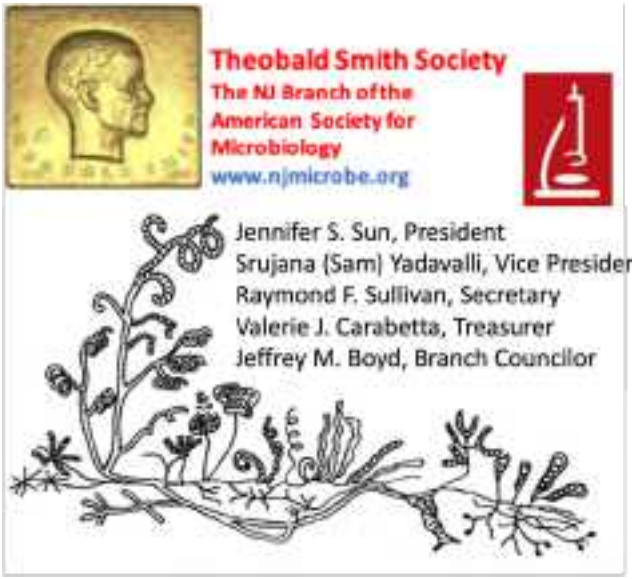

Fall 2024 Symposium Proceedings

8 November 2024

The Institute for Marine & Coastal Sciences
Rutgers University, New Brunswick, NJ

4:00pm Opening remarks by Theobald Smith Society President Jennifer S. Sun. Jenn's research combines her expertise in microbiology, entomology, neuroscience, and biochemistry to investigate how insects' sense of smell can be altered by the bacteria which reside in their gut. As a Presidential Postdoctoral Fellow, Dr. Sun employs an interdisciplinary approach to understand if and how endosymbionts enable insect vectors to locate suitable hosts, with the aim of providing better insight into the mechanism of olfaction across species through the study of a highly evolutionarily conserved interspecies relationship. Dr. Sun obtained her Ph.D. in Molecular, Cellular, and Developmental Biology from Yale University and postdoctoral training in infectious diseases from Princeton University.

4:10pm 2024 Theobald Smith Society Young Investigator Award Lecturer Srujana (Sam) Yadavalli, PhD. Sam earned her Ph.D. in microbiology from the Ohio State University and has postdoctoral training in bacterial physiology and stress response from the University of Pennsylvania. She is an Assistant Professor in the Department of Genetics and the Waksman Institute of Microbiology at Rutgers-New Brunswick. Research in the Yadavalli lab is focused on two emerging themes in bacterial gene regulation: (i) Small regulatory proteins – proteins with less than 50 amino acids that are translated from short open reading frames; (ii) Epitranscriptomic regulators – proteins that connect RNA modifications and translation to metabolism and stress response. Her research integrates tools from high throughput sequencing, genetic engineering, microbiology, biochemistry, and proteomics to address fundamental questions in bacterial stress response.

Sam presented recent work regarding the challenges of discovering and characterizing small membrane proteins (up to 50 amino acids) involved in *E. coli* stress response. Until recently, these small proteins were not annotated in genome databases and often overlooked using traditional protein analytical techniques. Using Ribo-RET libraries of E. coli cells grown in media with or without Mg^{2+}, her lab identified 17 small proteins involved in Mg^{2+} stress response. Retapamulin (RET) stalls ribosomes at their translation start sites and the subsequent ribosome protected mRNA fragments were surveyed by deep sequencing. Many of these small proteins were found to be membrane-bound where they can interact with larger membrane proteins/complexes such as two-component signaling systems. A preprint of the work was recently submitted https://www.biorxiv.org/content/10.1101/2024.09.13.612970v4

4:50pm American Society for Microbiology Distinguished Lecturer Sean Crosson, PhD, is the Professor Rudolph Hugh Endowed Chair at Michigan State University. He studies mechanisms by which bacteria adapt to diverse environments, including freshwater, soil, and the interior of mammalian cells. Dr. Crosson received his B.A. in biology from Earlham College, his Ph.D. in molecular biophysics from the University of Chicago and was a postdoctoral fellow at Stanford University. Prior to joining the faculty of Michigan State in 2019, he was a professor of biochemistry and molecular biophysics and of microbiology at the University of Chicago.

The Crosson lab seeks to understand molecular mechanisms that underlie the ability of bacterial cells to adapt to complex, dynamic environments, including mammalian hosts. Their research primarily focuses on the physiology of *Alphaproteobacteria*, including mammalian pathogens of the genus *Brucella* and the freshwater/soil bacterium *Caulobacter crescentus*. To this end, they utilize an interdisciplinary set of genetic, biochemical, and biophysical approaches to address these questions on multiple scales, from the cellular/systems level to the level of molecular structure. They also collaborate with clinicians and ecologists on studies of *Bacteroides fragilis* and other bacteria that reside in the human gut. Dr. Crosson will present recent work in this area including the discovery of novel bile resistance genes in the opportunistic gut pathogen *Bacteroides fragilis* and dissection of a multicomponent sensory system that is critical for bacterial cell adhesion to surfaces. This work is aimed at understanding the contribution of these microbes to inflammatory bowel diseases.

In the first portion of his presentation, Dr. Crosson talked about *Caulobacter crescentus,* a model organism that exhibits unique polar cell development, allowing it to mediate attachment to various surfaces, including abiotic and biotic interfaces, self-surfaces, and air-liquid boundaries. This attachment is facilitated by Wzy-dependent polysaccharides, which contribute to the formation of a polysaccharide-based adhesin known as holdfast—a specialized structure which enables tight attachment to exogenous surfaces (see https://journals.asm.org/doi/10.1128/jb.00276-19). Dr. Crosson's lab utilized barcoded mutants to calculate fitness, focusing mainly on enriching mutants that cannot adhere to surfaces like cheesecloth. This approach led to identifying potential holdfast biosynthesis genes, including ccna_02722, which encodes a protein that helps cells attach but does

not allow them to adhere to cheesecloth (see https://journals.asm.org/doi/10.1128/mbio.02273-18). They further investigated the holdfast biosynthesis pathway, revealing that the holdfast polysaccharide consists of common sugars, including glucose and mannose. Having identified key enzymes and the sugars involved, they hope to reconstitute the holdfast biosynthesis pathway in vitro to better understand its composition and assembly.

In the second portion of the presentation, Dr. Crosson presented their most recent research findings on *Bacteroides fragilis*, a key player in colitis and pouchitis, a condition that often arises following the surgical creation of a J-pouch after colon removal. By collaborating with clinicians and ecologists, Dr. Crosson's lab found the treatment of *B. fragilis* with physiologically relevant concentrations of the secondary bile acid deoxycholate reduced cell growth and remodeled transcription of one-quarter of the bacterium's genome, resulting in the discovery of novel bile resistance genes in *B. fragilis*. They also discovered the mechanisms behind resistance and the survival of *B. fragilis* inside the J pouch. He hopes their findings will contribute to understanding the causes of inflammatory bowel diseases (see https://journals.asm.org/doi/10.1128/mbio.02830-23).

There was not a hint of self-importance in Professor Crosson; he leads from the front. While most of the ASM Distinguished Lecturers we've had are anxious to get on the road after their talks, Sean was there with a big smile until the very end engaging with students, post-docs, faculty – with everyone. He volunteered to be a poster presentation judge. He was there putting away the poster easels and helping with the clean-up. He was the last one to leave. From the moment he showed up, he was there to serve.

Sean Crosson and Sam Yadavalli enjoying dinner.

Branch Councilor and Past President Jeff Boyd and Professor Sean Crosson.

Invited Speakers

Precious Newman, Department of Biomedical Sciences, Cooper Medical School of Rowan University, Camden, NJ: "Phage-antibiotic combination therapy: restoring antibiotic sensitivity in extensively drug-resistant Acinetobacter baumannii."

