)

Metagenomic sequencing (MGS) has become a cornerstone of modern microbiome characterizations; however, the resulting relative abundance data are inherently compositional, meaning the observed abundance of individual taxa are correlated with all other taxa and do not reliably scale with actual abundances. Herein, we report on the validation of an experimental design that incorporates internal standards to account for MGS compositionality and enable accurate analysis of individual taxa between samples. Following the addition of an internal standard to all samples, scaled abundances are calculated by normalizing each taxa's relative abundance by that of the internal standard.

Through a series of validation experiments, these scaled abundances were demonstrated to be independent of sample composition and directly proportional to actual biological abundance. When known amounts of exogenous taxa were added to human microbiome samples with varied compositions, the scaled abundance metric accurately reported taxa abundances with precisions similar to technical replicates. Furthermore, when a single human microbiome sample was diluted to systematically vary native taxa, scaled abundances exhibited high precision and good correlation with known variations in actual abundance, even for low-abundance taxa.

Ultimately, the strategy of systematically adding internal standards provides a straightforward approach for evaluating and correcting for the sample compositionality inherent to MGS. These findings, validated for both 16S and shotgun MGS analyses of human microbiome samples, confirm that scaled abundance measurements are directly proportional to biological actual abundance and independent of sample composition.

Reference: Forry SP, et al. 2026. A mathematical framework to correct for compositionality in microbiome data sets. Appl Environ Microbiol 92:e01126-25. https://doi.org/10.1128/aem.01126-25

Multiple Particle Tracking Reveals Vaginal Bacterial Extracellular Vesicle Diffusion through Cervicovaginal Mucus

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The composition of the vaginal microenvironment has significant implications for gynecologic and obstetric outcomes. A healthy vaginal microbiome consists of a homogenous population of Lactobacillus, which assists in maintaining epithelial barrier properties and a low pH environment. Alternatively, vaginal dysbiosis is characterized by a polymicrobial environment, and results in epithelial shedding, high vaginal pH, and discomfort. During pregnancy, vaginal dysbiosis has been implicated in preterm birth, miscarriage, and fetal hospitalization. However, the methods by which bacteria communicate with reproductive tissues and influence these outcomes are not understood. Initial speculation assumed whole bacteria ascended from the vaginal environment towards upper reproductive tract tissues. However, previous work demonstrates that the small pore size of cervicovaginal mucus (CVM, <300 nm), likely prohibits the diffusion of whole microbes (1-10 μm). Alternatively, bacterial extracellular vesicles (bEVs) are small, <200 nm particles which contain proteins and nucleic acid cargoes from host cells, facilitating long distance communication.

Vaginal bacterial strains were cultured in anaerobic conditions, and bEVs were isolated via ultracentrifugation. Using multiple particle tracking analysis to determine particle mean squared displacement, we demonstrate the increased mobility of these vaginal microbe-derived bEVs compared to their parent microbes in ex vivo CVM. We observed increased mobility by bEVs, as compared to whole bacteria, highlighting their potential role in microbe-host communication.

Furthermore, we characterize the uptake kinetics of bEVs by vaginal, endometrial, and placental cell lines. We determine differences in bEV uptake in reproductive tissues based on microbe strain, with L. crispatus- derived bEVs having higher internalization in endometrial and placental cells, while vaginal epithelial cells internalize a higher percentage of M. mulieris- and L. iners-derived bEVs.

Finally, we showcase their cellular effects, collectively supporting the role of bEVs as a primary mediator of microbe–host interactions in the reproductive tract. We show modulation of cytokine profiles based on microbe species. Specifically, G. vaginalis-derived bEVs showed an increase in

IL-6, IL-8, IL-10, IL-1β, TNFα, IP-10, MIPα, and MIPβ in vaginal epithelial cells, as well as IL-8 in endometrial cells, and TNFα in placental cells.

Resources, Richness, and Resistance: How Environment Drives Colonization Resistance

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Resident microbial communities, such as those inhabiting different parts of our body, can resist colonization by other microbes, a property called colonization resistance. Colonization resistance is important for protecting us from pathogens, bringing up the need to better understand the mechanisms that affect it.

We used a consumer-resource model to investigate how resources supplied in the environment can influence invasion outcomes including colonization resistance. We implemented a computational invasion assay and simulated many instances of resident communities encountering invaders to infer how different parameters such as the supply of resources or exchange of metabolites between species affect colonization resistance.

We found that colonization resistance is negatively correlated with both resource supply and resource diversity, except when resource supply is limited. We also showed that cross-feeding between species weakens colonization resistance by increasing the diversity of available resources, but this trend again disappears with limited resource supply. Collectively, our work highlights the impact of resources in shaping colonization resistance, offering useful insights that can guide future efforts to control colonization resistance.

Multi-year Survey and Compositional Analysis of Grape Microbiomes in Maryland Viticulture

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Microbes associated with grape vine production environments can contribute to the aroma and attributes of wine, which may influence the regional distinctiveness of certain wines. In addition, disruptions of certain microbial interactions may lead to potential host-plant vulnerabilities and the emergence of plant diseases (i.e., Powdery mildew, Pierce's disease), hence the importance of microbiomes to the health of grapes. As viticulture in Maryland is on the rise, innovative solutions for identifying effective biocontrols for phytopathogens are of increased interest. Establishing the key members of the microbiome in Maryland-grown wine grapes is a crucial step in identifying biobased solutions for improving local yields.

In a multi-year survey of wine grapes of three cultivars (i.e., chardonnay, cabernet franc, chambourcin) grown across four vineyards, grape musts were processed for ITS and 16S amplicon sequencing to characterize the associated fungal and bacterial communities. Beta diversity was assessed using Aitchison’s distance, and seasonal shifts were significant in both fungal and bacterial communities, while site variations were also significant in bacterial communities. Differentially abundant taxa were assessed using ANCOM-BC2. Fungal genera associated with late-season rots (i.e., Botryosphaeria, Vishniacozyma, Collectotrichum) were found to have a significant fold increases in samples taken close to harvest, while other rot-associated fungi were observed during the early growing season.

Ongoing analysis is investigating changes in bacterial and fungal taxa to determine linear shifts between taxa across vineyards. Year-to-year differences will also be evaluated to determine the stability of the microbiome over time.

Hierarchical Decomposition and Visualization of Metagenome Assembly Graphs

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Modern single- and meta-genome assembly projects often necessitate the manual inspection of sequence assembly graphs created by an assembler or scaffolder. The information contained in these graphs helps researchers understand both coarse information about an assembly (for example, distinguishing completely vs. incompletely assembled genomes) as well as fine-grained information about the details of an assembly (for example, observing single-nucleotide variations between some of the genomes being sequenced).

However, visualizing assembly graphs at both of these levels of detail—allowing the user to move back and forth between high-level overviews of the graph and small-scale details of interest—remains an open problem.

To this end we present MetagenomeScope, a tool that facilitates the multilevel exploration of assembly graphs using iterative pattern decomposition, hierarchical layout, and intuitive summary and path visualizations. The novel algorithms and features supported by MetagenomeScope simplify analyses of assembly graphs, lowering the barriers to meaningful inspection of assembly outputs.

Wastewater Surveillance for Candida Auris: Identification of Strong Biofilm-Forming and Multidrug-Resistant Environmental Strains

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Candida auris is an emerging multidrug-resistant fungal pathogen that represents a growing public health concern due to its persistence in healthcare environments, limited treatment options, and high transmissibility. Although closely related Candida species have been identified in diverse environmental habitats, the ecological niches and reservoirs of C. auris remain poorly understood. In particular, the environmental conditions that may facilitate its persistence, dissemination, and evolution of antifungal resistance are largely unknown.

We hypothesized that wastewater and harsh engineered environments may promote genetic adaptations enhancing the survival and drug resistance of C. auris. To investigate this, a 12-month wastewater surveillance study was conducted across five Maryland sewer sheds. Twice weekly, 24-hour composite wastewater samples (n = 425) were collected from pumping stations. Samples were enriched in Sabouraud Salt Dulcitol (SSD) broth for up to seven days, and C. auris was isolated using selective agar. Presumptive colonies were confirmed by qPCR targeting the ITS2 region and identified by MALDI-TOF MS. Antifungal susceptibility testing was performed following CLSI M27-A4 guidelines, and biofilm formation capacity was quantified using crystal violet assays. C. auris DNA was detected in 19.7 % (84/425) of samples (Cq < 37), while viable isolates were recovered from 4.2 % (18/425). Positive detections occurred year-round across all sampling sites. Notably, C. auris remained viable in wastewater-inoculated SSD cultures stored at 4 °C for more than two years, indicating remarkable persistence. All isolates exhibited resistance to fluconazole (100%, 18/18) and a high prevalence of amphotericin B resistance (83.3 %, 15/18), substantially exceeding resistance levels typically reported for clinical strains (~30%). Additionally, four isolates demonstrated significantly greater biofilm formation capacity than CDC 0385, the reference strain used in EPA antimicrobial efficacy testing.

This study provides evidence of viable C. auris in Maryland community wastewater, suggesting broader environmental than clinical surveillance alone indicates. Wastewater biofilms may represent a previously underrecognized ecological niche supporting survival and potentially facilitates the evolution of antifungal resistance in C. auris.

Integrative Multi-omics Modeling and Network Analysis Link the Gut Microbiome to Vital Status

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The gut microbiome plays a key role in aging through inflammation, metabolism, and immune signaling. However, most studies focus on microbial taxa alone, overlooking complementary molecular layers such as metabolites and proteins that reflect functional activity.

We analyzed participants from the Osteoporotic Fractures in Men Study (MrOS) with stool 16S microbiome, plasma metabolomics, and proteomics data to evaluate multi-omics contributions to predicting vital status (deceased vs. active) and to characterize cross-domain interactions associated with mortality.

A baseline random forest model using 14 clinical covariates (age, comorbidities, cognition, depression, and lifestyle factors) achieved modest discrimination (AUC = 0.693). Microbiome-based Elastic Net and XGBoost models yielded AUCs of 0.600 and 0.603. Ensemble models integrating clinical and microbiome predictions improved performance (AUC = 0.702 and 0.706), indicating incremental predictive value beyond clinical factors. SHAP identified Parasutterella, Angelakisella, Clostridium sensu stricto 1, and Roseburia as key contributors.

Metabolomics and proteomics were analyzed using the same framework with limma-based feature selection. Ensemble models again achieved the highest accuracy, supporting the additive prognostic value of multi-omics integration.

Network analysis of taxon–metabolite, taxon–protein, and metabolite–protein associations revealed substantial rewiring of inter-omic connectivity between deceased and active participants, suggesting altered coordination between microbial ecology, systemic metabolism, and host response in mortality-associated states.

Our findings demonstrate that integrating microbiome and complementary omics data with clinical variables improves prediction and provides a systems-level framework for microbiome-informed health modeling.

A Novel Quantitative Framework for Detecting Low-biomass, Growth-Independent Anaerobic Metabolism via Isothermal Microcalorimetry

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Anaerobic microbial communities frequently operate under energy-limited conditions in which cellular growth is minimal, yet metabolic activity persists. Quantifying low-level anaerobic microbial activity remains challenging because conventional metrics primarily rely on biomass accumulation or gene abundance.

This study assessed isothermal microcalorimetry (IMC) as a quantitative method for investigating low-biomass anaerobic metabolism and established empirical detection limits in controlled laboratory conditions. To estimate background heat-flow noise, repeated blank-vial assays were analyzed using a linear mixed-effects model accounting for assay grouping and ambient temperature effects. The limit of detection (LOD) and limit of quantification (LOQ)

were determined to be 1.00 μW and 3.33 μW, respectively. Subsequently, IMC was utilized to examine the metabolic dynamics of the sulfate-reducing bacterium Desulfovibrio vulgaris Hildenborough under growth-limited circumstances.

