Theobald Smith Society
The NJ Branch of the American Society for Microbiology
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Spring 2024 Symposium Proceedings

3 May 2024

Trayes Hall

Rutgers University, New Brunswick, NJ

Opening remarks by Jennifer S. Sun, President Elect, Theobald Smith Society. 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. Insects use their sense of smell to locate mates and food sources which, for biting insects, may include human hosts. Internal bacterial composition (endosymbionts) can alter insect behaviors like mating and digestion, but we do not have conclusive evidence that insect olfaction also changes. As a Presidential Postdoctoral Fellow, Dr. Sun will employ 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. Moreover, Dr. Sun will work on identifying novel mechanisms for keeping biting insects at bay, thereby promoting Health Equity by creating a more healthful physical environment via community-based disease prevention projects. Dr. Sun obtained her Ph.D. in Molecular, Cellular, and Developmental Biology from Yale University and postdoctoral training in infectious diseases from Princeton University. As a Presidential Postdoctoral Fellow, she will establish a research laboratory and develop a course curriculum based on immersive research experiences. Her years dedicated to teaching and mentoring for the enhancement of minority participation in STEAM, and the overall improvement of high school and college-level students' prowess, will inevitably be perpetuated in her role as future a Rutgers faculty.

Overview of ASM Journals by Adrianna Borgia, Managing Editor, American Society for Microbiology. Adrianna Borgia is the Managing Editor of Microbiology Spectrum and Microbiology Resource Announcements at the American Society for Microbiology, where she oversees the development and daily operations of the journals. Prior to ASM, she was a Production Team Manager at Elsevier, working with society-owned titles in the health and medical sciences fields. She is also a volunteer on

committees for the Society of Scholarly Publishing and is the Vice President of the International Society for Managing and Technical Editors. Outside of publishing, Adrianna loves running, caring for her 2 cockatiels, practicing embroidery, and watching the Phillies (even if they're losing).

ASM Journals cover all areas of microbial sciences (molecular, cellular, applied, environmental, clinical) focusing on all microbes – from viruses to bacteria and archaea to parasites and fungi. Our editorial vision is to be a home for research that advances science and to provide exceptional author services. Learn more about what's new with our journals program and how you can be involved.

INVITED SPEAKERS

Chao Li, Department of Chemistry and Environmental Science, New Jersey Institute of Technology, Newark, NJ: "Enriching biofilms for effective biodegradation of commingled emerging contaminants: optimal inoculation strategy and microbial community analysis."

Maxwell Akantibila, Department of Biomedical Sciences, Rowan University, Camden, NJ: "Evaluating the antimicrobial activity of silver oxides against Gram-negative and -positive patient isolates."

Garima Verma, Center of Advanced Biotechnology and Medicine, Rutgers University, Piscataway, NJ: "A tail for two proteins: regulation of chemotaxis in nitrogen fixing bacterium *Sinorhizobium meliloti."*

Nupur Tyagi, Dept. of Biochemistry and Microbiology, Rutgers University, New Brunswick, NJ: "Determining how itaconic acid modulates *Staphylococcus aureus* physiology."

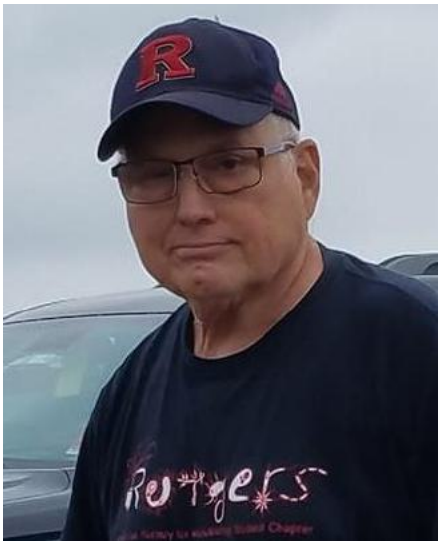
LeAnna Ross, Department of Biological Science, Seton Hall University, South Orange, NJ: "The molluscum contagiosum MC159 and MC160 immune evasion molecules: two great viral proteins better together."