Arkajyoti Dutta, Department of Plant Biology, Rutgers University, New Brunswick, NJ: "Small molecule and peptide inhibitors of ricin & Shiga toxin-ribosome interaction."

Liisa Veerus, Center for Advanced Biotechnology and Medicine, Rutgers University, Piscataway,NJ: The interplay between the maternal gut microbiome and breast milk sugars."

Amir George, Department of Pharmacology, Physiology and Neuroscience, Rutgers New Jersey Medical School, Newark, NJ: "The quantification of drug accumulation and metabolism within gram-negative bacteria."

Sarah Krisak and Naima Zaheer, Montclair State University, NJ: "Investigating the effectiveness of different artificial root exudate combinations on soil microbial function in metal-contaminated soil."

Duhita G. Sant, Center for Advanced Biotechnology and Medicine, Rutgers University, Piscataway, NJ: "The effect of cross feeding interactions on mutant fitness in communities of *E. coli.*"

POSTERS

1. Small protein YoaI connects distinct signaling systems – PhoQ-PhoP, PhoR-PhoB, and EnvZ-OmpR.

Sangeevan Vellappan[1,2,3], Junhong Sun[1], John Favate [2,3], Pranavi Jagadeesan[1], Debbie Cerda[1,2], Premal Shah [2,3], Srujana S. Yadavalli[1,2]

1. Waksman Institute of Microbiology, Rutgers University, Piscataway, NJ
2. Department of Genetics, School of Arts and Sciences, Rutgers University, Piscataway, NJ
3. Human Genetics Institute of New Jersey, Rutgers University, Piscataway, NJ

Signaling networks allow adaptation to stressful environments by activating genes that counteract stressors. Small proteins (≤ 50 amino acids long) are a rising class of stress response regulators. *Escherichia coli* encodes over 150 small proteins, most of which lack phenotypes and their biological roles remain elusive. Using magnesium limitation as a stressor, we identify stress-induced small proteins using ribosome profiling, RNA sequencing, and transcriptional reporter assays. We uncover 17 small proteins with increased translation initiation, several of them transcriptionally upregulated by the PhoQ-PhoP two-component signaling system, crucial for magnesium homeostasis. Most remarkably, we elucidate an unusual connection via a small membrane protein YoaI, between major signaling networks – PhoR-PhoB and EnvZ-OmpR in *E. coli.*

2. Cultivation of arsenic-respiring anaerobic bacteria from the rivers of Vietnam.

Angel G. Robinson, Nicole Asmod, Max M. Häggblom

Dept. of Biochemistry and Microbiology, Rutgers University, New Brunswick, NJ

Arsenic is a toxic metalloid of concern in many watersheds. This study aims to cultivate and characterize arsenic-respiring anaerobic bacteria from the Mekong and the Red River Deltas to address the pressing issue of arsenic contamination in Vietnam. Enrichment cultures of agricultural soils were established in anaerobic media with pyruvate, ferulic acid, or methanol as the carbon source and arsenate as the electron acceptor. Arsenate reduction occurred in the majority of enrichment cultures and subsequently after initial enrichment, bacterial colonies were isolated in agar media. Isolated colonies of arsenate-respiring bacteria will be identified and characterized in future advancements of this study.

3. The interplay between the maternal gut microbiome and breast milk sugars.

Liisa Veerus[1], Joyce E. Stuckey[2], Stephanie A. Archer-Hartmann[3], Sruthi Alampally[1], Margot J. Shumaker[1], Mary C. Lally[1], Laïlatou M. Bambara[1], Erica R. Levin[1], Yue Sandra Yin[1], Parastoo Azadi[3], Martin J. Blaser[1]

1. Center for Advanced Biotechnology and Medicine, Rutgers University, Piscataway, NJ
2. Comparative Medicine Resources, Rutgers University Animal Care, Piscataway, NJ
3. Complex Carbohydrate Research Center, University of Georgia, Athens, GA

Breast milk production is a conserved trait in mammals, providing nutrition for the offspring to support their growth and immunologic development. In addition to macromolecules, lactose and other small sugars—or oligosaccharides—are present in breast milk. Most oligosaccharides cannot be digested by the newborn but are metabolized by specific microbiota that support the healthy development of the infant. Previous research in humans has found that both the pregnant mother's gut microbiome and the sugars present in her breast milk are individual-specific; however, little is known about their interplay. We hypothesize that the mother's gestational gut microbiome informs which sugars are produced by the mammary glands. To investigate this, we used 20 conventional and 10 germ-free C57BL/6 female mice to determine the link between the gestational gut microbiome and mouse milk oligosaccharides (MMOs). The conventional dams received vancomycin or neomycin gavages to substantially change their gestational gut microbiome or water as a control. We confirmed the antibiotic effects with 16S rRNA gene sequencing. We developed a novel protocol for mouse breast milk collection, which was used on dams with pups from P7-P19. Untargeted LC-MS processing of milk samples detected >30 MMOs, of which several differed significantly in abundance between conventional and germ-free mice. Observing that most oligosaccharides found in murine milk were sialylated, using RT-qPCR, we measured the relative expression of 7 sialyltransferase genes in mouse mammary glands with Rpl13a and Gapdh as standards, comparing 22 conventional and 10 germ-free mice dissected across different reproductive stages. We found significantly different sialyltransferase expression patterns between the groups. Finally, we analyzed the relationships between the mother and pup gut microbiomes and the dam's MMOs. Using the mouse model, we found that oligosaccharide composition is linked to the gestational gut microbiome. This work provides further definition of the intricate mechanisms involved in the transgenerational inheritance of a healthy microbiome.

4. The effect of cross feeding interactions on mutant fitness in communities of *E. coli*.

Duhita G. Sant[1], Tomas Lio Grudny[1,2], Atharv Jayprakash[1], Bidong D. Nguyen[3], and Michael Manhart[1,2]

1. Center for Advanced Biotechnology and Medicine, Rutgers University, Piscataway, NJ
2. Institute of Integrative Biology, ETH Zurich, Zurich, Switzerland
3. Institute of Microbiology, ETH Zurich, Zurich, Switzerland

Ecological interactions between microbes, such as cross-feeding nutrients, are believed to be abundant in microbial communities. Empirical data has shown that ecological interactions affect evolutionary dynamics in these communities. However, this raises questions about the mechanisms that mediate interactions between the cells and how they affect evolution. The distribution of fitness values of spontaneous mutations available to the organism, also known as the distribution of fitness effects (DFE), is one of the key determinants of evolution. These distributions determine how fast the population will adapt and which genes are under selection, revealing the potential molecular pathways leading to evolutionary outcomes. Bacteria experience different kinds of ecological interactions, and these interactions may have different effects on the DFE. However, to systematically test the effect of cross-feeding interactions on the DFE, we must use a system where the interactions are precisely defined and controllable. Interactions between different species are difficult to interpret in this context since the species are so genetically different that they may interact in complex ways. Instead, engineered auxotroph strains are ideal for this question: their interactions can be precisely defined since they are identical besides their auxotrophy. Here, we have generated barcoded transposon insertion mutant libraries of >105 unique mutants for engineered isoleucine and methionine auxotrophs of *Escherichia coli.* We then competed each library in monoculture as well as in coculture combinations with its ancestor, testing the cross-feeding of amino acids. By sequencing these libraries and competition cultures, we have determined the DFEs of gene knockouts in these strains and how they are affected by cross-feeding. So far, our results indicate that the presence of the interaction altered the shape of the DFE by reducing the number of deleterious mutations and increasing the number of beneficial mutations. In particular, we have found that cross-feeding methionine and cross-feeding isoleucine reversed selection on some mutants from being strongly deleterious without cross-feeding to being the most beneficial mutants with cross-feeding. Altogether this work contributes to our understanding of how ecological interactions in microbial communities affect their adaptation.