The resulting thermograms revealed reproducible variations in metabolic onset and peak heat flow across inoculum sizes differing by as little as 0.2% (v/v). Despite observable shifts in metabolic rate and timing, total heat production remained statistically consistent across treatments ($p > 0.05$). Parallel quantification revealed greater than two-fold increases in metabolic heat flow and sulfate production, while Desulfovibrio 16S rRNA gene abundance showed no significant change ($p > 0.05$). Importantly, the continued coupling between heat flow and sulfide production beyond peak metabolic rate demonstrates that IMC effectively captures functional activity across the entire metabolic trajectory, including phases of metabolic slowdown.

These findings indicate that functional metabolic activity can be decoupled from measurable biomass growth, thereby challenging the reliance on growth-based proxies commonly employed to infer microbial activity in energy-limited systems. This framework establishes a quantitative foundation for elucidating physiological states, stress adaptation, and functional persistence within anaerobic microbiomes.

Oral Presentations II: Microbiome in Environment and Health

From Particles to Pathways: Electrostatic Air Sampling and Metagenomics to Locate and Mitigate Microbial Hotspots Indoors

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Most Indoor Air Quality (IAQ) programs track particles, not biology. This study utilized Poppy's discrete Electrostatic Precipitator (ESP) air samplers combined with a metagenomic framework to map how resuspended microbes spatially distribute across an occupied office space. At a commercial building, seventeen ESP monitors were deployed—drawing air at ~150 $L \cdot min^{-1}$ at ~15 dBA—alongside artificial saliva DNA-barcoded tracers to quantify bioaerosol resuspension across zones like reception, kitchens, and conference rooms. Air samples were profiled by RT-qPCR for SARS-CoV-2, 16S/ITS sequencing, and viable culture recovery, while floors and high-touch surfaces were processed through shallow shotgun metagenomics with AMR class prediction.

Results showed airborne communities were mold-dominant (e.g., *Cladosporium*), while surfaces enriched for human-associated taxa (e.g., *Cutibacterium acnes*). Resuspended air resembled floor microbiota and was phylogenetically distinct from surfaces (PERMANOVA $p=0.008$); AMR models predicted higher MLS and Fluoroquinolone classes on surfaces ($p=0.0001$; $p=0.0232$). The compact, quiet, low-ozone ESP platform enabled unobtrusive deployment, while tracer-informed airflow mapping connected sources, pathways, and sinks. By using select Reckitt products, the team introduced targeted surface cleaning and hand-hygiene that reduced alpha-diversity in treated spaces without destabilizing community structure, though resuspension links between floors and air persisted.

Ultimately, this study advances an operational microbiome framework for commercial buildings—moving beyond proxy IAQ metrics to decision-ready, microbiome-aware stewardship. The approach provides a practical, zone-resolved playbook linking airflow, resuspension, and touch points to measurable hygiene actions, product choice, and verification over time. This framework supports seasonally robust deployment and simplifies the communication of "where/when to clean" for non-specialists while maintaining normal operations and occupant acceptance.

Metagenomic and Quasimetagenomic Insights into Microbial and Antimicrobial Resistance Across a Cross-Zonal Watershed

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Surface water is essential for human health, agriculture, and environmental conservation. However, most studies assess microbial communities at isolated time points, overlooking temporal dynamics and spatial gradients.

This study evaluated the spatiotemporal dynamics of bacterial communities and antimicrobial resistance (AMR) in the Mapocho River watershed (Santiago, Chile) through monthly 10 L water sampling over 12 months at 15 sites representing natural, agricultural, and urban land uses, and different water sources (river, canal, pond). Shotgun metagenomic and quasimetagenomic sequencing were performed on an Illumina NextSeq 2000. To assess spatiotemporal dynamics, α-diversity (Shannon index) was modeled with an autoregressive temporal correlation structure, and β-diversity across Southern Hemisphere seasons—autumn (Mar–May), winter (Jun–Aug), spring (Sep–Nov) and summer (Dec–Jan)—was evaluated using Bray–Curtis dissimilarities alongside ANOSIM and PCoA in metagenomics data. The relationship between physicochemical parameters and seasonal factors to community composition was analyzed via redundancy analysis (RDA) and PERMANOVA (retaining only predictors with VIF ≤ 5) and co-occurrence network analysis explored interactions among key taxa. Antimicrobial Resistance genes from 15 AMR classes were determined from the quasimetagenomics data using the CARD database in the Galaxytrakr platform.

Overall, microbial diversity remained stable. Natural sites exhibited higher diversity while agricultural sites showed reduced diversity; urban sites did not differ significantly.

Community composition varied with water source, land use and season, though the latter two had moderate effects. Electrical conductivity and pH were significant but explained limited variation. Despite these fluctuations, certain bacterial taxa persisted and formed stable, intercorrelated core communities. AMR genes were detected in both approaches, with stable temporal abundance. Macrolides resistance genes predominated in metagenomics samples while both aminoglycosides and macrolides predominated in quasimetagenomics samples. These results underscore the value of long-term, spatially resolved microbial monitoring in revealing persistent core communities and environmentally responsive taxa—insights that are essential for designing effective water quality management strategies in dynamic, human-impacted watersheds.

Microbial Community Response and Recovery through an Aeration-Cessation Time Series in a Eutrophic Estuary

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Eutrophication driven hypoxia is an expanding threat to water quality in estuaries. Artificial aeration is used in aquatic systems to mitigate the threat of hypoxia through direct re-oxygenation of the water column. Despite its expanding use, the impact of aeration on the structure and function of microbial communities has not been well characterized.

Here we present a time series investigation of microbial community structure in response to cessation and restoration of aeration in a eutrophic tributary estuary of Chesapeake Bay. Samples for 16S rRNA gene analysis were collected over a month-long period during which time the aerators transitioned from being on to off/disrupted to on again. Amplicon sequence variants of 16S rRNA genes were clustered into representative modules of correlated abundance. These modules demonstrate several temporal patterns of peak abundance (i.e. pre-disruption, disruption, post-disruption, recovery). The taxonomic composition of each module is distinct, demonstrating community successional patterns during the time series. The time series data revealed an algal bloom dominated by Heterosigma akashiwo that began immediately following cessation of aeration. Restoration of aeration led to rapid bloom termination. It has been proposed that aeration may inhibit algal blooms by mixing the water column, transporting populations out of the photic zone.

Our results are consistent with this hypothesis, as we observe increased signals of community connectivity and exchange between surface and deep waters during periods of aeration. These findings demonstrate the physical effects of aeration likely influence microbial community structure, ultimately mitigating the risk of harmful algal bloom formation.

Oral Presentations III: Microbiome Engineering & Wet Lab

Designing High-Yielding and Resilient Synthetic Microbial Consortia for Medium Chain Carboxylic Acid Production

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There has been growing interest in transitioning to a circular economy that uses sustainable anaerobic biotechnologies to produce biochemicals such as medium chain carboxylic acids (MCCAs). MCCAs are traditionally produced through plant and petrochemical refining. Microbial production is an alternative that can reduce carbon emissions and reliance on finite resources. Traditional microbial approaches use naturally occurring mixed cultures; however, these systems are complex and unpredictable, resulting in low yields. In contrast, synthetic consortia composed of well-characterized species enable greater control and potentially enhanced MCCA yield. Despite this potential, few studies have evaluated synthetic consortia for MCCA production or their resiliency.

This work studied MCCA-producing consortia by examining the impact of consortium composition on MCCA production, comparing performance to a mixed culture, and assessing resiliency following a simulated disturbance. Batch experiments were conducted using two synthetic consortia, Megasphaera elsdenii paired with either Bifidobacterium animalis or Lactobacillus pentosus, and an MCCA-producing mixed culture as the control. Glucose served as the substrate and MCCA production was quantified regularly. A second experiment examined disturbance response, where individual cultures were grown to mid exponential phase at pH 5.5, subjected to stress by dropping the pH to 4.75 for 1 hour, then returned to pH 5.5 for 56 hours before viability was determined via anaerobic plate counts.

Both consortia achieved higher caproate concentrations (533.6 mM with B.animalis and 429.6 mM with L.pentosus) than the mixed culture (1.74 mM). The superior performance of the B.animalis consortium was likely due to the bifid shunt pathway, which reduces carbon loss compared to the pentose phosphate pathway used by L.pentosus. The mixed culture also accumulated undesirable intermediates, with propionate yields of 72.1% compared to 9.9% for the B.animalis consortium. All consortium members demonstrated resiliency and recovered after a pH disturbance. The M. elsdenii, L. pentosus, and B. animalis cultures recovered to 89 ± 4.7%, 84 ± 7.2%, and 73 ± 6.5% cell viability, respectively. These results demonstrate that synthetic consortia improve MCCA production compared to a traditional mixed culture and exhibit

resiliency to pH disruptions. Future work will verify co-culture resiliency and evaluate MCCA production after pH disturbances.

Engineering Bacterial Multicellular Structures for Therapeutic Applications

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Probiotic bacteria have broad therapeutic potential across mucosal and epithelial surfaces, yet their clinical translation is limited by poor persistence and unreliable colonization. Bacterial self-assembly is a key determinant of probiotic survival and adhesion with aggregate size critically influencing therapeutic success. For example, smaller assemblies could be used for gastrointestinal delivery as the size must remain below the threshold for intestinal clearance. Whereas larger assemblies could enable probiotic-based modalities such as anticavity pastes, topical treatments for vaginal infections, and wound dressings that inhibit pathogenic biofilm. Despite this importance, existing aggregation strategies offer limited control over aggregate dimensions.

Here, we present a programmable platform for bacterial self-assembly based on the surface display of elastin-like polypeptides (ELPs), self-binding polymers composed of repeating pentapeptides with a single variable guest residue. By systematically varying ELP guest-residue and pentapeptide repetitions, we demonstrate a correlation between amino acid sequence to assembly size. Using these principles, we generated bacterial assemblies spanning micron to centimeter-scale living structures. Notably, we found that ELPs containing aromatic residues consistently produce the largest assemblies, despite a comparable level of surface hydrophobicity among the difference sequences tested.

This work provides a generalizable strategy for engineering bacterial multicellularity with precise physical control, enabling enhanced colonization, and persistence across diverse host environments. Moreover, this platform supports integration of additional genetic functionalities, including tissue-specific adhesion and controlled therapeutic delivery. This positions programmable bacterial self-assembly as a foundation for the next-generation of engineered probiotics and living materials.

The Effects of PCR Spike-in Standards on Observed Microbiome Structure

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Microbial abundances in microbiome studies are often estimated using relative abundances derived from DNA sequencing data. There is increasing evidence that relative abundance estimates do not provide the information necessary to accurately conduct taxon-specific analysis (e.g., how a bacterial taxon responds to disease). Absolute quantification offers promise to improve estimating true abundance of microbial taxa. Methods, including qPCR/ddPCR, flow cytometry, and the addition of internal controls (or spike-ins), have all been proposed as means to quantify absolute abundance. After DNA sequencing, read counts are corrected by measured total counts using one of these methods. The process of including spike-ins relies on the addition of a quantified number of microbial cells or DNA during sample processing (e.g., DNA extraction, PCR) that are not present in the biological samples. However, few studies have examined the effects of the spike-in on observed microbiome structure of the biological samples.

Here, we spiked-in different concentrations of a DNA mixture representing five bacterial species into 16S rRNA microbiome library prep during PCR to determine the effects of spike-in concentration (0 – 80 % spike-in) on bacterial community structure. We used three sample types: (1) agricultural soil collected at USDA Beltsville, MD from a 30-year farm experiment, (2) urban deciduous forest soil collected at NIST campus in Gaithersburg, MD, and (3) wastewater samples collected at Loudon County, VA wastewater treatment facilities. In all sample types, we found that spiking in high percentages of exogenous DNA (to use for calculating absolute abundance) into samples at the PCR step led to changes in observed bacterial community structure. At average spike-in greater than 30%, spike-in samples all differed statistically in at least one measure of structure (bacterial richness and/or community composition) from control non-spiked samples. This corroborates findings in one other study that similarly demonstrated spike-in standards impacted the observed microbiome composition.