Geordan Stukey, Dept. of Food Science, Rutgers University, New Brunswick, NJ: "Identification of three conserved motifs in the HAD-like domain from Saccharomyces cerevisiae phosphatidate phosphatase Pah1."

2024 Young Investigator Award Presentation to Srujana (Sam) Yadavalli

Sam 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. Our research integrates tools from high throughput sequencing, genetic engineering, microbiology, biochemistry, and proteomics to address fundamental questions in bacterial stress response.

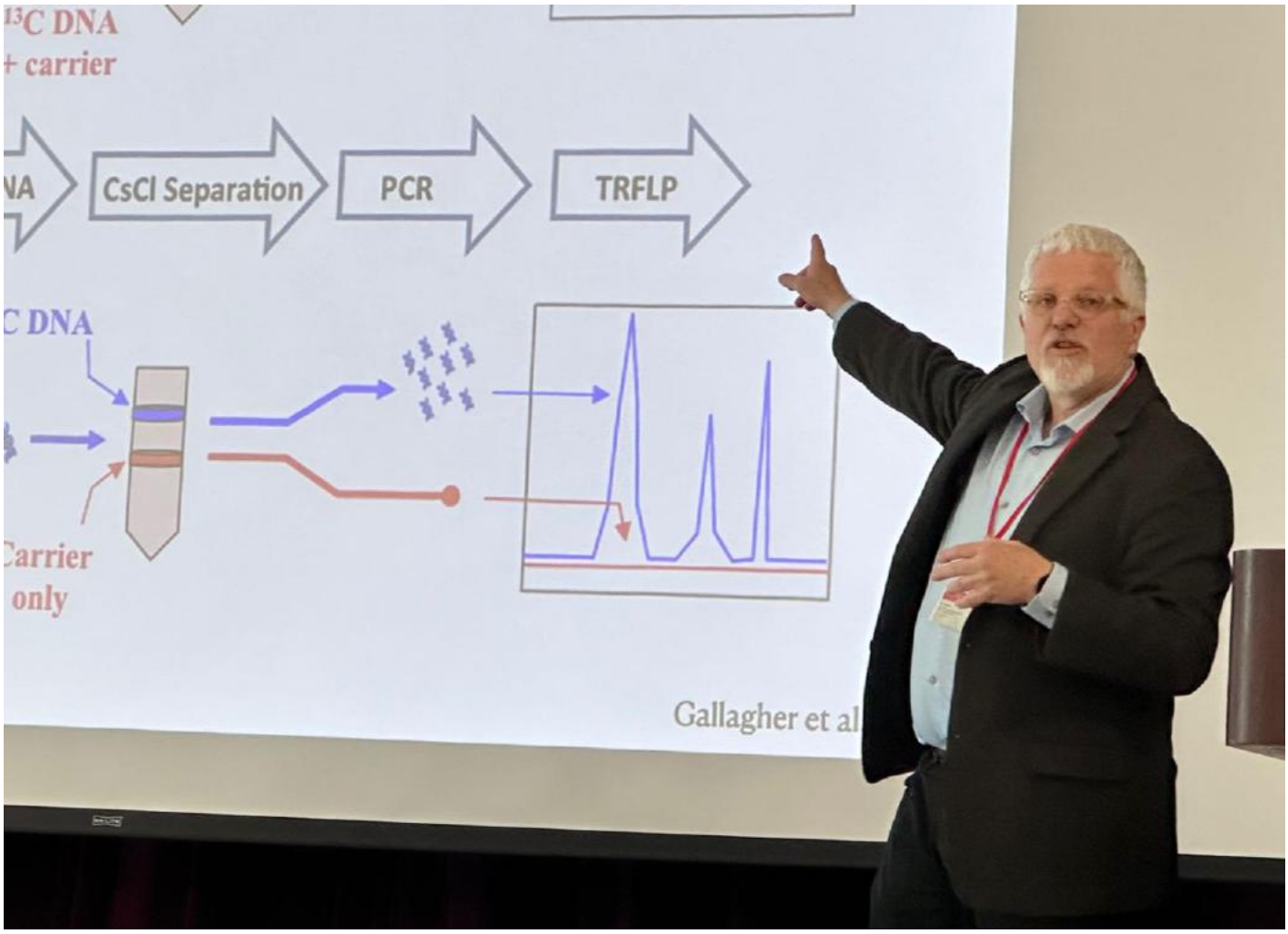
11:05AM 2024 The Andrew Marinucci Service Award Presentation to Dr. Andrew Marinucci Andy Marinucci led our society as president not for just one year, but for three years. He is the only three term president in the society's history. After his presidencies, he continued his support to the society as treasurer for the past 20 years. In recognition of his exemplary leadership, unwavering dedication, and outstanding service to the Theobald Smith Society, we are proud to present the first Andrew Marinucci Service Award to Andrew Marinucci. Throughout his tenure as president and treasurer, he has demonstrated exceptional commitment to advancing the field of microbiology and enriching the society's mission. Andy embodies the spirit of service and dedication that defines our society's mission.