5. The acetylation state of HBsu at lysine 41: a significant regulator of gene expression during the late stages of *Bacillus subtilis* sporulation.

Liya Popova, Hritisha Pandey, Olivia R. Schreiber, Valerie J. Carabetta

Department of Biomedical Sciences, Cooper Medical School of Rowan University

Sporulation is triggered by environmental stresses, such as nutrient starvation, resulting in the formation of highly resistant spores. It involves a complex series of reactions between the mother cell and the forespore, guided by sporulation-specific genes. It was proposed that the nucleoid-associated protein HBsu plays a role in chromosome compaction during the process of sporulation. Previously, we identified seven acetylation sites on HBsu. We examined the role of HBsu acetylation during sporulation, and found a reduction in sporulation frequency and resistance properties of mature spores of mutant strains that mimic the acetylated forms (glutamine substitutions) of HBsu. We hypothesized that the DNA packaging or the gene expression program during sporulation might depend on the acetylation state of HBsu. To understand whether the acetylated forms of HBsu affect the sporulation program, we analyzed the expression of the sigma factor genes and their regulons, serving as reporters for their activity, using quantitative real-time PCR (qRT-PCR). Out of all seven mutants, we found that the hbsK41Q mutant significantly overexpressed the early sporulation genes (spo0A, spoIIGA, spoIIE, spoIIAB,spoIID, spoIIQ, sigF) at the T2 time point. In addition, the late sporulation genes (sigG, cotE, cotH, lipC, asnO, spoVK, spoIVCA, spoVT, spoVAA, sspB) were continuously overexpressed at both T2 and T4, compared to wildtype. Since we observed overexpression of sigF and sigG mRNA, we analyzed their protein levels by western blot, and found overproduction of σF at both T2 and T4, and σG at T4. We also detected overexpression of the reporters for σK activity (late sporulation genes cotE, cotH, cotD, and lipC) at T2 and T4, and an increase in SigK protein levels at T4. Contrary to hbsK41Q, the opposite mutant mimicking the deacetylated state, hbsK41R, did not show a difference in the expression of the above-mentioned genes. Our findings suggest that the acetylation state of HBsu at K41 is critical for proper gene expression during sporulation. We propose that it regulates the transition between early and late sporulation phases, and that deacetylation of K41 is necessary to turn off expression of specific genes during the lates stages of sporulation.

6. Coastal soil salinization reduces cedar sapling survival and–combined with artificial root exudates–synergistically enhances soil phosphatase activity.

Nicolina Lentine[1], Nina Goodey[2], Sarah E. Krisak[2]

1. Department of Earth and Environmental Studies, Montclair State University, Montclair, NJ
2. Department of Chemistry and Biochemistry, Montclair State University, Montclair, NJ

Saltwater intrusion into coastal soils threatens New Jersey's coastal ecosystems. Increased soil salinity can impact soil function, seed germination, and plant vigor. At Cheesequake State Park in Matawan, New Jersey, saltwater intrusion from Raritan Bay via Cheesequake Creek affects plant life. In this study, we investigated how salinity and artificial root exudate additions impact Cheesequake soil function and determined whether their effects are additive, synergistic, or antagonistic. A three-by-three factorial design was used with varying concentrations of artificial root exudates and sodium chloride (NaCl), resulting in nine treatment combinations. The nine treatments included varying concentrations of NaCl and artificial root exudates: Control (C), Low Salt (LS), High Salt (HS), Low Exudate (LE), Low Salt and Low Exudate (LSLE), High Salt and Low Exudate (HSLE), High Exudate (HE), Low Salt and High Exudate (LSHE), and High Salt and High Exudate (HSHE). We measured soil phosphatase activity and microbial community composition at Day 39, which was the final day for this phase of the experiment. The addition of NaCl or artificial root exudates alone did not significantly change phosphatase activity. However, the HSHE treatment significantly increased phosphatase activity (1067 ± 278 nmol/(hr*g dry soil)) compared to the control (373 ± 29 nmol/(hr*g dry soil)) ($p < 0.0001$). On Day 39, the HS and HSHE treatments resulted in a dominant Bacillus population, a salt-tolerant genus, and an increased abundance of *Penicillium* and *Selemophoma* fungi, with *Penicillium* being a facultative halophile. High NaCl levels reduced microbial and fungal diversity, while the combination of NaCl and exudates synergistically boosted soil phosphatase activity. These shifts in microbial composition and phosphatase activity offer potential strategies to mitigate the negative impacts of saline soils.

7. Investigating the sub-zero active microbiome of the Arctic tundra and Antarctic coast using high osmotic pressure media.

Andrea Vizcarra[1], Max M. Häggblom[1], Neil Simmons[1], Lee Kerkhof[2]

1. Dept. of Biochemistry and Microbiology, Rutgers University, New Brunswick, NJ
2. Dept. of Marine and Coastal Sciences, Rutgers University, New Brunswick, NJ

The Arctic and Antarctic environments are heavily impacted by global warming; this study investigates how the microbial community shifts during temperature changes. High osmotic pressure media helps simulate low temperatures by lowering water availability. Soil samples from the Arctic tundra and Antarctic coast underwent DNA sequencing to obtain phylogenetic profiles and their relative abundance. Among the observed phyla, "*Candidatus Eremiobacterota*" and "*Verrucomicrobia*" increased in relative abundance after treatment, while "*Bacteroidetes*" decreased. Future advancements include a dilution series with growth and enrichment to select for organisms that favor low temperatures. Understanding the sub-zero active microbiome has larger implications in climate change research and astrobiology.

8. Investigating the effectiveness of different artificial root exudate combinations on soil microbial function in metal-contaminated soil.

Naima Zaheer[1], Sarah Krisak[2], Dr. Nina Goodey[2]

1. Department of Earth and Environmental Studies, Montclair State University, NJ
2. Department of Chemistry and Biochemistry, Montclair State University, NJ

Heavy metals are one of the main reasons for poorly functional soil. Because they interfere with the normal functioning of the microbes and can cause microbes to become dormant. Dormant microbes are not able to support plant life causing an area to lose vegetation and become barren and can lead to food insecurity. To mitigate this problem, the introduced strategies should be cost efficient and have no indirect effect on the environment. This is the reason we opted for artificial root exudates instead of fertilizers. The ideal exudate solution would be beneficial to a wide range of microbes, be inexpensive, and have minimal environmental impacts. Exudates are a natural source of food for microbes in the soil normally provided by healthy plants that promote mycorrhizal association between plant roots and microbes, mobilize nutrients in the soil and act as a chelating agent for heavy metals. Soil respiration rate and phosphatase activity are major indicators of functional soil and active microbes. We analyzed the effects of seven different combinations of exudates on soil respiration rate and phosphatase activity. Our results suggested that the presence of one sugar, one organic acid and one amino acid together are important to increase soil respiration rate and phosphatase activity. DNA analysis results revealed that one of our treatments having one sugar, one organic acid and one amino acid together recruited Eurotiomycetes. These results provide a guideline for selecting the optimal exudate source from any inexpensive waste products, they may play a key role in revitalization of any contaminated soil.