Based on our previous studies, we hypothesize that using low PCR spike-in amounts (< 5 %) or using spike-ins at the DNA extraction step do not affect observed microbiome structure compared to controls. In the future, we plan to test these hypotheses to evaluate spike-in utility across diverse sample types and microbiome library preps in quantifying absolute abundance in microbiome analyses.

Closing Keynote

Amy Willis, associate professor of biostatistics, University of Washington

Talk: Model Misspecification and Differential Abundance in Microbiome Studies

Microbial communities are often studied via marker gene and shotgun sequencing. However, the relationship between the output of these techniques and the sequenced communities is nontrivial: sequencing is largely uninformative with respect to cell or DNA concentrations, and microbes are systematically over- and under-represented relative to their true abundances. To enable comparisons of communities in the presence of these challenges, we present a model to estimate fold-changes in microbial abundances. Our method is fast, scalable, and does not require a "reference taxon" nor pseudocounts. We contrast our approach with other common differential abundance methods in a number of examples, and argue that our approach allows for superior deconvolution of biology from sampling artefacts.

Bio: Amy Willis is an associate professor of biostatistics at the University of Washington (UW). The Willis Lab develops statistical methods for the analysis of ecological data obtained from high-throughput sequencing, including microbial diversity, abundance, and phylogenetics. Willis began as an assistant professor at UW in 2017 after completing her Ph.D. in statistics at Cornell University. Her research focuses on bridging the gap between advanced mathematical theory and biological insight to improve the accuracy of microbiome characterizations.

Willis has held several prestigious positions, including the Genentech Endowed Professorship in Biostatistics (2018–2019) and roles within the Center for Microbiome Sciences and Therapeutics (CMiST) and the UW eScience Institute. She has served on the Scientific Advisory Board for Takeda Pharmaceuticals' Microbiome Biomarker Program and is a faculty member for the Quantitative Ecology and Resource Management (QERM) and Computational Molecular Biology programs.

Willis is the recipient of an NIH Outstanding Investigator Award, as well as both the Outstanding Faculty Mentor Award and Outstanding Faculty Teaching award from UW.

Poster Presentations

1. Internal Standards Improve Metagenomic Sequencing Data

Sam Forry[1], Stephanie Servetas[1], Jason Kralj[1], Monique E. Hunter[1], Jennifer N. Dootz[1]

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Metagenomic sequencing (MGS) has become a cornerstone of modern microbiome characterizations; however, the resulting relative abundance data are inherently compositional, meaning the observed abundance of individual taxa are correlated with all other taxa and do not reliably scale with actual abundances. Herein, we report on the validation of an experimental design that incorporates internal standards to account for MGS compositionality and enable accurate analysis of individual taxa between samples. Following the addition of an internal standard to all samples, scaled abundances are calculated by normalizing each taxa's relative abundance by that of the internal standard.

Through a series of validation experiments, these scaled abundances were demonstrated to be independent of sample composition and directly proportional to actual biological abundance. When known amounts of exogenous taxa were added to human microbiome samples with varied compositions, the scaled abundance metric accurately reported taxa abundances with precisions similar to technical replicates. Furthermore, when a single human microbiome sample was diluted to systematically vary native taxa, scaled abundances exhibited high precision and good correlation with known variations in actual abundance, even for low-abundance taxa.

Ultimately, the strategy of systematically adding internal standards provides a straightforward approach for evaluating and correcting for the sample compositionality inherent to MGS. These findings, validated for both 16S and shotgun MGS analyses of human microbiome samples, confirm that scaled abundance measurements are directly proportional to biological actual abundance and independent of sample composition.

Reference: Forry SP, et al. 2026. A mathematical framework to correct for compositionality in microbiome data sets. Appl Environ Microbiol 92:e01126-25. https://doi.org/10.1128/aem.01126-25

2. Multiple Particle Tracking Reveals Vaginal Bacterial Extracellular Vesicle Diffusion through Cervicovaginal Mucus

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The composition of the vaginal microenvironment has significant implications for gynecologic and obstetric outcomes. A healthy vaginal microbiome consists of a homogenous population of Lactobacillus, which assists in maintaining epithelial barrier properties and a low pH environment. Alternatively, vaginal dysbiosis is characterized by a polymicrobial environment, and results in epithelial shedding, high vaginal pH, and discomfort. During pregnancy, vaginal dysbiosis has been implicated in preterm birth, miscarriage, and fetal hospitalization. However, the methods by which bacteria communicate with reproductive tissues and influence these outcomes are not understood. Initial speculation assumed whole bacteria ascended from the vaginal environment towards upper reproductive tract tissues. However, previous work demonstrates that the small pore size of cervicovaginal mucus (CVM, <300 nm), likely prohibits the diffusion of whole microbes (1-10 μm). Alternatively, bacterial extracellular vesicles (bEVs) are small, <200 nm particles which contain proteins and nucleic acid cargoes from host cells, facilitating long distance communication.

Vaginal bacterial strains were cultured in anaerobic conditions, and bEVs were isolated via ultracentrifugation. Using multiple particle tracking analysis to determine particle mean squared displacement, we demonstrate the increased mobility of these vaginal microbe-derived bEVs compared to their parent microbes in ex vivo CVM. We observed increased mobility by bEVs, as compared to whole bacteria, highlighting their potential role in microbe-host communication.

Furthermore, we characterize the uptake kinetics of bEVs by vaginal, endometrial, and placental cell lines. We determine differences in bEV uptake in reproductive tissues based on microbe strain, with L. crispatus- derived bEVs having higher internalization in endometrial and placental cells, while vaginal epithelial cells internalize a higher percentage of M. mulieris- and L. iners-derived bEVs.

Finally, we showcase their cellular effects, collectively supporting the role of bEVs as a primary mediator of microbe–host interactions in the reproductive tract. We show modulation of cytokine profiles based on microbe species. Specifically, G. vaginalis-derived bEVs showed an increase in

IL-6, IL-8, IL-10, IL-1β, TNFα, IP-10, MIPα, and MIPβ in vaginal epithelial cells, as well as IL-8 in endometrial cells, and TNFα in placental cells.

3. Multi-year Survey and Compositional Analysis of Grape Microbiomes in Maryland Viticulture

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Microbes associated with grape vine production environments can contribute to the aroma and attributes of wine, which may influence the regional distinctiveness of certain wines. In addition, disruptions of certain microbial interactions may lead to potential host-plant vulnerabilities and the emergence of plant diseases (i.e., Powdery mildew, Pierce's disease), hence the importance of microbiomes to the health of grapes. As viticulture in Maryland is on the rise, innovative solutions for identifying effective biocontrols for phytopathogens are of increased interest. Establishing the key members of the microbiome in Maryland-grown wine grapes is a crucial step in identifying biobased solutions for improving local yields.

In a multi-year survey of wine grapes of three cultivars (i.e., chardonnay, cabernet franc, chambourcin) grown across four vineyards, grape musts were processed for ITS and 16S amplicon sequencing to characterize the associated fungal and bacterial communities. Beta diversity was assessed using Aitchison's distance, and seasonal shifts were significant in both fungal and bacterial communities, while site variations were also significant in bacterial communities. Differentially abundant taxa were assessed using ANCOM-BC2. Fungal genera associated with late-season rots (i.e., Botryosphaeria, Vishniacozyma, Collectotrichum) were found to have a significant fold increases in samples taken close to harvest, while other rot-associated fungi were observed during the early growing season.

Ongoing analysis is investigating changes in bacterial and fungal taxa to determine linear shifts between taxa across vineyards. Year-to-year differences will also be evaluated to determine the stability of the microbiome over time.

4. Wastewater Surveillance for Candida Auris: Identification of Strong Biofilm-Forming and Multidrug-Resistant Environmental Strains

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Candida auris is an emerging multidrug-resistant fungal pathogen that represents a growing public health concern due to its persistence in healthcare environments, limited treatment options, and high transmissibility. Although closely related Candida species have been identified in diverse environmental habitats, the ecological niches and reservoirs of C. auris remain poorly understood. In particular, the environmental conditions that may facilitate its persistence, dissemination, and evolution of antifungal resistance are largely unknown.

We hypothesized that wastewater and harsh engineered environments may promote genetic adaptations enhancing the survival and drug resistance of C. auris. To investigate this, a 12-month wastewater surveillance study was conducted across five Maryland sewer sheds. Twice weekly, 24-hour composite wastewater samples (n = 425) were collected from pumping stations. Samples were enriched in Sabouraud Salt Dulcitol (SSD) broth for up to seven days, and C. auris was isolated using selective agar. Presumptive colonies were confirmed by qPCR targeting the ITS2 region and identified by MALDI-TOF MS. Antifungal susceptibility testing was performed following CLSI M27-A4 guidelines, and biofilm formation capacity was quantified using crystal violet assays. C. auris DNA was detected in 19.7 % (84/425) of samples (Cq < 37), while viable isolates were recovered from 4.2 % (18/425). Positive detections occurred year-round across all sampling sites. Notably, C. auris remained viable in wastewater-inoculated SSD cultures stored at 4 °C for more than two years, indicating remarkable persistence. All isolates exhibited resistance to fluconazole (100%, 18/18) and a high prevalence of amphotericin B resistance (83.3 %, 15/18), substantially exceeding resistance levels typically reported for clinical strains (~30%). Additionally, four isolates demonstrated significantly greater biofilm formation capacity than CDC 0385, the reference strain used in EPA antimicrobial efficacy testing.

This study provides evidence of viable C. auris in Maryland community wastewater, suggesting broader environmental than clinical surveillance alone indicates. Wastewater biofilms may represent a previously underrecognized ecological niche supporting survival and potentially facilitates the evolution of antifungal resistance in C. auris.

5. Integrative Multi-omics Modeling and Network Analysis Link the Gut Microbiome to Vital Status

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The gut microbiome plays a key role in aging through inflammation, metabolism, and immune signaling. However, most studies focus on microbial taxa alone, overlooking complementary molecular layers such as metabolites and proteins that reflect functional activity.

We analyzed participants from the Osteoporotic Fractures in Men Study (MrOS) with stool 16S microbiome, plasma metabolomics, and proteomics data to evaluate multi-omics contributions to predicting vital status (deceased vs. active) and to characterize cross-domain interactions associated with mortality.

A baseline random forest model using 14 clinical covariates (age, comorbidities, cognition, depression, and lifestyle factors) achieved modest discrimination (AUC = 0.693). Microbiome-based Elastic Net and XGBoost models yielded AUCs of 0.600 and 0.603. Ensemble models integrating clinical and microbiome predictions improved performance (AUC = 0.702 and 0.706), indicating incremental predictive value beyond clinical factors. SHAP identified Parasutterella, Angelakisella, Clostridium sensu stricto 1, and Roseburia as key contributors.

Metabolomics and proteomics were analyzed using the same framework with limma-based feature selection. Ensemble models again achieved the highest accuracy, supporting the additive prognostic value of multi-omics integration.

Network analysis of taxon–metabolite, taxon–protein, and metabolite–protein associations revealed substantial rewiring of inter-omic connectivity between deceased and active participants, suggesting altered coordination between microbial ecology, systemic metabolism, and host response in mortality-associated states.

Our findings demonstrate that integrating microbiome and complementary omics data with clinical variables improves prediction and provides a systems-level framework for microbiome-informed health modeling.

6. A Novel Quantitative Framework for Detecting Low-biomass, Growth-Independent Anaerobic Metabolism via Isothermal Microcalorimetry

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Anaerobic microbial communities frequently operate under energy-limited conditions in which cellular growth is minimal, yet metabolic activity persists. Quantifying low-level anaerobic microbial activity remains challenging because conventional metrics primarily rely on biomass accumulation or gene abundance.

This study assessed isothermal microcalorimetry (IMC) as a quantitative method for investigating low-biomass anaerobic metabolism and established empirical detection limits in controlled laboratory conditions. To estimate background heat-flow noise, repeated blank-vial assays were analyzed using a linear mixed-effects model accounting for assay grouping and ambient temperature effects. The limit of detection (LOD) and limit of quantification (LOQ) were determined to be 1.00 μW and 3.33 μW, respectively. Subsequently, IMC was utilized to examine the metabolic dynamics of the sulfate-reducing bacterium Desulfovibrio vulgaris Hildenborough under growth-limited circumstances.