Dr. Marinucci is a retired microbial ecologist. He was a research scientist in the Site Remediation and Waste Management Program within the New Jersey Department of Environmental Protection for 22 years providing technical support to the Department. He has worked and published for nearly 30 years on the fate and degradation of natural and anthropogenic compounds in the environment.

2024 Waksman Award Presentation and Lecture by Lee Kerkhof: A Retrospective on the Use of Molecular Tools to Study Microbial Communities Nucleic acid-based methods have become the most widely accepted way to characterize microbial communities in the last 35-40 years. Most effort has been focused on investigating ribosomal RNA genes to document the diversity and discern the members of the community. However, there is much more which can be gleaned about microbial communities by analyzing different pools of nucleic acids from newly synthesized genomic DNA to ribosomal RNA. Professor Kerkhof discussed efforts to monitor functional genes involved in biogeochemical cycling, ascertain which microorganisms are respiring specific substrates, and which microbes are actively growing under different environmental conditions. His studies have primarily focused on aeolian systems, aquatic systems, in association with eukaryotic hosts, and in sediments/soils spanning a continuum from pure cultures, to engineered systems, to field measurements. Additionally, he described how we can use these approaches to ask more fundamental questions regarding resource partitioning and specific predation in the field to help explain the mechanisms driving the microbial diversity in natural systems. Finally, he presented on efforts for microbiome characterization in the field using the Oxford Nanopore MinION- a small, affordable hand-held DNA/RNA sequencer that can provide strain-level resolution by profiling rRNA operons.

Lee Kerkhof has been a Professor at Rutgers for nearly 30 years. His research has focused on employing a variety of nucleic acid-based analyses to understand the mechanisms driving diversity and biogeochemical processes in complex environments.



Poster Session Winners

Sangeevan Vellappan, Waksman Institute of Microbiology, Rutgers University, Piscataway, NJ: "Identification and characterization of low Mg^{2+} stress-induced small proteins in *E. coli"*

Grace Bustamante, Department of Biology, William Paterson University of New Jersey, Wayne, NJ: "Cloning and expression of a caspase-3 candidate from the harmful algal bloom species, *Karenia brevis"*

Allie Spector and Torri Burghoffer, Department of Biology, William Paterson University of New Jersey, Wayne, NJ: "Examination of the roles of the mrp and pha genes in the marine bacterium *Marinobacteria adhaerens*."