9. Soil microbial composition is affected by SRE treatments in barren, contaminated soils.

Sarah E. Krisak[1], Bhagyashree P. Vaidya[2], Adam Parker[3], Jennifer Adams Krumins[3], Nina M. Goodey[1]

1. Department of Chemistry and Biochemistry, Montclair State University, Montclair, NJ
2. Department of Earth and Environmental Science, Montclair State University, Montclair, NJ
3. Department of Biology, Montclair State University, Montclair, NJ

A well-functioning soil microbiome is crucial to soil health and quality. Soil microbes aid in important processes such as nutrient cycling, which in turn supports plant life. Brownfield soils typically exhibit low levels of microbial functioning from contaminant-resistant microbial communities. The low level of microbial functioning plays a significant effect on natural vegetation growth, leaving areas barren. Without plants, soils lack structure and stability. Soil microbial function has been shown to increase when primed with a simulated root exudate (SRE) solution, creating an environment within the soil that is able to support plant growth. This study investigates how different SRE dosing strategies influence the soil microbial communities in barren, contaminated soils. We compared the effects of a single SRE addition, a repeated addition added 14 times over 30 days, and a control which received sterile tap water only. We also compared the effects of these SRE additions in a contaminated, vegetated soil. The DNA of the bacterial and fungal communities within the soil was extracted and sequenced using Next Generation Sequencing at two time points to observe changes within the microbial community in response to SRE priming. Using targeted sequencing methods (16S and ITS), we gained a deeper understanding of how SREs affect soil microbial composition and function. On day 0 of the experiment, the bacterial communities from the single addition, repeated addition, and control soils were relatively similar and grouped together graphically when Principal Coordinates Analysis is performed. On day 120 of the experiment, the bacterial communities from the single and repeated additions differed from the control, as well as from the communities on day 0. All single addition and control fungal communities from both time points remained relatively similar, while repeated addition fungal communities highly differed at both day 0 and day 120. This understanding is crucial in the efforts to revitalize poorly functioning soils practically and effectively.

10. The quantification of drug accumulation within *Mycobacterium smegmatis*.

Shivangi[1], Joel S. Freundlich[1,2]

1. Department of Pharmacology, Physiology, and Neuroscience, Rutgers University – New Jersey Medical School, Newark, NJ

2. Department of Medicine, Center for Emerging and Re-emerging Pathogens, Rutgers University – New Jersey Medical School, Newark, NJ

For decades, *Mycobacterium tuberculosis*, the etiological agent of tuberculosis, has posed a threat to global health. Antitubercular drug efficacy may be significantly impeded by the composition of the mycobacterial cell wall, necessitating a comprehensive and quantitative understanding of drug accumulation within the targeted bacterial cell. Our intrabacterial drug accumulation and metabolism (IBDM) assay has enabled the assessment of drug levels and the identification of probable drug metabolites within the bacterium. *Mycobacterium smegmatis* has served as the model organism in this application of the IBDM method. We began with a single compound format and then adapted it to a high-throughput format (96-well plate). We assert that the *M. smegmatis* IBDM platform will prove valuable in both drug development and basic mycobacterial biology research.

11. The quantification of drug accumulation and metabolism within gram-negative bacteria.

Amir George, Shivangi, Joel S. Freundlich

Department of Pharmacology, Physiology and Neuroscience, Rutgers New Jersey Medical School, Newark, NJ

Herein, we present an intrabacterial drug accumulation and metabolism (IBDM) assay with Gram-negative bacteria. The IBDM assay was employed to study the accumulation of a range of antibacterial agents in different *E. coli* strains while also being exemplified with *K. pneumoniae*, *A. baumannii*, and *P. aeruginosa*. Our findings support a direct correlation between intrabacterial accumulation and whole-cell activity. In addition, by combining insights from our IBDM assay with flow cytometry studies, we identified a synergistic interaction between indacaterol and rifampicin mediated through permeabilization of the bacterial cell membrane by indacaterol. Finally, the assay was translated to a 96-well plate format to allow for high-throughput quantification of intrabacterial drug accumulation. In summary, our IBDM assay provides a simple and cost-effective approach to measuring drug accumulation, metabolism, and drug-drug interactions in Gram-negative bacteria and the potential for high-throughput applications with diverse chemical libraries and/or bacterial strains.

12. Small molecule and peptide inhibitors of ricin & Shiga toxin-ribosome interaction.

Arkajyoti Dutta[1], Nilgun E. Tumer[1], Xiao-Ping Li[1], Anastasiia Tsymbal[2], Jacques Roberge[2], Hakan Guven[2], Zoltan Szekely[2], John McLaughlin[1], Benjamin Algava[1] and Michael Rudolph[3]

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2. Molecular Design and Synthesis Core, Rutgers University, Office of Research, Piscataway, NJ
3. New York Structural Biology Center, 89 Convent Ave, NY

Ricin, one of the most potent toxins known, and Shiga toxins (Stxs) produced by *Escherichia coli* (STEC) bind to the C-termini of ribosomal P-stalk proteins to depurinate the sarcin/ricin loop at a distant location on the large subunit of ribosome. No effective therapy exists for ricin or Shiga toxin intoxication. Ribosome binding sites have not been targeted so far by small molecules. We set up fragment-based ligand discovery (FBLD) using surface plasmon resonance (SPR) with Biacore T200 and identified CC10501 that binds at the P-stalk binding site of ricin A subunit (RTA) and reduces the catalytic activity. Using a structure-based design with fluorescence anisotropy (FA)-based competitive binding assay we obtained RU-NT-192 and RU-NT-206, which bind P-stalk pocket of RTA with over 50-fold higher affinity and inhibit catalytic activity with sub-micromolar potency. We measured the binding affinity of the P-stalk peptides for RTA and Stx2A1 using the FA assay and compared it to the affinities determined by SPR with Biacore T200. The rank order of the peptides was the same by both methods. However, the FA assay could differentiate better between peptides that showed nonspecific interactions with RTA by SPR. The identification of peptides and small molecules that inhibit depurination at a physically remote site underscores the importance of the P-stalk binding site as a novel target for allosteric inhibitors against ricin.

14. Playing with power: increasing ATP synthase activity.

Truman Dunkley[1], Daniel Shain[1,2], Eric Klein[1,2]

1. Center for Computational and Integrative Biology, Rutgers University-Camden, Camden, NJ
2. Biology Department, Rutgers University-Camden, Camden, NJ

Mesenchytraeus solifugus (a psychrophilic glacial ice worm) contains a histidine rich, C-terminal extension on the mitochondrially encoded ATP6 (subunit a) of ATP synthase. We made a genomic fusion of this extension to the orthologous subunit in *Escherichia coli* (exAtpB) and found that this fusion increased ATP synthase maximum velocity by around 20% and Km by around 30%. This difference persisted in the phylogenetically distant alphaproteobacterium *Caulobacter crescentus* where fermentation is not possible and aerobic respiration necessary. *E. coli* tightly regulate the expression of ATP synthase to optimize growth rate, and we found that the higher production of ATP by the fusion strain (exAtpB) led to reduced expression of Complex V genes. As a function of [NADH], the difference in activity between WT and exAtpB was highest at intermediate concentrations, suggesting a higher binding affinity between the half-pore and protons, while exAtpB-facilited proton flux appears to be unidirectional with little effect seen in the direction of ATP hydrolysis.

15. How does nutrient availability affect bacterial susceptibility to antibiotics?

Rachana Rao Battaje[1], Joelle N. Reich[1], Noelle A. Held[2] and Michael Manhart[1]

1. Center for Advanced Biotechnology and Medicine, Rutgers University, Piscataway, NJ

2. Department of Biological Sciences: Marine and Environmental Biology Division, University of Southern California, Los Angeles, CA