The resulting thermograms revealed reproducible variations in metabolic onset and peak heat flow across inoculum sizes differing by as little as 0.2% (v/v). Despite observable shifts in metabolic rate and timing, total heat production remained statistically consistent across treatments ($p > 0.05$). Parallel quantification revealed greater than two-fold increases in metabolic heat flow and sulfate production, while Desulfovibrio 16S rRNA gene abundance showed no significant change ($p > 0.05$). Importantly, the continued coupling between heat flow and sulfide production beyond peak metabolic rate demonstrates that IMC effectively captures functional activity across the entire metabolic trajectory, including phases of metabolic slowdown.

These findings indicate that functional metabolic activity can be decoupled from measurable biomass growth, thereby challenging the reliance on growth-based proxies commonly employed to infer microbial activity in energy-limited systems. This framework establishes a quantitative foundation for elucidating physiological states, stress adaptation, and functional persistence within anaerobic microbiomes.

7. Evolving in a Glass House: Diversification & Ecology of Deep-Sea Glass Sponge Microbiomes

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Sponge-microbial symbioses are critical for enhancing biodiversity in shallow and deep-sea reefs, yet deep-sea microbial symbiont genome diversity remains largely obscure. In shallow sponges, microbiome community variance captures even subtle variations in host sponge diversity missed by host marker genes, making microbiome diversity a promising diagnostic for untangling difficult aspects of sponge evolution. However, deep-sea sponges, especially deep-sea glass sponges (class Hexactinellida), remain understudied because they only live in difficult-to-sample deep and polar sea habitats.

Recently, The Smithsonian National Museum of Natural History's Invertebrate Zoology Department has performed low-coverage whole-genome shotgun sequencing (genome skimming) on hundreds of sponge specimens from across a large geographic range in the deep Atlantic and Pacific Oceans to derive markers for barcoding and systematics analyses. Although these metagenomic datasets include both sponge, animal associate, and microbial DNA, 99% of these data remained unexplored.

In an analysis of over 50 of these deep-sea metagenomes, I assembled and annotated genomes of microbial associates, yielding the largest metagenomic survey of glass sponge microbial symbionts to-date. Using cophylogeny testing, we identified archaeal and bacterial clades evolving in parallel with host phylogeny and host geographic distribution. By using an evolutionarily-informed metagenomics approach, this work reveals microbial symbiont genome features associated across host diversity, yielding new insights into the roles glass sponges play in shaping their deep-sea environments.

8. Integrating Graph-based Alignment and the Pangenome into the MetaCompass Pipeline

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Previous research developed MetaCompass [1], a reference-guided metagenomic assembly tool. Recent advances have also highlighted pangenomes as a powerful way to represent sequence variations as graph structures. In particular, PanGraph [2] is a scalable method for constructing pangenome graphs from whole-genome FASTA inputs.

We investigated the integration of pangenome graphs into the MetaCompass pipeline to improve reference-guided metagenomic assembly. We constructed pangenome graphs from clustered bacterial reference genomes and evaluated their structural properties and runtime across varying cluster sizes and references. We developed multiple pruning methods to reduce graph complexity, and we evaluated the resulting mapping rate, MAPQ, and build and alignment time across clusters and pruning thresholds. Our work demonstrates a framework for incorporating pangenome graphs into the MetaCompass pipeline.

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2. Noll N, Molari M, Shaw LP, Neher RA. PanGraph: scalable bacterial pan-genome graph construction. Microbial Genomics. 2023;9(6):001034. doi:https://doi.org/10.1099/mgen.0.001034

9. Modeling Phage-Antibiotic Combination Therapy: Assessing Inhibitory and Synergistic Treatment Effects in Clinically Relevant Contexts

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Novel approaches are needed to combat infections by multi-drug resistant (MDR) ESKAPE pathogens, including Pseudomonas aeruginosa (PA). One approach is to combine conventional antibiotics with bacteriophage (phage) to eliminate target pathogens and reduce the risk of the evolution of drug-resistance. However, optimal combination treatment configurations for effective bacterial elimination remain poorly characterized. For example, a recent study using Hollow-Fiber Infection Models (HFIMs), mimicking physiologically relevant concentration profiles of antibiotics in patients, showed that simultaneous administration of phage and ciprofloxacin initially reduces the total density of PA which is then followed by the resurgence of PA. In contrast, administering phage first, and delaying the antibiotic by four hours, results in local elimination of PA.

This HFIM study suggests the potential for both synergistic and inhibitory eco-evolutionary dynamics in combination therapy. Here, we analyze a nonlinear dynamic model of phage-antibiotic combination treatment, based on the aforementioned HFIM study, to evaluate how phage dosage and timing can affect treatment outcome. Leveraging our computational framework, we identified potential adverse effects of ciprofloxacin on phage dynamics.

We show that simultaneous administration of phage and ciprofloxacin can lead to treatment failure, as phage infection opportunities are ‘wasted’ when jointly applied with antibiotics. In contrast, moderate delays in the administration of antibiotics can lead to bacterial clearance, even at low initial phage densities. We further utilize the model to explore a broad range of scenarios, including variation in densities and timing in shaping the robustness of combination therapy in elimination of target PA and prevention of the proliferation of double-resistant bacterial mutants of clinical relevance.

10. Detecting Distributed Microbiota Shifts in a Dietary Intervention for Gulf War Illness: Analytical Challenges in Human Research Trials

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Engineering and computational advances are essential for detecting subtle microbiota changes in small human intervention studies. We evaluated gut microbiota shifts during a one-month low-glutamate dietary intervention in veterans with Gulf War Illness (GWI), a chronic multisymptom condition associated with depression and neuroinflammation.

Stool samples (N=41 paired intervention; N=13 waitlist controls) underwent long-read 16S rRNA sequencing (PacBio Revio; V1–V9 HiFi reads). Amplicon sequence variants were inferred using DADA2 and classified with SILVA 138. To address compositionality and sparsity, low-prevalence taxa were amalgamated into an “Other” category to preserve relative abundance structure while reducing zero inflation. Community-level shifts were evaluated using Bray–Curtis dissimilarity with PERMANOVA, and differential abundance was tested using ALDEx2 using a scale-model framework (γ=0.5) to reduce false discovery inflation in a modestly powered dataset.

The dietary intervention was associated with significant community-level compositional change (PERMANOVA q=0.004) without detectable alpha diversity shifts. Taxon-level changes were modest and broadly distributed across the community, with no species surviving FDR correction. However, ALDEx2 overlap values suggested widespread, low-magnitude shifts rather than isolated taxon-specific effects.

These findings highlight a central challenge in microbiome science: clinical improvements may correspond to distributed ecosystem-level restructuring that is difficult to capture using taxon-by-taxon significance testing in small samples. Careful engineering of sequencing pipelines, compositional modeling, and multi-omic integration will be necessary to improve detection and mechanistic resolution in human intervention studies.

11. Extracellular Vesicles Isolated from Human Cervicovaginal Mucus affect Inflammation in Female Reproductive Tract Cells

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The vaginal microbiome is a critical mediator of female reproductive health. A Lactobacillus-dominated vaginal microenvironment is considered optimal, whereas a dysbiotic polymicrobial vaginal environment, marked by the presence of G. vaginalis, is associated with increased risk for adverse gynecologic and obstetric outcomes. Vaginal bacteria produce a variety of signaling moieties, including small molecules, proteins, and extracellular vesicles. Extracellular vesicles are membrane-bound, nano-sized particles that carry diverse biological cargoes, serving as a key mode of microbe-host communication. Despite a growing body of work that investigates the role of vaginal extracellular vesicles in reproductive outcomes, the precise function of extracellular vesicles produced in vivo is not well understood.

Here, we collected human cervicovaginal mucus (CVM) samples according to Kuali-IRB 0121-3. CVM samples were determined to be L. crispatus or G. vaginalis-dominant following genomic analysis. Extracellular vesicles were isolated from CVM samples via size exclusion chromatography to investigate their origin and function. First, we compared the concentration and size distribution of extracellular vesicles via nanoparticle tracking analysis. There was no significant difference in total particle concentration, though G. vaginalis-dominant extracellular vesicles did exhibit a greater size distribution range as compared to L. crispatus-dominant extracellular vesicles, which were more concentrated in their distribution. Next, human-derived strains of L. crispatus and G. vaginalis were cultured in anaerobic conditions. Extracellular vesicles were isolated via ultracentrifugation and used to generate a PCR-based approach to determine the origin of CVM-derived extracellular vesicles. Finally, we compared the in vitro cell response to extracellular vesicles isolated from bacterial monocultures or CVM.

This work supports the hypothesis that extracellular vesicles contribute to microbe-host communication in the female reproductive tract, and sets the stage for future work using mucus-derived extracellular vesicles as useful biomarkers of female reproductive tract diseases.

12. Enrichment of Mucosa-Associated Sutterella Spp. Characterizes Biofilm-Positive Colorectal Cancer

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Colonic biofilms (BF) are invasive bacterial aggregates that promote colorectal cancer (CRC). BF may also predate or co-evolve with CRC precursor lesions. Determining if an individual is BF+ may help identify those at increased risk for CRC and inform strategies to prevent disease onset and progression. BF are currently identified using fluorescent in situ hybridization (FISH); but this method is time-intensive, qualitative, and requires invasive sampling. Additionally, FISH does not allow for broad genus and species-level characterization. We examined the bacterial composition and predicted functions of BF in CRC using next-generation sequencing.

Samples included colon tumor and paired normal tissues (n = 83 total) from people with CRC undergoing colectomy at the Universiti Malaya in Malaysia (n = 25 people) and at the Johns Hopkins Hospital (n = 19 people). Half of the samples were BF positive (BF+). Groups were matched on all available clinical and demographic data. 16S rRNA gene amplicon sequencing and analysis, including differential analysis, were performed. Random forest (RF) models were trained and tested (80:20 split) with five-fold cross-validation. PICRUSt2 was used for predictive functional analysis. The impact of methodological choices (e.g., tissue fixation, contaminant removal) was also assessed.

Sutterella relative abundance and percent positivity were higher in BF+ than BF negative (BF-) tissues ($q = 7.28e\text{-}4$, $p = 1.52e\text{-}6$). Sutterella had the highest importance score in RF models and an AUC of 0.75. A model that included bacteria with the 25 highest importance scores and tumor location had an AUC of 0.92. In predictive functional analysis, formaldehyde assimilation was upregulated in BF+ samples and samples in which Sutterella was present ($q < 0.01$, log fold-change > 2).

Sutterella was enriched in BF+ compared to BF- tissues and predictive of BF, suggesting a potential role in BF formation and/or maintenance. Research indicates that Sutterella may degrade IgA, thereby disrupting the colonic mucosal barrier, a process important for BF formation. Additionally, Sutterella and BF+ tissues share predicted functional pathways like

formaldehyde metabolism. Formate, a derivative of formaldehyde, can be produced by gut bacteria, promotes CRC development, and increased utilization relates to BF formation. Thus, Sutterella may be a candidate marker for CRC BF and formate may contribute to CRC BF formation. Validation by PCR and RNA-seq is underway.

13. How do Antimicrobial Resistance Genes Change Over Time in a River System? A Quasi-Metagenomic Approach

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Antimicrobial resistance (AMR) is a growing global public health concern, and surface waters serve as environmental reservoirs for antibiotic resistance genes (ARGs). Although studies investigate how AMR spreads, we still do not fully understand how ARGs behave over time in surface waters. Quasimetagenomic sequencing is a modified metagenomics approach that culture-enriches selected microbes within samples before sequencing. This approach may improve the detection of public health-relevant genes overlooked in typical metagenomic sequencing.