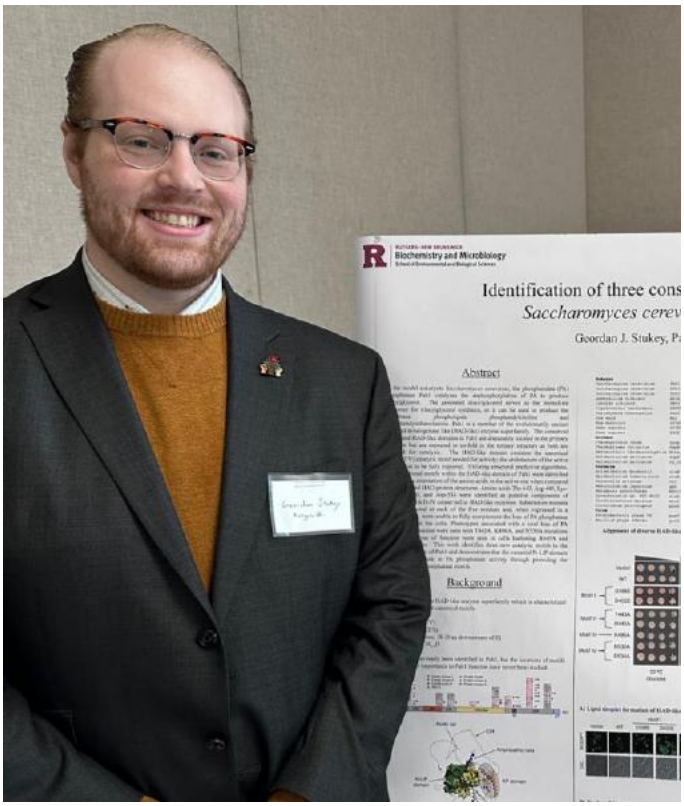
POSTERS

1. Department of Food Science and New Jersey Institute for Food, Nutrition and Health, Rutgers University, New Brunswick, NJ
2. Rutgers Center for Lipid Research and Center for Nutrition, Microbiome, and Health, Rutgers University, New Brunswick, NJ

1. Identification of three conserved motifs in the HAD-like domain from *Saccharomyces cerevisiae* phosphatidate phosphatase Pah1.