Rising antimicrobial resistance demands urgent action, particularly through optimizing the use of existing antibiotics. Bacterial susceptibility to antibiotics is influenced by both physiological states and environmental conditions, yet standard tests often overlook this variability, leading to inefficient treatment regimes. Previous studies suggest that bacterial tolerance to antibiotics increases in nutrient-poor environments. However, these studies focus on the quality of the nutrients or on the quantitative limitation of a single nutrient. Bacteria require a variety of nutrients whose abundances often co-vary in nature, leading to nutrient colimitation, where multiple resources simultaneously limit microbial growth. There remains a significant knowledge gap in understanding how nutrient colimitation, specifically, variations in nutrient availability rather than quality, affects bacterial adaptation to antibiotic stress and the molecular mechanisms that drive this process. In this project, we address this gap through quantitative assessment of the variability in antibiotic response of *E. coli* grown in varied availability of two essential nutrients for growth, carbon and nitrogen. We performed high-throughput growth-curve measurements of *E. coli* under single nutrient limitation versus colimitation states by simultaneously varying concentrations of glucose and ammonium, along with sub-MIC concentrations of antibiotics that allow growth. We quantified the bacterial growth by fitting the biomass yield into a model to extract parameters and examined how antibiotics altered nutrient colimitation. The glucose-to-ammonium stoichiometry in biomass yield varied distinctly with treatment with different antibiotics, suggesting that antibiotics fundamentally alter nutrient uptake, depending on their mechanism of action. Further, treatment with a translational inhibitor – kanamycin – shifted colimitation dynamics towards single nitrogen limitation for a narrow range of ammonium concentration, demonstrating an all-or-nothing growth response that is both quantitatively and characteristically different from the control. Evidence that nitrogen limitation increased kanamycin susceptibility in *E. coli* challenges the prevailing notion that nutrient limitations generally enhance bacterial tolerance to antibiotics. These results add complexity to the current understanding of how nutrient conditions influence antibiotic resistance. By outlining a conceptual approach to quantify the overall growth limitation contributed by antibiotic and nutrient stresses, this research aims to fill a critical knowledge gap on how quantitative fluctuations in the environment affect antibiotic susceptibility. Clinically, the findings could lead to strategies for manipulating nutrient environments and improving treatment outcomes in combating drug-resistant infections.

16. Sisters, not twins: comparing the average effect of transition mutations on protein stability of two *Escherichia* coliphages.

Victoria A. Sharp[1], Siobain Duffy[2]

1. Dept. of Biochemistry and Microbiology, Rutgers University, New Brunswick, NJ
2. Dept. of Ecology, Evolution, and Natural Resources, Rutgers University, New Brunswick, NJ

Using PopMusic3.0, the average effects of single point transition mutations on the protein stability of two closely-related *Escherichia* coliphages, phiX174 and alpha3, were compared. These coliphages have solved, non-overlapping protein structures for proteins F, G, and H.

17. Plant root microbiome response to herbivory - potential insecticidal activity.

Anthony Barrera, Jennifer Adams Krumins

College of Science and Mathematics Biology, Montclair State University, Montclair, NJ

Rhizospheric bacteria of the soil have been shown to have insecticidal features and produce plant growth promoting compounds such as indole acetic acid. We propose that under herbivore stress, plants will recruit a consortium of bacteria that will confer herbivore defense to the host plant. Subsequently, this established community structure can be transferred to another plant, either of the same or different species, to promote growth of the plant and deter herbivory. To investigate these ideas, we are growing tomato and cucumber plants with or without herbivore stress and in sterile or non-sterile conditions in full factorial (2 x 2) fashion to observe the rhizospheric bacterial community structure. The soils from this initial set up will be collected and used to inoculate a second phase of plants with the same parameters as previously stated. We will characterize the community with next generation sequencing using 16S primers. The outcome of this experiment will provide a deeper understanding of the rhizosphere community in plants affected by herbivory. This can help to inform agricultural practices and reduce the demand for exogenous pesticide application of agricultural soils.

18. Plant and soil microbial community structure: resolving relationships in the field.

William Cartelli, Jennifer A. Krumins

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Invasive species are among the largest threats to ecosystems worldwide alongside climate change and habitat destruction. In regard to plant and soil communities, many hypotheses have explored the conditions that lead to invasive success. Among them is The Facilitative Microbial Warfare hypothesis which stipulates that invasive plants support soil microbial communities which can outcompete native microbiomes. As studies continue to show that a plant's native microbiome predicts the vitality of itself and nearby neighbors, we ought to characterize the soil microbiomes of native and invasive plant species. This effort may provide insight into the dynamics that lead to plant success in invasion. The study at hand utilizes two flowering species of the Asteraceae family from the same Old Field (Hutchinson Memorial Forest): The invasive species *Centaurea stoebe* L. and the native species *Solidago rugosa* & *Solidago canadensis*. Preliminary analysis of above ground plant community data has revealed that the above-ground communities do not differ between *Centaurea* and *Solidago* plants. Sequencing of fungal and bacterial soil populations will reveal whether these communities are correlated with and determined by the above ground plant community as a whole, or alternatively only associated with the target plant. My research asks the following questions: (1) Is there a difference in the soil microbial community composition among different species (one native and one invasive) within the family Asteraceae? (2) Does the microbiome of *Centaurea* correlate with the community composition of *Solidago* and vice versa? And finally (3) Do seasonality and phenology affect the plant microbe dynamic relationship?

19. Investigating the roles of residues N189 in *Mycobacterium tuberculosis* IGP synthase.

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Tuberculosis (TB) ranks as the second most devastating infectious disease in the world. TB has become increasingly resistant to antibiotic treatment translating to the projected 10 million deaths annually by 2050 if no new solutions are found. The fourth step in the tryptophan biosynthesis pathway involves the catalysis of CdRP by IGP synthase to form IGP which then forms tryptophan. Tryptophan is an essential amino acid necessary for the survival of *Mycobacterium tuberculosis*, the bacteria that causes TB. By studying the enzyme IGP synthase from *Mycobacterium tuberculosis* we can discover an inhibitor that will halt the production of IGP. To study how the enzyme and substrate interact we expressed *Mycobacterium tuberculosis* in LB Broth followed by protein purification to elude the IGPS from the *E. coli* cells using a His6-tag. Using the purified protein, we determined the Michaelis-Menten kinetic parameters. Kcat (1/S) can tell us the rate at which the enzyme and substrate react to form IGP. Km (uM) can tell us whether the enzyme and substrate have a high or low affinity. The mutants N189D, N189S, and N189Q all saw a change in Km and kcat from the wildtype meaning that they serve some catalytic importance and substrate binding effect in the production of IGP. Studying and understanding how *Mycobacterium tuberculosis* interacts with the substrate, CdRP, is crucial in learning how to halt the production of IGP to hopefully then halt its conversion of tryptophan. Halting the production of tryptophan will in turn halt the production of *Mycobacterium tuberculosis* and save millions of people infected with TB.

20. No clean sweep for the sweepoviruses: existing begomovirus species demarcation criteria often fail to create monophyletic species.

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Sweepoviruses are ssDNA species in the genus Begomovirus which infect sweetpotato (Ipomoea batatas). The International Committee for the Taxonomy of Viruses (ICTV) specifies a percent nucleotide identity threshold for species demarcation, but these criteria have not been systematically applied across sweepoviruses. Simultaneously, ICTV aims for species (and all levels of viral taxonomy) to be monophyletic. 398 full genome sequences of sweepoviruses (GenBank) were used to construct a Maximum Likelihood (ML) phylogeny. The ML tree only supports monophyly of five of 14 ICTV-recognized species. Another species has a well-supported paraphyly, which is a legitimate biological possibility for these viruses. These analyses revealed a distinct difference in Sweet potato leaf curl Hubei virus (SPLCHbV) versus the other species, a result which is substantiated by previous findings that this species is the product of recombination with a non-sweepovirus begomovirus (Crespo-Bellido, *et al.* in press). The Recombination Detection Program (RDP) found extensive recombination among the sweepoviruses, which is at odds with the goal of monophyletic species. We propose extensive taxonomic revisions to currently accepted sweepovirus species based on the ICTV-specified species delineation, cutting the number of sweepovirus species in half. Comprehensively, our analyses reveal a general failure of this percent nucleotide identity threshold to create monophyletic species. The threshold also conflates lineages that are mostly separately evolving into a single species, leading to average percent nucleotide identities within species that are <91%.

21. RMAPS, a novel thermophilic, chemolithoautotrophic, thiosulfate-oxidizing gammaproteobacterium isolated from a deep-sea hydrothermal vent.