This study aims to characterize the dynamics of public health-relevant ARGs across the Mapocho river system over time. Ten liters of water from 15 surface water sites were filtered in situ through modified Moore swabs (MMS). For the quasimetagenomics approach, Moore swabs were incubated with 200 mL of Buffer Peptone Water for 24 hrs at 37°C, and 1 ml of the enriched broth was used for DNA extraction. Shotgun metagenomics and quasimetagenomics sequencing were performed on an Illumina NextSeq 2000 platform. ARGs identification and their classification into classes were performed in the GalaxyTrackr platform using the CARD database. Data were standardized to reads per kilobase per million mapped reads (RPKM), and ARGs were grouped into 15 antimicrobial classes. Median ARG class abundances were calculated across months and sites, log-transformed [ln(x+1)], and visualized using heatmaps and bar graphs.

Findings showed an apparent dominance of aminoglycoside and macrolide resistance genes across sites and seasons. ARG abundance seemed spatially heterogeneous, with select sites showing recurrent spikes of specific classes, including winter-associated tetracycline elevations

and summer-associated oxazolidinone surges. However, these increases looked to be episodic and class-specific rather than indicative of sustained multi-year escalation. Similarly, metagenomic analysis appeared to find the resistome to be spatiotemporally stable, with macrolides being the visually dominant antimicrobial class. This work helps to characterize the Mapocho watershed's ARG dynamics in Chile and highlights the utility of quasi-metagenomics as a One Health approach and in future AMR surveillance efforts.

14. Why are Core-genome Phylogenies Robust to Recombination?

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Phylogenetic analysis of concatenated conserved loci (i.e., the core genome) is frequently used to reconstruct the ‘clonal’ (i.e., vertical) evolutionary history of prokaryotic genomes. Many such analyses ignore the potential impacts of homologous recombination, a phenomenon that is widespread and should, in theory, disrupt the ‘clonal’ signal and the accuracy of phylogenetic estimates based on whole genomes. Yet, the reasons why core-genome phylogenies are generally robust to recombination has only been assumed, and not fully interrogated.

We used simulations of core genomes with mutation and recombination to investigate the reasons why recombination often does not impact whole-genome phylogenetic inference. We find that most polymorphisms generated by recombination are observed late in the diversification process, where they have few descendants and conflict less with non-recombined sites.

By examining individual site patterns, we find that recombined sites remain largely compatible with the ‘clonal’ phylogeny and lend greater statistical support to the ‘clonal’ phylogeny over alternative phylogenies. This work provides a biological and technical explanation for the prevailing wisdom and could help researchers determine when they need to consider the impact of homologous recombination in phylogenetic analysis.

15. Transitions in the Canine Gut Microbiome After Neutering

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Neutering can assist population control, adjust behaviors, and reduce the risk of reproductive diseases. However, it may affect metabolism, immune responses, gut microbiome composition, and more. The gut microbiome plays roles in digestion, nutrient absorption, and immune function, and disruptions in its stability may impact overall health.

We investigated how neutering affects the gut microbiome in dogs of various ages, sex, and diets before and three months after neutering. Fecal samples were analyzed using full length 16S rRNA sequencing to analyze microbial diversity and taxonomic composition. Our findings will help identify microbiome trends associated with neutering and contribute to the discussion of how surgical sterilization shapes microbial ecology in the gastrointestinal tract of canines.

16. How does Quasi-Metagenomics Influence Observed Bacterial Diversity in River Surface Waters?

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Surface waters used in agriculture host diverse microbial communities, which may impact human health and the environment. To enrich bacteria of interest, quasi-metagenomics uses selective culturing techniques before sequencing, affecting the sample's microbial composition.

We aimed to evaluate the impact of quasi-metagenomics on the composition and diversity of microbial communities in surface water samples. Using modified Moore swabs, monthly water samples (2 x 10L) were collected from January 2021 to January 2022 across 15 sites in the Mapocho watershed (Chile). Quasimetagenomic samples were incubated in buffered peptone

water for 24 hours at 37 °C and DNA was extracted from 1 mL. Shotgun metagenomic sequencing was performed on the Illumina NextSeq 2000 platform. After trimming for quality, Kraken2/Braken was used to classify reads. We calculated the Shannon, Simpson, Pielou Evenness, and Berger-Parker α-diversity indices for samples having both sets of data (metagenomic and quasi-metagenomic; n=84). β-diversity was measured using ANOSIM on Bray-Curtis sample distances grouped by approach (metagenomic and quasi-metagenomic; 999 permutations). Comparisons of α-diversity indices variations between approaches were determined using Wilcoxon signed rank-sum tests in RStudio, and diversity indices were calculated with the vegan package in R (4.5.2). Metagenomic samples yielded significantly higher Shannon, Simpson, and Pielou Evenness indices than quasi-metagenomic samples ($p<0.001$), indicating greater diversity. The Berger-Parker index was significantly higher in quasi-metagenomic samples ($p<0.001$), showing reduced diversity due to dominance by a single family.

Further sample analysis revealed that the Comamonadaceae family was heavily dominant in quasi-metagenomic samples (71/84), and the rest were dominated by only four families. By contrast, only 47/84 metagenomic samples were dominated by Comamonadaceae, and the rest were dominated by seven other families, including Flavobacteriaceae (12/84) and Microcoloeaceae (9/84). Significant approach-based differences were also found in β-diversity as reflected by ANOSIM assessments ($R=0.916$, $p=0.001$). The quasimetagenomic approach used in this experiment decreased sample diversity and increased homogeneity across samples. Alternate enrichment procedures may be used to selectively modify bacterial communities and create conditions to enhance detection of targets of interest in surface water samples.

17. Phyllosphere Microbiome Profiling in Field-Grown Lettuce Affected Under Mulch and Temporal Factor

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Mulch is applied in-field vegetable row crops to suppress weeds, regulate soil temperature and retain soil moisture. Studies have also shown that plastic mulch may increase food safety risk by protecting enteric pathogens under and favouring their dispersal above plastic mulch. Presently, how mulch influences the phyllosphere microbiome of lettuce, a product intended for raw consumption, remains understudied.

A field trial was conducted at the University of Maryland Wye Research and Education Center in fall 2023 to assess phyllosphere microbial shifts under four ground treatments – biodegradable

plastic (BP), polyethylene plastic (P), straw (S) and bare ground (BG) using a randomized complete block design (16 beds; 4/treatment) to which loose-leaf lettuce cv. 'Magenta' seedlings were transplanted. Samples, collected 10 (T1) and 20 (T2) days post-transplant, consisted of two replicates/treatment bed/timepoint (5 heads/replicate) for phyllosphere microbiome analysis by washing leaves and filtering wash through 0.45 µm sterile filters. The V1-V3 hypervariable region of the 16S rRNA gene in the samples was amplified and DNA libraries prepared for sequencing on Illumina NextSeq1000. Qiime2 was used in data analysis.

PCA showed that PC1 and PC2 explained 16.5% and 11.0% of the total variance, respectively. Plastic and bare ground samples that mostly clustered together converged primarily by timepoint along PCI. The straw treatment (T2) diverged from all other samples along PC2, forming a distinct cluster. Straw (T1) did not yield sufficient reads to be included in the analysis. At the phylum level, the Proteobacteria and Actinobacteria dominated all mulch treatments at both time points. On the other hand, bare ground (T2) was dominated by Firmicutes (>74%). The Pseudomonadaceae, Oxalobacteraceae, Microbacteriaceae, Rhizobiaceae and Erwiniaceae were among the top five families. Alpha diversity varied significantly across treatments and time points (Kruskal–Wallis, $H = 14.29$, $p = 0.0266$); post hoc pairwise comparisons revealed that the BG (T1) group differed significantly from multiple groups across both time points P (T1), BG (T2), BP (T2), and S (T2). Additionally, alpha diversity differed significantly at T2 between P and S ($p=0.03$). Findings show that time of harvest and mulch treatment may influence the microbial composition of lettuce. This study can help assess agricultural practices that support sustainable practices, as well as crop and consumer health.

18. Assessing the Impact of Phthalates on Vaginal Bacteria Function and its Implications for Bacterial Vaginosis

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The vaginal microbiome is crucial in regulating female reproductive health. Compared to other microbiomes, the vaginal microbiome is unique in that it is considered healthy when it is single-species dominated (Lactobacillus spp.). Lactobacilli are essential for maintaining strong barrier properties in the female reproductive tract. The vaginal microbiome can shift into dysbiosis, where commensal organisms are displaced by pathogenic bacteria. Vaginal dysbiosis, known as bacterial vaginosis (BV), is characterized by a polymicrobial environment with impaired mucosal and epithelial barrier function. 30% of women in the United States are affected by BV and these individuals have increased susceptibility to infection, inflammation, and

disease. Clinical associations have been determined between BV and sexual practices, environmental factors, and stress have been determined, however the mechanisms promoting a dysbiotic environment remain not well understood.

Prior work has correlated systemic phthalate exposure and the composition of the vaginal microbiome, suggesting some phthalates are associated with BV. Phthalates are classified as endocrine disrupting chemicals known to interfere with estrogen and progesterone signaling. Phthalates are used as plasticizers in cosmetics and feminine hygiene products, directly exposing women to these chemicals in the vaginal environment. Despite this correlation, the mechanisms by which local phthalate exposure influence the onset of vaginal dysbiosis remains undefined. Taking together the presence of phthalates in feminine hygiene products and the lack of defined BV pathogenesis mechanisms, the role of phthalates in BV warrants investigation.

We hypothesize that tampon-derived phthalates disrupt the vaginal microbiome, contributing to adverse reproductive health. Here, we utilize optical density measurements to investigate the impact of phthalates on the growth of key commensal and pathogenic vaginal bacteria species (L. crispatus, L. iners, and G. vaginalis). We also evaluate the impact of phthalates on G. vaginalis, the “first invader” of BV, biofilm mass through a crystal violet assay. This work aims to understand mechanisms by which phthalates alter microbial composition in the vagina, with important implications for reproductive health and disease.

19. Modeling Skin Microbial Communities with Rewilded Laboratory Mice

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Laboratory mice have been widely used as model organisms in scientific research and have contributed greatly to medical advancements. Traditionally, these mice have been housed in clean, specific-pathogen-free (SPF) environments to prevent undesired infections that could confound research results. However, exposure to various microbes during early development and throughout life is critical to mature host immunity. Limited or lack of exposure to a variety of microbes in SPF mice leads to immature immunity that contributes to major differences in drug response of mice during pre-clinical research phases, compared to humans in clinical trials. Creating a model organism that reflects human biology is essential in advancing science and facilitating therapeutic discoveries, which has led to recent interest in understanding how SPF lab mice differ from wild mice in microbial composition, immunity, metabolism, and response to

various infections.

Exposing laboratory mice to a semi-natural outdoor enclosure, or “rewilding” them, allows us to study their immunological responses to various environmental changes. These rewilded mice are exposed to a variety of microbes (including bacteria, fungi, and viruses), varying ranges of temperature and humidity, and different sources of food and water. They are also able to burrow.

Our goal is to understand microbial changes in the skin of rewilded mice and to characterize immunological changes in the skin that may shed light on the host-microbe interaction in the natural environment. We performed 16S and ITS rRNA sequencing of skin microbes and discovered that there are significant differences in bacterial and fungal compositions between lab mice and rewilded mice. In addition, we recovered various bacterial and fungal isolates from rewilded mice.

In the future, we hope to understand how these environmental changes affect mouse skin by characterizing immune cells and investigating RNA signatures in the skin. We are also exploring how skin microbiota changes over time when returning these rewilded mice to a clean laboratory environment and evaluating whether they can be “dewilded” or whether the exposure to various microbes provides long-term immunological changes.

20. Clonal-Level Engraftment and Strain Tracking

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Engraftment of live biotherapeutics and probiotics is increasingly recognized as a key driver of clinical efficacy, yet most clinical microbiome studies still rely on species-level readouts that cannot distinguish donor or product strains from endogenous microbiota. Species resolution obscures which strains truly colonize, at what abundance, and for how long, limiting our ability to relate microbiome dynamics to therapeutic response and safety. Here we apply StrainQTM, a clonal-level metagenomic pipeline, to resolve engraftment and persistence of individual bacterial strains in recipients of live biotherapeutics and probiotics with high precision, enabling accurate quantification of multiple strains of the same species in microbiota-based interventions.