Geordan J. Stukey, Parth Sharma, Gil-Soo Han, George M. Carman

Dept. of Food Science, Rutgers University, New Brunswick, NJ

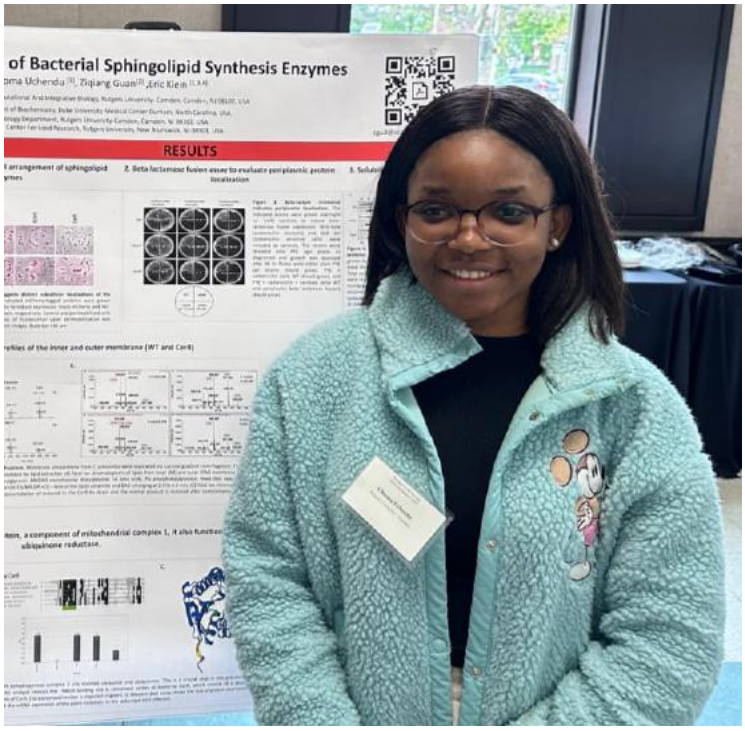


In the model eukaryote *Saccharomyces cerevisiae*, the phosphatidate (PA) phosphatase Pah1 catalyzes the dephosphorylation of PA to produce diacylglycerol. The generated diacylglycerol serves as the immediate precursor for triacylglycerol synthesis, or it can be used to produce the membrane phospholipids phosphatidylcholine and phosphatidylethanolamine. Pah1 is a member of the evolutionarily ancient haloacid dehalogenase like (HAD-like) enzyme superfamily. The conserved N-LIP and HAD-like domains in Pah1 are disparately located in the primary structure, but are expected to co-fold in the tertiary structure as both are required for catalysis. The HAD-like domain contains the canonical DXDX(T/V) catalytic motif needed for activity; the architecture of the active site has yet to be fully explored. Utilizing structural predictive algorithms, three additional motifs within the HAD-like domain of Pah1 were identified based on the orientation of the amino acids in the active site when compared to other solved HAD protein structures. Amino acids Thr-443, Arg-445, Lys-496, Asn-530, and Asp-534 were identified as putative components of catalytic motifs II-IV conserved in HAD-like enzymes. Substitution mutants were constructed at each of the five residues and, when expressed in a pah1Δ mutant, were unable to fully complement the loss of PA phosphatase function within the cells. Phenotypes associated with a total loss of PA phosphatase function were seen with T443A, K496A, and N530A mutations while partial loss of function were seen in cells harboring R445A and D534A mutations. This work identifies three new catalytic motifs in the HAD-like domain of Pah1 and demonstrates that the essential N-LIP domain does not contribute to PA phosphatase activity through providing the conserved HAD phosphatase motifs.

2. Spatial organization of sphingolipid synthesis enzymes.

Chioma Uchendu[1], Ziqiang Guan[2], Eric Klein[1,2,3,4]

1. Center for Computational and Integrative Biology, Rutgers University- Camden, NJ
2. Dept. of Biochemistry, Duke University Medical Center, Durham, NC
3. Biology Department, Rutgers University-Camden, NJ
4. Rutgers Center for Lipid Research, Rutgers University New Brunswick, NJ