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RMAPS (Riftia Mount Alvinellid Purse Slurry) is a newly isolated gammaproteobacterium of the family Chromatiales. During a research expedition on the R/V Atlantis to the East Pacific Rise, an experimental colonizer was retrieved from the Riftia Mound vent site using the deep-submergence vehicle Alvin. A biofilm-covered fragment of mesh from the colonizer was suspended in anaerobic artificial seawater to create a slurry, which was then used to inoculate various types of media for enrichment cultures. After performing multiple end-point dilutions, a pure culture was obtained and named RMAPS. This bacterium is a thermophile that grows at 55°C, oxidizes thiosulfate, reduces nitrate, and fixes carbon dioxide. The 16S rRNA gene was amplified by polymerase chain reaction from the genomic DNA of RMAPS, and the amplicons were subsequently purified and sent out for Sanger sequencing. Following Blast searches on EzBioCloud and NCBI, the closest relative to RMAPS was identified as Thiolapillus brandeum, whose 16S rRNA gene shares 94% sequence similarity with that of RMAPS. These findings suggest that RMAPS belongs to a new genus. The genome of RMAPS was then sequenced on Illumina and Nanopore platforms, providing insights into the biochemical capabilities of this bacterium. This information will be used to conduct a series of experiments to further characterize the metabolism of RMAPS and to investigate its taxonomy within the Chromatiales.

22. Modulating ATP synthase activity as a therapeutic strategy for mitochondrial diseases.

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Mitochondrial dysfunction due to mutations in the MT-atp6 gene is a key factor in diseases like Leigh syndrome, characterized by impaired ATP production. Our study aims to investigate the potential of a C-terminal extension from *M. solifigus* ATP6 to rescue mitochondrial function in a Leigh syndrome cell model harboring the m8993T>G mutation. We use a cybrid cell line, GM16530, derived from the fusion of enucleated B-lymphocytes from a Leigh syndrome patient with the 143BTK- osteosarcoma cell line, which is depleted of mtDNA. We have created a plasmid containing the C-terminal extension fused to human ATP6 and will employ mitochondrial targeting sequences from COX10 or LOC100282174 to facilitate mitochondrial translocation. Following transient transfection of this construct into the cybrid cell line, we will assess mitochondrial localization using immunofluorescence and evaluate functionality by comparing cell growth on glucose (glycolysis) versus galactose (OXPHOS) media. To further evaluate the extension's effectiveness, we will generate stable cell lines via retroviral infection and assess growth rates and mitochondrial function. Key assays will include ATP detection, and oxygen consumption measurements using the Seahorse XF Extracellular Flux Analyzer.

23. Effects of bacterial sphingolipids on the properties of synthetic liposomes.

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Liposomal drug delivery has been at the forefront of nanoparticle drug delivery systems for nearly 30 years. These liposomes and their components, including lipids, must be highly characterized to provide the ideal conditions for effective drug delivery. Among the commonly used lipids in liposome formation are ceramides and sphingolipids. Recent discoveries regarding bacterial ceramide synthesis have created opportunities to incorporate novel, previously uncharacterized, bacterially derived sphingolipids into these synthetic membranes. Here, we report on the development of various analyses to characterize the effects that novel sphingolipids have on membrane biophysical properties, as well as our efforts to purify and characterize the novel *C. crescentus* lipid, Ceramide Poly-Phosphoglycerate (CPG2).

24. How predictable is microbial mutant fitness in stress and non-stress environments?

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Microbial populations are constantly shaped by the environmental pressures they encounter, and understanding how different environments influence their evolutionary pathways is essential to predicting adaptive outcomes. A principal candidate of influence on microbial population evolution is stress, which can come in the form of high pH, high salinity, antibiotic presence, and more. Specifically, when separating environmental conditions along the lines of stressful and non-stressful, the effect on microbial population fitness is expected to be distinct between these two groups. There is also an ambiguity about what makes an environment stressful, and whether a predetermined definition of stress is supported by a population's evolutionary response. This study aims to sharpen the definition of stress in the context of environmental dependencies based on the relative fitness of mutations in *E. coli* populations. We analyzed *E. coli* fitness data in 168 environmental conditions from previously published work. This dataset has fitness values in each condition calculated as the change in log abundance, over one batch growth cycle, of mutant strains generated by a transposon-induced knockout library. We ran hierarchical clustering models on the fitness data and expected a similar but not complete overlap in the clustering; by locating the environments that make up the discrepancies in the overlap, we hoped to better tailor the definition of stress. Our observations indicated that non-stressful environments affect mutant fitness in a similar way while stressful environments affect mutant fitness in many different ways, and fitness effects of sulfur environments aligned more with non-stress environments. This analysis intends to reduce the dimensionality of environmental dependency and discover larger trends in evolutionary response to stress, which can be utilized to predict the outcome of population dynamics and cell physiology in stressful and non-stressful conditions.

25. Evolution of begomoviruses in the laboratory and at geological timescales.

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Begomoviruses infect a wide variety of plants and cause devastating diseases in crops such as tomato and cassava. These single-stranded-DNA viruses, named for bean golden mosaic disease, have one or two circular genomic segments (~2.7 kb each). We have explored the phylogenetic history of the longest coding genes on the virion-packaged and complementary strand for both segments: DNA-A (Crespo-Bellido, *et al.* 2024 doi.org/MJGC) and DNA-B (Dubey, *et al.* 2023 doi.org/JHZ5). For all four genes there are two main geographic clusters, one for viruses discovered in the Americas and a second for the rest of the world. Phylogenetic discordance within these clusters indicates substantial genetic exchange via recombination. Notable sequence outliers include two bipartite viruses discovered in *Corchorus* species in Vietnam and a cluster of fourteen monopartite sweetpotato-infecting viruses found across the world. Our phylogenetic analysis of the coat protein suggests possible co-evolution with whitefly vectors, particularly for a cluster found primarily in Africa. This cluster includes a synergistic pair of viruses that caused devastating cassava losses and local famine beginning in 1988. Another virus from the cluster, tomato yellow leaf curl virus, has spread worldwide and recombined with at least one virus in the Americas. The Americas clade of viruses is defined by a ~120-nucleotide deletion directly upstream of the coat protein gene, which appears to promote dependence on DNA-B. In the most prominent subclade within this group, part of the replication-associated protein was replaced via recombination with an unknown virus. One member of this subclade is particularly tractable to study: cabbage leaf curl virus infects *Arabidopsis thaliana* and has known deleterious nucleotide variants. Our analysis of spontaneous mutation in cabbage leaf curl virus (Hoyer, *et al.* 2022 doi.org/HKXQ) complements other recent analyses in cassava and tomato, strengthening our understanding of the evolutionary potential of begomoviruses.

26. Does climate change promote antibiotic resilience?

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Microbial evolution is driven largely by changing environmental conditions, and understanding the effects of these shifts is crucial for predicting evolution. One of the most pressing examples of this is the rise of antimicrobial resistance (AMR), which poses a growing threat to public health, with estimates predicting up to 10 million deaths annually from resistant bacterial infections. Despite extensive research into the mechanisms and spread of AMR, the influence of environmental factors—especially climate change—on the evolution of resistance remains underexplored. In this study, we analyze previously published fitness data to assess the effect of climate change stressors on the emergence of antibiotic resilient mutants in model organism *Pseudomonas stutzeri.* The dataset used contains *P. stutzeri* mutant fitness data in 176 unique conditions. The fitness value assigned to each mutant in each condition is calculated as the change in log abundance, over one batch growth cycle, of mutant strains generated by a transposon-induced knockout library. We selected thermal, salinity, and metal stress environments to emulate climate stressors, as these variables have been significantly altered by climate change. We hypothesized that these climate change stressors will afford fitness advantages to antibiotic resilient mutants (ABR). The analysis reveals that there exist many antibiotic resilient mutants that are granted fitness advantages in the presence of a climate stressor. When we examined which of these ABR mutants become more fit in these stressors, we were able to postulate several of the molecular mechanisms that are responsible for the relationship between AMR and climate change. Additionally, across climate stress conditions, we find that the fitness cost associated with being antibiotic resilient is reduced due the climate stressor. These results suggest climate stress is "rescuing" these antibiotic resilient mutants. The findings indicate that AMR mutants can more readily evolve and take root in microbial populations as climate change effects continue to manifest, which can exacerbate the already-worsening AMR crisis.