StrainQTM provides the clonal-level resolution needed to answer an important questions of probiotic development: does the product strain engraft, how abundant is it, how long does it persist, and how does it behave alongside endogenous strains? By distinguishing strains from endogenous background strains with high sensitivity and specificity, our pipeline turns shotgun metagenomic data into clear, actionable readouts of probiotic colonization. These quantitative

strain trajectories enable assessment of live biotherapeutics, support data-driven decisions on strain selection, formulation, and dosing, and de-risk clinical programs by directly linking engraftment and persistence to target engagement and clinical outcomes.

21. Metabolites in Urine Differ Among Isoflavone Metabolizing Metabotypes for Women Who Participated in the SWAN Study

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Daidzein, a soy isoflavone, is metabolized to O-desmethylangolensin (ODMA) by select gut bacteria, resulting in distinct ODMA producer and non-producer metabotypes. Evidence suggests producers have unique health associations, such as lower systolic blood pressure and percent body fat, alongside distinct gut metabolomes. This study aimed to characterize differences in metabolomics profiles across ODMA metabotypes to further our knowledge of the gut microbiome's influence on human health.

Using urine and serum samples from 191 participants in the Study of Women's Health Across the Nation (SWAN), researchers classified metabotypes via HPLC-MS/MS based on ODMA/daidzein ratios: non-producers (n=29), low-producers (n=117), and high-producers (n=45). Untargeted metabolomics data were processed via MS-DIAL, and differences across groups were compared using PCA and PLS-DA via Metaboanalyst.

PCA revealed a significant difference in urinary metabolite profiles between ODMA high-producers and non-producers ($R^2=0.049$, $p=0.023$), with PLS-DA explaining 17.4% of the total variance ($Q^2=0.45$) in urine samples; however, these differences were not evident in serum. Among key discriminating metabolites, high-producers exhibited higher concentrations of certain polyphenols and polyphenol metabolites (VIP > 5). These findings indicate that ODMA metabotype groups have significantly different metabolite clusters in urine, which may help explain previously observed health associations, though further research is needed to examine the specific mechanisms behind these unique health impacts.

22. Programmable Surface Adhesion Enables the Formation of Biofilm-Based Living Materials

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Engineered living materials (ELMs) that exhibit autonomy, growth, self-organization, and self-repair hold promise for applications in energy production, bioremediation, and healthcare. However, a central challenge in advancing ELMs has been developing strategies to reliably self-assemble living cells into structured, functional materials. Biofilms provide a compelling biological foundation for such systems due to their intrinsic capacity for self-assembly through coordinated cell–cell interactions and matrix-mediated cohesion. Marine adhesive proteins such as CP19K are known for their robust adhesion in natural systems; however, their potential to program self-assembly within biofilm-based living materials remains largely unexplored.

Here, we engineered the surface display of the barnacle-derived adhesive protein CP19K in Escherichia coli to drive programmable biofilm-based assembly using a plasmid-encoded genetic circuit. Confocal microscopy confirmed successful surface localization of CP19K. Under static conditions, CP19K promoted dual-mode aggregation, forming spheroidal assemblies in the bulk phase while simultaneously generating structured pellicles at the air–liquid interface within 24 hours. In contrast, shaking conditions introduced shear forces that suppressed interface colonization and favored filamentous, mesh-like assemblies in the bulk phase. These assemblies were less cell-dense and more protein-rich than those formed under static conditions, demonstrating that CP19K-driven aggregation is mechanically tunable and responsive to defined physical constraints. To identify the domains responsible for this behavior, we evaluated twelve discrete CP19K segments. Two segments recapitulated the full-length phenotype under both static and shaking conditions, revealing key domains required for CP19K-mediated assembly.

Together, these findings establish surface-displayed CP19K as a programmable strategy to control microbial self-assembly and provide a foundation for rational protein design in biofilm-based engineered living materials.

23. Gut Microbiome-Derived Agmatine Associates with Epinephrine Use and Adverse Events During Peanut Oral Immunotherapy

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Peanut protein allergy (PA) is the leading cause of food-induced anaphylaxis in children, and Peanut Oral Immunotherapy (POIT) is the only FDA-approved treatment for PA. Although POIT induces immune desensitization and remission in most patients, a substantial proportion of treated individuals experience adverse reactions requiring epinephrine to prevent anaphylaxis, with many participants requiring multiple doses during the IMPACT clinical trial (NCT01867671). Given the protracted nature of POIT and the high risk of severe adverse events in treated peanut-allergic children, identifying biomarkers to predict treatment responsiveness and to identify individuals at high risk for adverse events prior to therapy initiation would have substantial clinical utility.

Building on our previous work demonstrating that POIT efficacy is associated with gut microbiome metabolic capacity, we applied a machine learning framework to longitudinal fecal metabolomic and metagenomic data from 58 children undergoing POIT to identify microbiome determinants of adverse events, and epinephrine dose requirements in the IMPACT clinical trial.

By using logistic regression and random forest models, we found that agmatine, a byproduct of microbial arginine metabolism, could serve as a biomarker of epinephrine usage during POIT. The relative abundance of agmatine was significantly lower in the fecal samples of children who required at least one dose of epinephrine ($p = 0.00091$). Moreover, agmatine abundance was significantly depleted in POIT nonresponders ($p = 0.038$) and negatively correlates with peanut specific IgE levels at baseline ($p = 0.01$, $r = -0.32$). Together, our data suggest that agmatine, a

metabolite of gut microbial arginine metabolism, is associated with anaphylaxis risk and may play a protective role against POIT-related adverse events. Our future work will use in vivo and in vitro models to investigate the molecular mechanisms linking gut microbial arginine metabolism to food-induced anaphylaxis development.

24. Isolation of Antifungal Resistant Candida from Vineyards Across Multiple Wine Grape Production Seasons

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Fungicide usage in crop production can be a driving factor in the propagation of fungi with multi-drug resistance (MDR). The U.S. CDC recognizes Candida auris, a rapidly spreading MDR yeast implicated in nosocomial infections, as a critical health threat with environmental and potentially agricultural origins.

As grapevine production requires routine fungicide applications to mitigate crop loss due to phytopathogens, this study aimed to screen for emergence of C. auris in vineyards across Maryland over two grape production seasons. Grape clusters were collected at four MD vineyard sites during berry ripening in 2024 and 2025 (n=12 sampling events). Following a modified CDC protocol, harvested grape clusters were juiced and enriched in Salt Sabouraud Dulcitol Broth containing fluconazole at 37°C for up to one week. Samples with microbial growth were plated on CHROMagarTM Candida agar and incubated at 37°C for 48 hours. Putative Candida isolates with unique morphologies (n=18 in 2024, n=7 in 2025) were processed for DNA extraction, PCR for C. auris, and ITS2 amplicon sequencing for taxonomic identification. All grapevine isolates were negative for C. auris, however ITS sequencing revealed several key Candida relatives. Candida tropicalis, Meyerozyma guilliermondii, and Hanseniaspora uvarum were identified in 2024 and 2025, and Pichia kudriavzevii (formerly Candida krusei) and Zygoascus meyerae were also identified in 2024.

This work will be extended with whole genome sequencing to provide additional insights regarding potential health risks of the identified strains. Agricultural sites such as vineyards can be a valuable model system for exploring emergence of MDR Candida with public health implications.

25. Lactobacillus Crispatus-Dominated Cervicovaginal Microbiomes Coupled with Distinct Immune Profiles are Associated with Spontaneous Clearance of Chlamydia Trachomatis

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Up to 32% of urogenital Chlamydia trachomatis (CT) infections in women spontaneously clear without antibiotics between screening and treatment. The contributions of the cervicovaginal microbiome and immune milieu to clearance are unclear. Identifying drivers of spontaneous clearance can inform antibiotic-sparing strategies to reduce CT and long-term sequelae, like infertility.

The Longitudinal Study of Vaginal Flora (1999-2002) followed women quarterly for 1 year and collected endocervical swabs and cervicovaginal lavages at each visit. Baseline CT screening was started in 2001 based on commercial availability of ligase chain reaction assays; untested swabs were screened retrospectively. Empiric treatment and symptomatic CT testing were implemented throughout the study. We excluded visits with prior CT-active antibiotics and designed a nested case-control study, defining persistence as 2 consecutive CT+ visits (N=310) and spontaneous clearance as a CT+ followed by CT- visit (N=301). We used stored lavages for metagenome sequencing and quantifying lactic acid isomers and 21 cytokines.

Compared to optimal Lactobacillus crispatus-dominated microbiomes, bacterial vaginosis-associated microbiomes predominated by “Candidatus Lachnocurva vaginae” or specific Gardnerella species were associated with CT persistence at the next visit 3 months later. Low D- and L-lactic acid levels were also associated with persistence. Hierarchical clustering

revealed 5 cervicovaginal immune profiles: intermediate chemoattractant levels (CCL20, CXCL1, CXCL10, IL-8), intermediate IL-1a/b levels, high chemoattractant levels, high IL-1a/b levels, and high cytokine levels. Compared to the high IL-1a/b or high cytokine profiles, the intermediate chemoattractant profile was associated with subsequent spontaneous CT clearance, and it was largely composed of Lactobacillus-dominated microbiomes that were compositionally unique from Lactobacillus-dominated microbiomes assigned to other immune profiles. The high IL-1a/b and high cytokine profiles co-occurred with Gardnerella- and "Ca. L. vaginae"-predominated microbiomes.

This work offers new insight into in vivo host-microbiome interactions that likely influence CT natural history. Lactobacillus-dominated microbiomes coupled with intermediate chemoattractant responses may promote spontaneous clearance, while bacterial vaginosis-associated microbiomes with pro-inflammatory states may hinder clearance.

26. Oyster Microbiome Insights: Exploring Relationships between Bacterial Communities and Host Health Metrics

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Microbiomes have the potential to provide beneficial services to bivalve hosts through antimicrobial and probiotic activity, immune defense, and response to environmental stressors which can impact host performance. Ongoing work aims to classify core members of oyster microbiomes to better establish bacterial symbionts that serve as markers of oyster health.

This talk will synthesize two 16S metabarcoding field studies of oyster gills - a survey and a reciprocal transplant - to provide insight into microbial members contributing to Crassostrea virginica health and performance in Chesapeake Bay. Oyster gills surveyed in the field maintained distinct bacterial communities from the water, varied by site, and revealed an association between Endozoicomonas and the absence of Perkinsus spp. infection. No ASVs were shared by all oysters, but 103 ASVs were shared across surveyed sites spanning 4 tributaries. During transplant experiments, oyster condition depended on disease status and recovery location rather than deployment/source location. Oyster gills shared 4 core ASVs and bacterial community composition clustered by source location with shifts in composition for oysters transplanted between rivers.

These results indicate the current environment and infection status are more important for oyster health than oyster source, but shifts in microbial community vary based on both source and

current environment. Further synthesis across studies will explore shifts in gill microbiomes with ploidy, site-specific features, and oyster health metrics.

27. Exploring Interactions Between Cholesterol and the Gut Bacterial Genus, Turicibacter

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Bile salts, produced from cholesterol in the liver, play a crucial role in regulating cholesterol levels by emulsification of dietary fat and cholesterol in the intestine. Excess cholesterol or hyperlipidemia in circulation can eventually lead to plaque buildup narrowing blood vessels, ultimately resulting in stroke and/or heart disease- leading causes of death globally. Human metagenomic data has previously demonstrated reduced abundance of the bacterial genus Turicibacter in the gut microbiomes of individuals with obesity, and experimental models have shown reduced circulating cholesterol in mice colonized with Turicibacters. However, specific mechanisms by which Turicibacter may reduce or respond to host cholesterol specifically have yet to be identified.