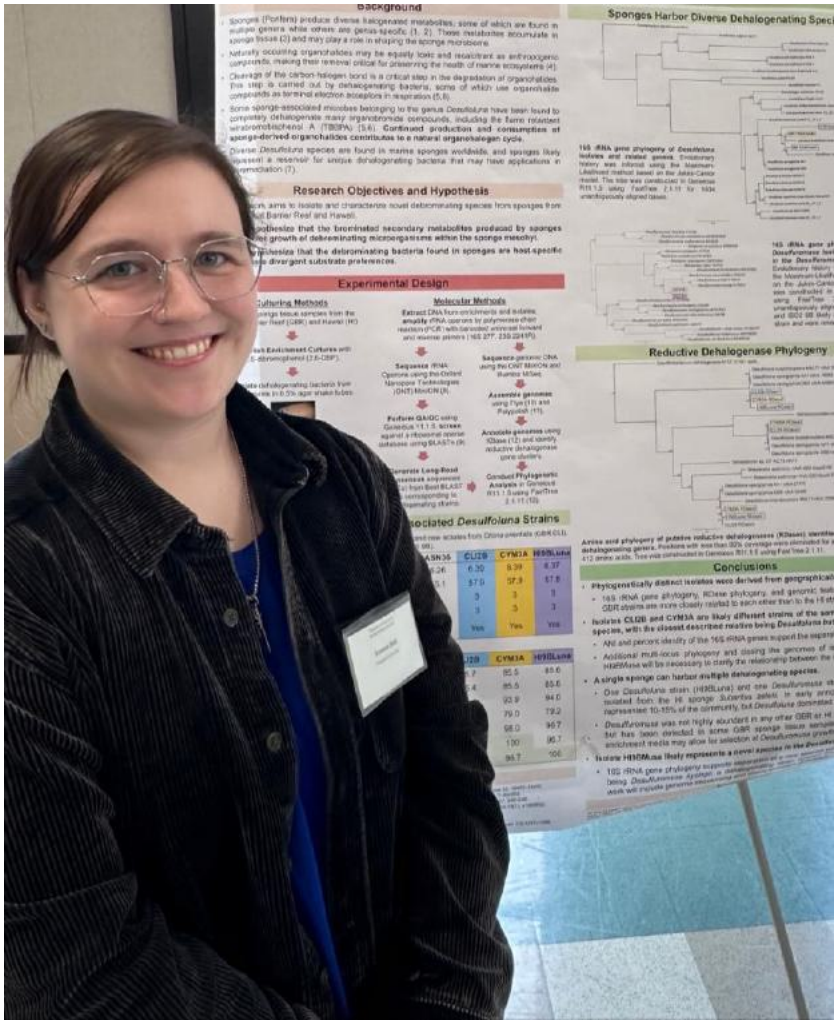


Sphingolipids are essential constituents produced by most eukaryotes, playing significant roles in cellular process such as cell growth, programmed cell death, angiogenesis, and inflammation. Despite the previous belief that sphingolipids were uncommon in bacteria, recent bioinformatic analysis of identified bacterial synthesis genes suggest a broader production among microbial species. The sphingolipid synthesis pathway involves three key enzymes: serine palmitoyl transferase, which catalyzes the condensation of serine with palmitoyl-CoA; ceramide synthase, responsible for adding the second acyl chain; and a reductase, which reduces the ketone on the long-chain base. While the identity of these bacterial enzymes is generally agreed upon, the precise mechanism and order of chemical reactions for microbial sphingolipid synthesis remains quite unclear, two proposed mechanisms include following the well-characterized eukaryotic pathway, where the long-chain base is reduced before the addition of the second acyl chain, or an alternative model where the second acyl chain is added before the reduction of the long-chain base. To distinguish between these models, we investigated the subcellular localization of the three key enzymes. Our findings reveal that serine palmitoyl transferase and ceramide synthase are located in the cytoplasm, while ceramide reductase is found in the periplasmic space. This supports our previous model, suggesting that the second acyl chain is added in the cytoplasm before export to the periplasm, where the lipid molecule undergoes reduction.

3. Isolation of novel dehalogenating bacteria from marine sponges.

Lauren A. Hall[1], Kaitlin A. Decker[1], Katie Scott[1], Max Dvinskikh[1], Kayla Ventura[1], Nicole S. Webster[2], Lee J. Kerkhof[3], Max M. Häggblom[1]

1. Dept. of Biochemistry and Microbiology, Rutgers University, New Brunswick, NJ
2. University of Tasmania, Hobart TAS, Australia
3. Dept. of Marine and Coastal Sciences, Rutgers University, New Brunswick, NJ



While anthropogenic organohalides are toxic and recalcitrant environmental contaminants of concern, thousands of organohalide compounds are of natural origin. For example, marine sponges produce diverse brominated secondary metabolites, creating a selective environment for debrominating microorganisms. The sponge-associated bacterium *Desulfoluna spongiiphila* AA1 uses organobromide compounds as terminal electron acceptors in organohalide respiration, during which the carbon-halogen bond is cleaved. We hypothesize that sponges harbor several host-specific dehalogenating bacterial species and strains with divergent substrate preferences. This work aims to isolate and characterize novel dehalogenating bacterial species and strains in sponges from the Great Barrier Reef (GBR) and Hawaii (HI) to determine host specificity of dehalogenating strains. Sponge samples were collected, homogenized, and inoculated into anaerobic enrichment media containing lactate, acetate, and propionate as carbon sources and 2,6-dibromophenol as the terminal electron acceptor. Debromination activity was monitored by HPLC. Pure cultures were isolated using anaerobic semi-solid shake tubes. DNA was extracted from active isolates and rRNA operons were sequenced using the Oxford Nanopore Technologies MinION for strain identification. Complete genomes were sequenced by Illumina and Nanopore platforms. Dehalogenating bacteria were enriched from every GBR and HI sponge tested, and several new strains were obtained in pure culture. Diverse dehalogenating *Desulfoluna* spp. were isolated from the HI and GBR sponges, including two GBR isolates which likely represent a novel species in the *Desulfoluna* genus. Both isolates possess two unique reductive dehalogenase gene clusters and genes for cobalamin biosynthesis, similar to other members of the genus. Both a *Desulfoluna* sp. and a *Desulfuromusa* sp. were isolated from one HI sponge. Phylogenetic analysis of the rRNA operon suggests that the *Desulfuromusa* isolate is a novel species. These results indicate that diverse *Desulfoluna* species are found in marine sponges worldwide, and sponges likely represent a reservoir for unique dehalogenating bacteria.