27. Co-metabolic biodegradation of 1,4-Dioxane and co-occurring chlorinated aliphatic hydrocarbons by psychrophilic propanotrophs enriched with a new cluster of Group-6 soluble di-iron monooxygenases.

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Commingled contamination of 1,4-dioxane (dioxane) and chlorinated aliphatic hydrocarbons (CAHs) are prevalent in groundwater at impacted sites. Here we enriched and characterized two psychrophilic consortia that were capable of degrading dioxane and a wide spectrum of CAHs (e.g., trichloroethene, 1,1-dichloroethene, 1,2-cis-dichloroethene, and vinyl chloride) under aquifer-relevant temperature (14 °C) when propane was supplemented. Functional gene-targeted sequencing further revealed the dominance (>70%) of Group-6 soluble di-iron monooxygenases (SDIMOs) in both consortia, crucial for concurrent propane consumption and co-oxidation of dioxane and CAHs. Sequence data mining and phylogenetic analysis uncovered three distinct clusters (I, II, and III) within Group-6 SDIMOs, with the most abundant SDIMO (48~59%) in Cluster III, sharing partial sequence identity with the α subunit of a Group-6 SDIMO in a propanotroph Mycobacterium sp. ENV421. Additionally, two other SDIMOs, closely related (>93%) to that of the archetypical dioxane metabolizer *Mycobacterium dioxanotrophicus* PH-06, were classified into Cluster I, collectively constituting 14% and 23% of total recovered SDIMOs in both consortia. Sequence comparison highlighted the divergence of Group-6 Cluster-III SDIMOs, exhibiting amino acid features (e.g., prolines avoidance and glycine substitution) in proximity of the active sites that are conducive to psychrophilic activity, emphasizing the necessity to characterize their catalytic behaviors and develop sensitive biomarkers for field assessment. This research sheds light on the unique adaptations of psychrophilic consortia and their potential for remediation strategies in cold groundwater environments contaminated with dioxane and CAHs.

Synopsis Psychrophilic consortia with newly discovered SDIMOs offer promise for degrading dioxane and CAHs in cold groundwater, aiding remediation efforts in contaminated environments.

28. Substrate-mediated biodefluorination of 6:2 fluorotelomer carboxylic acid by *Rhodococcus jostii* RHA1.

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Relatively high concentrations of PFASs have been found in landfill leachates and soil and groundwater that have undergone years of aqueous film-forming foams (AFFFs) use. Of the PFASs in AFFFs, 25% of them are fluorotelomer-based, which is why this study focused on fluorotelomer-carboxylic acid (FTCA) biodegradation. This study will investigate how carbon co-substrates can impact the product composition created from FTCA biodegradation or encourage cometabolism by *Rhodococcus jostii* RHA1 (RHA1). RHA1 is of interest for the biodegradation of PFASs because of its robust enzymatic capabilities. RHA1 has one of the largest bacterial genome sequences, and it is known to contain many oxygenase and ligase enzymes capable of degrading a wide range of organic compounds and other toxic chemicals like polychlorinated biphenyls, which it is known to co-metabolize. Various targeted and non-targeted analytical techniques will be used to create a collection of data quantifying fluoride release, the products generated by each microcosm, and their abundance. This data will then be synergized to create potential biotransformation pathways. Laboratory techniques will also be used to analyze the expression and regulation of targeted RHA1 enzymes.

29. Development of a proximity labeling method to study small protein interactions.

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Escherichia coli adapts to environmental stress through well-known regulatory mechanisms. While some regulatory processes such as transcriptional control and small RNAs are extensively studied, other mechanisms that drive stress responses remain poorly understood. Recent evidence suggests that small proteins (≤50 amino acids) play a crucial role in regulating bacterial stress response pathways. However, their precise expression patterns under stress conditions and their interaction partners are poorly characterized. While there have been great strides made in the identification of hundreds to thousands of small proteins across different model organisms, their biological functions remain largely unknown. Proximity labeling has become a widely used method, especially in eukaryotic systems, to identify proteins that form complexes with or are in close proximity to a target protein. One such method uses APEX2, an engineered ascorbate peroxidase, which enables the rapid labeling of proteins by catalyzing the formation of biotin-phenoxyl radicals in the presence of hydrogen peroxide, which leads to biotinylation of neighboring proteins. These biotinylated proteins are identified using mass spectrometry. The rapid nature of APEX2 labeling and it's applicability to living cells make it an ideal tool for detecting transient protein complexes. For the first time, we are adapting and developing the proximity labeling technique using APEX2 to identify protein binding partners of small proteins in vivo in *E. coli.* This approach will help us identify the precise target(s) of an individual small protein and further characterize its function.

30. Comparative biodegradation of 1,4-dioxane by bioaugmentation with *Azoarcus* sp. DD4 at a Superfund site using 1-propanol and propane as auxiliary substrates.

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1,4-dioxane is a persistent contaminant of concern due to its high solubility and resistance to degradation in aquatic environments, posing significant environmental and public health risks. Recent studies have identified several microorganisms capable of degrading dioxane, paving the way for microbial-based remediation strategies. *Azoarcus* sp. DD4, a novel propanotrophic bacterium, has demonstrated an enhanced ability to cometabolize and degrade dioxane using propane or 1-propanol as carbon sources. In this study, we evaluated DD4's efficiency in dioxane degradation, comparing three remediation approaches: monitored natural attenuation (MNA), biostimulation, and bioaugmentation. Bioaugmentation with DD4 reduced dioxane concentrations below the detection limit (0.13 µg/L) in both propane and 1-propanol treatments, starting from initial concentrations of 41 ± 0.5 µg/L in sample MW-A and 12.3 ± 0.2 µg/L in sample MW-B. Dioxane degradation was faster in propane-amended treatments, with degradation rates of 2.23 and 0.72 µg/L/day in MW-A and MW-B, respectively. For 1-propanol, degradation rates were 1.94 and 0.57 µg/L/day in MW-A and MW-B, respectively. MNA and biostimulation showed minimal dioxane removal compared to abiotic losses. Although total biomass remained constant before and after DD4 treatment, DD4 proliferation became dominant during incubation with both propane and 1-propanol. Microbial community analysis revealed that *Azoarcus* sp. DD4 was abundant in bioaugmented samples, constituting 48.9%, 9.8%, 45.1%, and 20% in MW-A (DD4+propane), MW-A (DD4+propanol), MW-B (DD4+propane), and MW-B (DD4+propanol), respectively. These findings were supported by tmoA biomarker analysis via qPCR. Principal coordinate analysis (PCoA) highlighted substantial shifts in microbial community structures post-DD4 inoculation, influenced by the substrate used.