We have previously demonstrated that monocolonization with individual Turicibacter strains significantly alters host bile acid profiles and engineered expression of bile modifying Turicibacter proteins is sufficient to reduce serum cholesterol, triglycerides, and adipose tissue mass. We performed microscopy using fluorescent cholesterol and found that cholesterol may be taken up by Turicibacters. Using RNAseq, we identified several candidate Turicibacter genes that are upregulated upon exposure to cholesterol. Further, we identified several candidate Turicibacter proteins that bind to cholesterol using a click chemistry-based pull down assay. Interestingly, data from both experiments orthogonally found identified proteins related to an ATP-binding cassette (ABC)-transporter. ABC transporters are a large family of membrane proteins used to pump a diverse range of different substrates, including cholesterol, across membranes.

We designed several plasmids containing cholesterol-responsive candidate genes from Turicibacter sanguinis and are currently purifying and biochemically characterizing these proteins to identify Turicibacter proteins responsible for cholesterol utilization. Future work will involve targeted lipidomics to identify cholesterol metabolites and breakdown products in addition to genetic knock-out models.

28. Microbial Food Safety in Integrated Crop and Pig Agroecosystems in South Africa

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Integrated farming systems (IFS), which combine crop and livestock production, are globally promoted for enhancing resource recycling and food security. However, the interconnectedness of these food production systems may create pathways for transmission of bacterial pathogens and antimicrobial resistance (AMR) between animals, crops, and the surrounding environment.

This study evaluated the presence and prevalence of Escherichia coli, Shiga toxin-producing E. coli (STEC), ESBL and AmpC-producing Enterobacterales (AMR-Ent), Salmonella, and Listeria monocytogenes across IFS pig production systems in South Africa that varied in scale (n=3 small-scale, n=3 commercial farms). Samples of soil, irrigation water, animal feed, drinking water, animal waste (fertilizer), and spinach or maize crops were collected at each site (n=5 per sample type). The AMR profiles of the bacterial isolates were determined using Kirby-Bauer disk diffusion method (n=17 antibiotics) for phenotypic characterization. Two-way ANOVA was used to evaluate differences in bacteria concentrations and AMR profiles as a factor of farm site and sample type.

E. coli was detected in all sample types at all farms, with higher concentrations in irrigation water at commercial farms ($P<0.05$). In total, 162, 3, 74, and 4 isolates of E. coli, PCR-confirmed STEC, presumptive AMR-Ent, and Salmonella were recovered. AMR phenotypes varied across sites and sample types. For example, resistance to beta-lactams was more prominent in isolates from commercial farms and linked to soil and irrigation water. In contrast, resistance to (fluoro)quinolones was elevated in animal feed and crops at small farms, where multidrug resistance (i.e., >3 antibiotics) was higher as well ($P<0.05$).

Farm management practices, resource availability, and production scale collectively influence microbial transmission within IFS. These factors have important implications for developing effective food safety interventions, targeted risk mitigation, and improved agricultural practices.

29. Strainify: Strain-Level Microbiome Profiling for Short-Read Metagenomic Datasets

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Strain-level microbiome profiling provides key insights into microbial composition and dynamics, yet accurate analysis remains challenging for a variety of reasons but especially for short-read metagenomic data. The problem is especially challenging when a large number of strains may be present due to the lower per-strain abundance amplifying the relative estimation error.

We present Strainify, a method for accurate strain-level abundance estimation from short-read metagenomes with as little as 1x genome coverage (at the species level). Strainify identifies uses a maximum likelihood model to estimate strain abundances based on core genome alignments and read counts for informative variants. Across simulated communities of varying complexity, Strainify consistently outperformed existing approaches and was able to successfully estimate strain-level abundances with as many as 30 distinct strains for a given species in simulated data. In mock community data, its estimates closely match reference abundances. Together, these results establish Strainify as a robust and versatile solution for accurate strain-level abundance estimation in short-read, low-coverage microbiome studies.

30. Microbial Community Dynamics During Conversion of Dairy Waste to Medium-Chain Carboxylic Acid at Ambient Temperature

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The transition toward a circular bioeconomy demands innovative biotechnologies that convert organic waste streams into valuable products while minimizing energy inputs. Microbial chain elongation achieves this by converting low-value organic substrates into medium-chain carboxylic acids (MCCAs, C6–C8) through the reverse β-oxidation pathway. MCCAs, including caproate and caprylate, are platform chemicals with higher market value than short-chain acids due to their greater hydrophobicity, higher energy density, and easier phase separation. These properties reduce downstream recovery costs and expand their utility in animal feed additives, antimicrobial formulations, sustainable aviation fuels, and bioplastic precursors. In addition, producing MCCAs from dairy waste such as cheese whey and skimmed milk valorizes

lactose-rich effluents that would otherwise contribute to high chemical oxygen demand (COD) disposal burdens, thereby improving waste management economics and reducing greenhouse gas emissions.

Time-series 16S rRNA amplicon sequencing using DADA2 identified 1,247 ASVs across 312 genera. Two functional guilds dominated: lactic acid bacteria (LAB; 12–87%) and chain elongators (Clostridium spp.; <1–45%). Maximum MCCA yield (0.40 g $COD \cdot g^{-1}$) occurred at HRT=15d with cheese whey feedstock. During process intensification (OLR=10, HRT=7.5d), pH critically determined product selectivity. The pH of 5.25 achieved highest caproate (C6 MCCA) selectivity (58.7%), while pH 5.0 shifted toward butyrate (C4) dominance (85.6%). Linear regression revealed a highly significant correlation between Clostridium abundance and caproate production ($r=0.924$, $R^2=0.854$, $p=0.0001$), indicating that 85.4% of caproate variance was explained by chain elongator abundance. This relationship enables predictive process monitoring using microbiome data. One-way ANOVA confirmed significant differences across experimental phases for butyrate ($p<0.001$), caproate ($p=0.003$), and caprylate ($p<0.001$). Notably, Caproiciproducens, which was dominant in previous mesophilic systems, was absent (<0.02%), suggesting cold-adapted Clostridium species perform chain elongation at ambient temperature.

These findings demonstrate that stable MCCA production is achievable without heating, with microbiome composition serving as a reliable performance indicator, advancing data-driven approaches for sustainable waste valorization.

31. Viral Impacts on Prochlorococcus Biogeography, Primary Productivity, and Biogeochemistry in a Model Ocean

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Photosynthetic microorganisms drive marine primary production and ocean biogeochemical cycles. Yet viral lysis is generally absent from large-scale ocean ecosystem models despite its documented role in regulating phytoplankton mortality and primary production.

In this study, we developed a computational analytics framework to quantify viral regulation and control in complex ocean microbiomes. First, we constructed and parameterized a viral lysis model in a non-spatial configuration for multiple phytoplankton types, enabling stable coexistence between a single phytoplankton, virus, and zooplankton population. Second, we embedded this formulation within a large-scale ocean ecosystem model. We evaluated the regional impacts of viral processes on Prochlorococcus, a widespread and abundant photosynthetic cyanobacterium in tropico-equatorial waters.

Our results reveal distinct effects of viral lysis - and particularly the viral shunt, which redirects lysate to the dissolved organic pool - on standing stocks and primary production. This framework offers a foundation for integrating viral processes into predictive ocean ecosystem models and provides a scalable approach to quantifying viral control in complex microbial systems.

32. Growth Phase Dictates Function of Gardnerella Vaginalis-Derived Bacterial Extracellular Vesicles

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Bacterial extracellular vesicles (bEVs) have emerged as a potential tool for targeted drug delivery. bEVs are membrane-bound nanoparticles loaded with bioactive cargos that mediate microbe-host interactions and modulate host-immune responses to microbiome-associated diseases. (Moore et al, Nanoscale, 2024) Our recent work demonstrates how endogenous properties of bEVs support their development as a vaginal drug delivery platform for targeting and treating female reproductive tract diseases. (Steinman et al, NPJ, 2026) Here, we investigate effects of growth phase on bEV biogenesis and host-immune interaction to inform their use for vaginal drug delivery.

Colony forming unit (CFU) analysis, optical density (OD600), and biocalorimetry identified times to collect bEVs reflective of the lag, exponential, stationary, early death, and late death phases. Transmission electron microscopy visualized morphology of isolated G. vaginalis-derived bEVs, while nanoparticle tracking analysis characterized the bEV concentration, size, and ζ-potential. Interactions between phase-specific bEVs and vaginal epithelial and endocervical cells were evaluated using uptake assays, viability assays, and electrochemiluminescence immunoassays. Furthermore, meta-omics analysis was performed to analyze changes in bacterial gene expression and protein and metabolite cargo of bEVs.

Bacterial growth phases were defined as: lag phase (0 to 12 h), exponential phase (12 to 24 h), stationary phase (24 to 36 h), early death phase (36 to 48 h), and late death phase (48 to 60 h). Presence of bEVs across the growth phases were confirmed by TEM and significant differences in bEV number, size, and ζ-potential were observed across growth phases ($n = 6$, $p < 0.0041$). No significant difference in the total protein content of bEVs was observed across growth phases. We observed significant differences in viability, uptake, and cytokine secretion of vaginal epithelial and endocervical cells in response to bEVs from distinct growth phases ($n \geq 4$, $p < 0.0134$). Further, we identified changes across growth phases in bacterial gene expression and the protein and metabolite cargo.

Together, these studies define growth phase-dependent differences in both bEV characteristics and their effects on modulating host cell immune pathways within the female reproductive tract. This work provides the framework for developing microbiome-targeted therapeutics to promote female reproductive health.

33. De Novo Engineered Living Materials via Elastin-like Polypeptide-Mediated Self-Assembly

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Microbiomes, from the human gut to soil ecosystems, encompass a diverse range of microbial communities that perform essential functions across various environments. However, their long-term resilience and functionality are often compromised by a lack of robustness, primarily due to factors such as environmental stress and instability. One promising solution lies in mimicking nature's own strategy of biofilm formation, where microbial populations create more stable and resilient communities by promoting intercellular adhesion.

This study presents a novel approach to enable bacterial self-assembly in E. coli through the surface display of adhesive proteins, elastin-like polypeptides (ELPs), creating ELP-based engineered living materials (ELMs) that mimic biofilm formation. We engineered E. coli cells with a plasmid-based, inducible genetic circuit to enable the surface display of ELPs. The extracellular localization of ELPs was confirmed via confocal microscopy and Western Blot analysis. To elucidate the design principles behind cell viability and material size, we developed three ELP variants by substituting the standard guest residue alanine with lysine (ELP10-LYS, positively charged) or glutamic acid (ELP10-GLU, negatively charged). Among these variants, the ELP10-LYS outperformed the others in terms of cell viability, as confirmed through confocal imaging after propidium iodide staining. Additionally, ELP10-LYS led to the formation of larger, macroscopic material assemblies, observable at the centimeter scale through image analysis. As smaller assemblies may be beneficial for certain microbiome applications, ELP10-GLU showed reasonable cell viability while providing consistent material formation below the centimeter scale.

These ELP-based ELMs offer a versatile platform for controllable microbial self-assembly. By mimicking biofilm-like structures, ELP-based ELMs could enhance microbial colonization in challenging environments such as the gut or soil and protect against external stressors, ultimately increasing the overall robustness of the microbiome. Moreover, having the cells contained within a biopolymer matrix offers stricter biocontainment than free cell solutions.

34. Microbiome Meta-Analysis: Single-Command Generation of Merged ASVs

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Publicly available and independently generated 16S rRNA sequencing datasets represent a valuable resource for microbiome research. However, cross-study comparison is frequently limited by heterogeneous preprocessing workflows, inconsistent parameter selection, and legacy OTU-based approaches. In ocular surface microbiome research, there is an opportunity for standardized ASV-level meta-analysis to enhance reproducibility and statistical power. The objective of this work was to develop a harmonized, automation-ready R framework enabling unified reprocessing and merging of independent 16S datasets.

An existing instructional 16S analysis workflow in R was iteratively refactored into a dataset-agnostic processing framework through systematic troubleshooting, structural redesign, and automation engineering. Development emphasized crash resistance, parameter harmonization, standardized sample handling, and explicit analytical provenance via structured logging and validation checkpoints. Cross-step parameter logic was encoded such that quantitative outputs (e.g., read retention, quality distributions, control prevalence) programmatically inform downstream thresholds, minimizing manual decision-making. The framework was progressively hardened for portability across heterogeneous datasets. Code refactoring and modular redesign were accelerated using AI-assisted programming tools to enhance structural robustness and universality.