4. Playing with power: modulating ATP synthase in *E. coli.*

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

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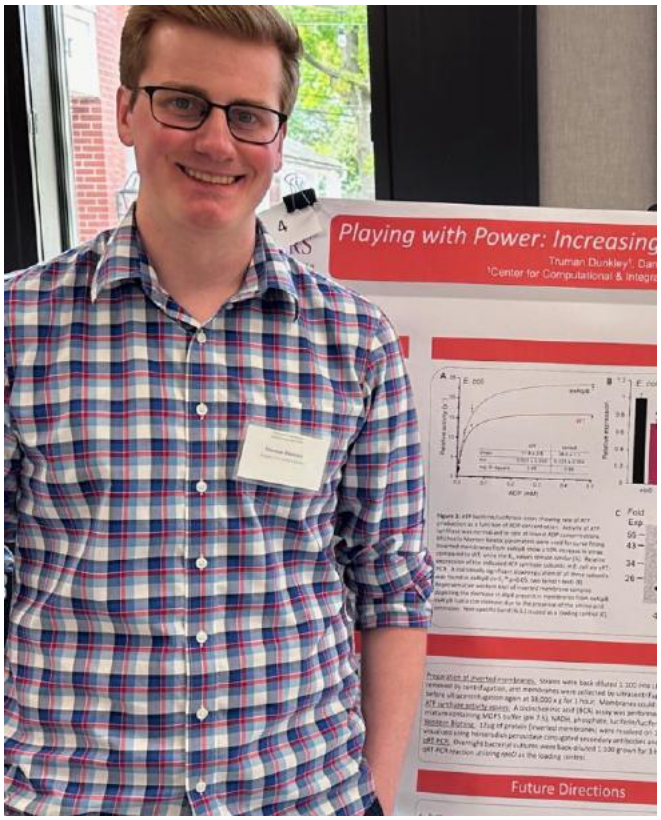
2. Biology Department, Rutgers University, Camden, NJ



The ubiquity of adenosine triphosphate (ATP) as the universal energy currency across almost all species underscores its pivotal role in facilitating life. For mesophilic organisms, steady-state ATP levels increase with temperature as one would expect from the Arrhenius relationship. Surprisingly, psychrophiles from all domains of life show an inverse relationship where cellular ATP levels increase as temperatures drop. Though the mechanism underlying this phenomenon is unknown, increased ATP levels likely contribute to psychrophily in these organisms by increasing the probability of molecular collisions with ATP—the universal currency of energy—under conditions of reduced molecular motion (e.g., low temperature). Indeed, genetic manipulation of *E. coli* to increase intracellular ATP enhanced cold tolerance up to 10-fold, and others have shown the equivalent in plants. We sequenced the transcriptome of the glacial ice worm *Mesenchytraeus solifugus*, which elevates ATP almost 2-fold over a ~10˚C temperature drop, and identified an 18 residue C-terminal extension of the mitochondrially encoded ATP6 subunit of the ATP synthase complex, which forms the proton pore that drives ATP synthesis. This sequence is not found on any other ATP6 protein in GenBank but is present on a variety of bacterial ion channels. Since manipulation of this mitochondrially-encoded gene is impossible in *M. solifugus*, we investigated the impact of this extension by making a chromosomal knock-in of this fusion into the homologous gene atpB in *E.coli* (exAtpB). Expression of exAtpB significantly increased the Vmax of the ATP synthase complex without affecting K_m. Remarkably, this enhanced activity persisted in the bacterium *Caulobacter crescentus* despite structural differences in the ATP synthase complex. The broad efficacy of this C-terminal extension across species underscores its potential as a simple yet potent modulator of ATP synthase catalytic efficiency.