31. Phage-antibiotic combination therapy: restoring antibiotic sensitivity in extensively drug-resistant *Acinetobacter baumannii.*

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The emergence of extensively drug-resistant (XDR) *Acinetobacter baumannii* poses a serious threat to global healthcare, due to resistance to most conventional antibiotics. Bacteriophage therapy, by itself or in conjunction with antibiotics presents a promising new treatment strategy to combat highly-resistant infections. Here we examined the re-sensitization potential of eight antibiotics (Ceftazidime, Cefepime, Piperacillin-tazobactam, Ampicillin-sulbactam, Meropenem, Imipenem, Ciprofloxacin, Levofloxacin) in combination with an *A. baumannii* specific bacteriophage (Phage C1) against clinical isolates of XDR *A. baumannii.* Our main objective was to identify combinations of bacteriophage and antibiotics that display potential synergy, thereby improving susceptibility and decreasing resistance to traditional antimicrobial agents. Previously, we identified combinations of bacteriophage and drugs that were promising, using standard checkerboard assays. We then performed growth curves, where we evaluated the effect of each antibiotic separately, with and without added bacteriophage. Preliminarily, specific combinations of phage and antibiotics decreased or inhibited the growth rate or prolonged the period of growth suppression in comparison to antibiotic monotherapy. Ceftazidime, Piperacillin-tazobactam, Meropenem and Ciprofloxacin combinations with phage were promising, inhibiting growth better than monotherapy. The present study underscores the potential of combining antibiotics with phages as a novel therapeutic strategy to combat multidrug-resistant bacterial infections. This strategy combines the bactericidal properties of bacteriophages, which may weaken the bacteria and make them more susceptible currently available antibiotics. Additional research is required to better understand the mechanisms underlying the synergy between phage and antibiotics and to improve treatment regimens for use in clinical settings.

32. Metabolically flexible iron-cycling bacteria dominate microbial communities in bog iron seeps in the NJ Pine Barrens

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The New Jersey Pine Barrens is the largest remaining example of the Atlantic coastal pine barrens ecosystem and a distinguishing feature of the coastal plain of southern New Jersey. The region is characterized by its nutrient-poor, sandy soil and iron-rich, acidic waterways. The delivery of reduced iron from underlying anoxic aquifers to the oxygenated surface leads to the precipitation of iron (III) oxyhydroxide or "bog iron" deposits, which supported a thriving ironworks industry throughout the 18th and early 19th centuries. While bog iron formation in the Pine Barrens is a well-documented phenomenon, microbial iron-cycling mechanisms and community dynamics in Pine Barrens waterways remain poorly characterized. In this two-year study, we collected iron oxyhydroxide microbial mat samples from three historic iron forges within three watersheds in the New Jersey Pinelands National Reserve: Martha's Furnace/Harrisville Furnace (Oswego/Wading River), Batsto Village (Mullica River), and Weymouth Furnace (Great Egg Harbor River). Metagenomic data revealed a diverse community of iron-oxidizing and iron-reducing bacteria coexisting within the fluctuating redox conditions that characterize bog iron seeps. Metatranscriptomic data obtained in year two of the study revealed that the active microbial community in most samples was dominated by members of the families *Gallionellaceae* (*Gallionella*, *Sideroxydans*, *Ferriphaeselus*, and *Ferrigenium*) and *Comamonadaceae* (chiefly *Rhodoferax* and *Leptothrix*). Metagenomes were also binned into metagenome-assembled genomes (MAGs) and metatranscriptomic reads were mapped to the bins to identify the most active MAGs. The annotated MAGs and metatranscriptomic data suggest that some of the most active members of the bog iron community may switch between iron oxidation and iron reduction as their needs and the environment dictates, however these findings will require further experimental verification. Genes connected to anoxygenic photosynthesis carried out by *Rhodoferax* were among the most abundant transcripts in nearly all samples, and these organisms may contribute in part to the characteristic orange color of the microbial mats by way of carotenoid production. Our results demonstrate that the Pine Barrens offers a historically significant and understudied habitat to explore the biogeochemical relationships between iron-cycling bacteria and their environment.

33. Bacterial leader peptides: more than just transcriptional regulators

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Small proteins are essential in responding to stress and environmental changes around hosts. In the *Escherichia coli* genome, over 150 small proteins have been identified, serving functions such as modulators of regulatory responses, leader peptides, and components of the toxin-antitoxin defense system. Leader peptides are short sequences encoded by the leader sequences of mRNA. They play a crucial role in transcription attenuation by forming secondary structures in response to the availability of biomolecules. This study focuses on investigating the functions of the leader peptide – MgtL as a small protein in E. coli and related organisms. The research aims to elucidate the role of MgtL under magnesium-replete conditions, where it is likely to accumulate and interact with potential protein partners. In this work, we seek to identify the cellular binding targets of MgtL and investigate the molecular determinants (including the conserved proline codons) that influence its role beyond transcription attenuation. As a first step towards the characterization of this small protein, the mgtL gene was cloned by epitope-tagging with either a hexahistidine, Flag, or green fluorescent protein (GFP) in an arabinose-inducible plasmid. Our preliminary data shows that MgtL is a membrane-bound protein in *E. coli* MG1655 cells, suggesting that it may bind and influence the activity of a large membrane protein or complex. Currently, we are using pull-down assays and cross-linking methods to identify the in vivo targets of MgtL. These findings will advance our understanding of small proteins as an emerging class of regulators, potentially leading to novel therapeutic strategies for microbial resistance mechanisms.

34. Sustainable soundproof mycelium panels.

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The linear life cycle of clothing, characterized by production, use, and disposal, ranks among the most polluting and wasteful industrial processes. This project aimed to explore the potential of repurposing second-hand fabrics with varying cotton content to create sustainable soundproof panels, utilizing fungal mycelium grown on different substrates. Several variables were tested to optimize the growth protocol for mycelium-based sound-absorbing panels, including environmental conditions (temperature, humidity, moisture content, light exposure), cotton content percentage, and types of nutrients and media. Two fungi were evaluated: *Ganoderma lucidum* (Reishi) and *Pleurotus citrinopileatus* (yellow oyster). Adding coffee grounds as a nitrogen source helped to promote growth but also made contamination easier, from coffee grounds posed creating significant challenges for this project and left the final protocol incomplete. However, Reishi exhibited faster growth at 27°C with minimal water, using oats as the primary nutrient source and ground coffee for nitrogen, in combination with 100% cotton fabric. Future work should focus on employing liquid culture mycelium and further testing different fungi and coffee ground quantities to better manage contamination.

35. Analyzing combinatorial antibiotic sensitivity in *Acinetobacter baumannii* isolates through disk stacking.

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Antibiotic resistance in *Acinetobacter baumannii*, a Gram-negative bacterium known for developing multidrug resistance, has become a serious challenge, complicating treatment for infections and posing risks in healthcare settings worldwide. As new antibiotics are slow to the market, developing novel treatment strategies is imperative. One such strategy is using a combination of antibiotics, that together display additive or synergistic effects. In this study, we explored combinations of the newer drug Durlobactam-sulbactam and used a disc stacking method to screen for potential synergistic interactions with other antibiotics against different *A. baumannii* patient isolates. Drugs included in our screen were Cefiderocol, Minocycline, Tobramycin, Ceftriaxone, Tigecycline, Piperacillin, Ciprofloxacin, Amikacin, Tazobactam, Omadacycline, and Rifampin. Using both the standard disk diffusion method for single drugs, a simple yet effective test for checking bacterial resistance, and a stack of discs for combinations, we measured the zones of inhibition produced on agar plates inoculated with bacteria and looked for larger zones of inhibition for the combinations (stacked discs). Preliminary results reveal varied responses across strains, with some combinations showing promising, possible synergistic interactions. These combinations will be further tested using checkerboard assays against the entire collection of strains. Our results demonstrate that this methodology is effective for screening antibiotic combinations quickly to identify possible drug synergy, which could be useful to find effective antibiotic therapies to combat the escalating threat of multidrug-resistant pathogens in clinical settings.

36. Sphingolipid synthesis and its trafficking in *Caulobacter crescentus*.

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Sphingolipids are a class of lipids containing a sphingoid base. These lipids are produced by eukaryotes and have been well studied in eukaryotes, where they have a role in host immunity modulation. For years, it was believed that these lipids were not produced by bacteria; however, with recent bioinformatics advancements, it has been observed that bacteria have the genes required to produce sphingolipids as well as those that code for transporters that transfer the lipids from the inner to outer membrane. Here, we are studying the same lipid transport system in our model organism, *Caulobacter crescentus*.