The resulting framework performs harmonized ASV inference using DADA2 and implements automated primer detection, adaptive quality filtering, denoising, chimera removal, contamination identification (decontam), taxonomic assignment, and phyloseq object construction under unified analytical conditions. Root-level orchestration enables batch processing across multiple project directories via single-command execution. Independent datasets are reprocessed under standardized inference conditions and merged into a unified ASV matrix suitable for ecological modeling and cross-study meta-analysis.

By reducing methodological heterogeneity at the sequence inference stage and enabling reproducible generation of merged ASVs with preserved provenance, this framework provides a scalable foundation for cross-study microbiome integration across both public and locally generated 16S datasets.

35. Microbiome Discovery and the Conservation of Reptiles and Amphibians

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Microbiomes are known to be important contributors to host fitness, interacting with their immune systems, physiology, and behavior. Most microbiome work to date has been in human and other model species, yet lessons learned from these can be applied to the conservation of lesser-known taxa. One such group is reptiles and amphibians, the most threatened vertebrate clades on the planet.

In our lab, we try to deepen our understanding of reptile and amphibian microbiomes in order to contribute to the management of these threatened species. For example, we are characterizing the microbiomes of two threatened turtle species, Eastern Box Turtles and Northern Map Turtles, to provide reference intervals and to begin understanding environmental interactions with the microbiome. Interactions or factors being studied include the effects of geography, host personality, captivity, age, sex, habitat type, pollution, and the presence of a hydroelectric dam.

In this poster, we highlight several ongoing lab projects on amphibian and reptile microbiomes and their implications for conservation. We also discuss how such research can be communicated to the public through outreach.

36. Microbiomes of Lettuce Grown Adjacent to CAFO Reveal Presence of Pen Soil

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Concentrated animal feeding operations (CAFOs) and fresh produce growing fields are in proximity with each other in some agricultural settings, creating the potential for transfer of STEC to fresh produce by direct aerial deposition of contaminated cattle pen soil.

Metagenomics based microbial source tracking was applied to outer romaine lettuce leaf microbiomes to evaluate the impact of distance on produce contamination from fugitive dust deposition from a large CAFO.

Metagenomic sequencing was performed on soil (n=449), irrigation water (n=70), and composite outer lettuce leaf (n=199) samples collected from five experimental plots in different growing seasons and located 0.26 to 1.97 miles from a CAFO (>100,000 cattle) in the southwestern US, as well as on cattle pen soil (n=8) from a cattle feedlot in the southwest. For each field separately, proportions of source bacterial communities (pen soil, field soil, and water) were computed for individual lettuce bacterial communities using SourceTracker2. Lettuce metagenomes were also queried for cattle mitochondrial DNA (mtDNA) using exact matching with a privately curated database.

The median percent lettuce bacterial communities similar to cattle pen soil ranged from 1.6% to 35.9%, with lettuce grown a greater distance from the CAFO having lower proportions of pen soil (Wilcoxon rank, $P<0.0001$). In addition, the relative abundance (RA) of Corynebacterium maris, a bacterial species associated with cattle, found in the lettuce metagenomes followed the same trend (Wilcoxon rank, $P<0.0001$) with median RA values of 1.0% and 22.9% for 1.97 and 0.26 miles, respectively. While cattle mtDNA was detected in 65.6% of the lettuce samples located 0.26 miles from the CAFO, a prevalence rate of only 7.8% was observed at 1.97 miles.

This work highlights the importance of including the potential for airborne transmission of pathogens in risk assessments for contamination of fresh produce grown in proximity to CAFOs.

37. Characterizing Fatty Acid Uptake and Metabolism by Gut Bacteria from the Genus Turicibacter

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The gastrointestinal microbiota is a potent mediator of host lipid interactions and metabolism. Gram positive bacteria from the understudied Turicibacter genus live in the guts of mammals, birds, and fish and are predicted to interact with lipids in human and animal hosts. Monocolonization of mice with different strains of Turicibacter alter host serum lipids with a notable decrease in serum triglycerides, particularly those containing saturated and monounsaturated fatty acids (FAs).

To understand how Turicibacters directly interact with FAs, we performed RNAseq on Turicibacter cultures preincubated with FAs and found that saturated, monounsaturated, and polyunsaturated FAs elicit distinct transcriptional responses in these cells. These results led us to identify putative Turicibacter gene homologs of a recently discovered Gram positive FA uptake system called the fatty acid kinase (Fak) system. Our RNAseq data indicated that different FA substrates prompted a different regulation pattern for the four FA-binding (FakB) homologs possessed by Turicibacters, and later amino acid sequence analysis revealed that three of these homologs are distinct from all previously studied Fak proteins.

To study these unique homologs, we have purified Fak kinase and FakB homologs identified in Turicibacters and are probing their ability to phosphorylate FAs (a crucial first step during metabolism). In complementary experiments to understand the fate of fatty acids taken up by Turicibacter, we used fluorescent labeled FAs as probes to determine that Turicibacters incorporate exogenous FAs into polar and neutral lipids. Further imaging analysis revealed the presence of lipid-labeled structures within Turicibacter cells that are not well studied in gut bacteria.

Further studies will lay a foundation for future research into how FA metabolism by Turicibacters and related bacteria influences host lipid metabolism and inform production of microbiota-based therapeutics for metabolic disorders.

38. Metagenomic Analysis of the Microbial Community of an Experimental Hydroponic System Growing Leafy Greens

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Hydroponics is gaining popularity for the growth of fresh produce due, in part, to the idea that hydroponic growth improves food safety outcomes. However, recent recalls of hydroponically grown lettuce due to contamination with foodborne bacterial pathogens highlight knowledge gaps regarding the microbiology of hydroponic systems.
This study explored the diversity and development of hydroponic microbiomes from inputs (seeds, nutrient solution, water) to harvest, and sought to identify food safety risks.

Romaine and butterhead lettuce were sprouted in rockwool and then grown in a benchtop deep water culture hydroponic system. Metagenomic sequencing was performed on samples from the hydroponic system and its inputs to characterize the microbiome and to identify potential food safety hazards. Samples were collected from the hydroponic system from the root zone, leaves, nutrient solution, and the interior surfaces during the 85-day growth period, as well as from the seeds (pelleted and naked), sprouts, rockwool, and nutrient solution inputs. Samples underwent shotgun metagenomic sequencing.

The microbial communities were taxonomically distinct, demonstrating variation by sampling location, lettuce type, and timepoint. The hydroponic system retained over 90% of the original seed genera, with half of all genera originating from the seeds. No foodborne pathogens were detected in the metagenomes; however, Legionella, a human pathogen often found in water, was amongst the top ten most abundant genera. Functional profiling of the metagenomes revealed a high abundance of metal resistance genes for copper, mercury, and nickel, suggesting that heavy metals might build up in such closed loop systems and need to be monitored in future work.

This study highlights that the inputs to a hydroponic system have a large and lasting impact on its microbiome. Future work will assess how the inputs might introduce and lead to the persistence of pathogens in hydroponic systems.

39. Predictive Modeling of Lupus Nephritis using Gut Microbiome Signatures

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Identifying early microbiome signatures that predict autoimmune disease progression remains a critical challenge in translational microbiome research. Here, we evaluated whether gut microbial composition at 7 weeks of age (pre-disease) could predict renal disease severity at 15 weeks (late disease) in the lupus-prone MRL/lpr mouse model. A total of 12 female mice were used as lupus has strong female bias.

16S rRNA amplicon sequence variant counts were normalized using the DESeq2 median-of-ratios method and aggregated at the family level. We applied supervised multivariable linear regression to model the relationship between early microbial features and subsequent proteinuria scores. A four-family model explained a substantial proportion of variance in later proteinuria ($R^2 = 0.76$, $p = 0.024$), with no evidence of multicollinearity (VIF < 3). Model refinement identified the families Oscillospiraceae and Ruminococcaceae as the primary contributors. The simplified two-family model remained significant ($R^2 = 0.59$, $p = 0.019$). Model robustness was evaluated using leave-one-out cross-validation (LOOCV), yielding moderate predictive performance ($r = 0.49$, RMSE = 1.50). To assess statistical stability, we performed 5,000 permutation tests, confirming that the observed explanatory power was unlikely under random label assignment ($p = 0.018$).

Together, these findings indicate that early gut microbiome composition contains measurable predictive information for lupus nephritis in mice. Elevated Oscillospiraceae and Ruminococcaceae abundance at 7 weeks forms a predictive microbial signature for subsequent proteinuria severity, supporting a potential role for specific microbial families in early disease programming. This work highlights the use of supervised modeling approaches in identifying disease-relevant microbiome features and provides a foundation for mechanistic investigation of microbiota-driven modulation of autoimmune kidney injury.

Future work will extend this framework using non-linear machine learning models, including random forest approaches to capture higher-order microbial interactions, and Markov chain–based state transition modeling to quantify probabilistic progression between microbiome states.

40. Comparative Functional Genomics and Systems-Level Metabolic Modeling of Three Commensal Clostridium Species Reveals Divergent Butyrate Biosynthetic Potential

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Comparative Functional Genomics and Systems-Level Metabolic Modeling of Three Commensal Clostridium Species Reveals Divergent Butyrate Biosynthetic Potential

Although multiple Clostridium species inhabit the human gut, their metabolic specialization and probiotic potential remain incompletely defined. We performed integrative comparative genomics and metabolic modeling of C. celatum, C. disporicum, and C. vincentii to characterize traits associated with gut adaptation and butyrate biosynthesis. These species were identified in metagenomic studies as inversely correlated with obesity, and in vitro screening confirmed their probiotic properties via bile salt hydrolase activity, hemolytic potential, and colonization capacity. Butyrate regulates intestinal barrier integrity and immune homeostasis.

Genomes were annotated using eggNOG-mapper v2 to identify genes linked to stress tolerance, bile resistance, and Clusters of Orthologous Groups (COGs). CAZyme repertoires were profiled using dbCAN3 to assess substrate utilization. Following confirmation of conserved butyrate biosynthesis pathways, we reconstructed a genome-scale metabolic model for C. celatum within the AGORA2 framework. FBA and FVA were applied to predict growth fluxes and SCFA production under defined nutrient constraints.

Approximately 400 genes per species were linked to probiotic-relevant functions. COG analysis revealed distinct metabolic distributions among species, reflecting divergent adaptive strategies. C. celatum encoded the highest number of genes for stress tolerance (n=38) and bile resistance (n=24), suggesting enhanced gastrointestinal resilience. CAZyme profiling showed enriched glycoside hydrolase repertoires, with C. celatum harboring 27 CBM32 domains, consistent with increased glycan-binding capacity. Modeling predicted a growth rate of 0.328 h^{-1} and butyrate production of 5.71 mmol gDW^{-1} h^{-1} under glucose. Acetate co-utilization enhanced butyrate synthesis, and FVA confirmed sustained production across optimal solutions (1.6–7.0 mmol gDW^{-1} h^{-1}).

While all three species share conserved biosynthetic pathways, C. celatum exhibits distinct genomic and metabolic features consistent with mucosal adaptation and efficient butyrate production, justifying its prioritization for experimental validation and next-generation probiotic development.

41. Biotransformation and Toxicity of PFAS Precursors

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Per- and polyfluoroalkyl substance (PFAS) precursors are increasingly detected in environmental systems, where biological and abiotic transformation processes can generate persistent and toxic terminal perfluoroalkyl acids (PFAAs). However, the understanding on pathways governing PFAS precursor biotransformation and the potential toxicity effects associated with transformation intermediates and released fluoride ions are limited.

This study aims to investigate the biotransformation behavior of PFAS precursors and evaluate their impacts on microbial activity and toxicity during degradation processes. Microcosm experiments will be used to examine precursor transformation under environmentally relevant conditions while monitoring microbial metabolic responses.

Results from this study will provide insight into the environmental fate of PFAS precursors and contribute to improved assessment of risks associated with their transformation in contaminated systems.