5. The effects of simulated root exudates on soil microbial composition in a barren, metal-contaminated soil.

Sarah E. Krisak[1], Adam Daniel Parker[2], Bhagyashree P. Vaidya[3], and Nina M. Goodey[1]

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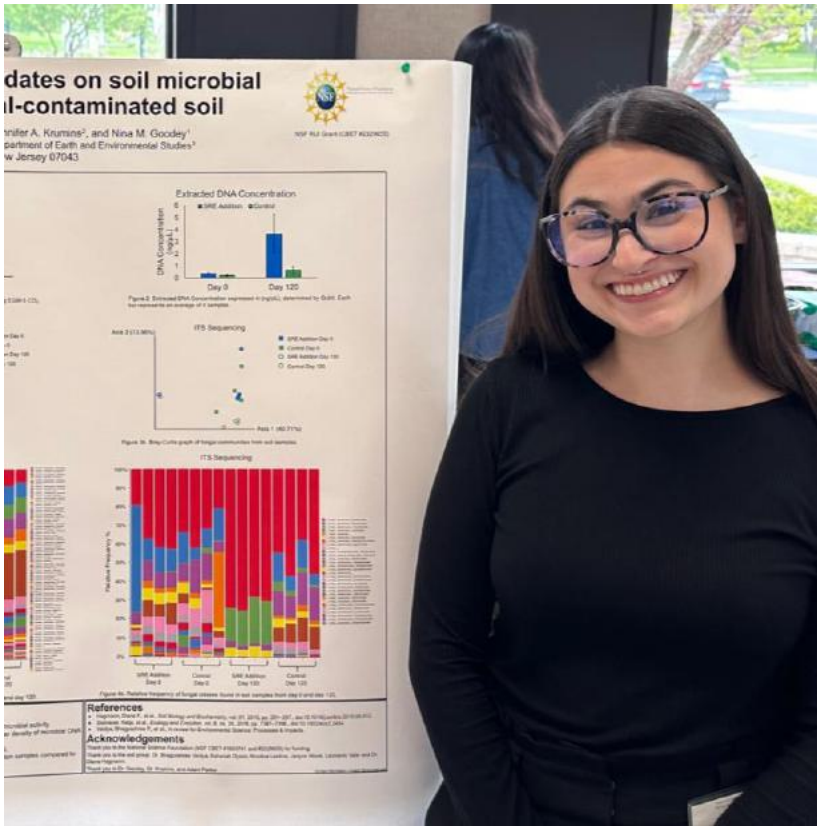


A well-functioning soil microbiome is crucial to soil health. Soil microbes facilitate nutrient cycling, which supports plant health and productivity. Brownfield soils typically exhibit low levels of microbial functioning, resulting in low plant productivity. Without plants, soils lack structure and stability. They also lack nutrient inputs in the form of simple metabolites, which are exuded by plant roots to feed soil microbes. We asked whether priming soils with a laboratory-prepared SRE solution stimulates native soil microbial function and how long the functioning is sustained after a single or repeated SRE addition(s) to a barren, contaminated soil. We also questioned how the soil contaminant-resistant microbial community changes in response to SREs and what types of microbes become more or less prevalent. We collected soil from a barren, metal contaminated, abandoned industrial rail yard. SRE-enriched barren, metal-contaminated soil showed a significantly higher soil respiration rate than the control, to which no SREs were added. Phosphatase activities were significantly higher in SRE-enriched barren, brownfield soil than its control. The DNA of the bacterial and fungal communities within barren, contaminated soil was extracted and sequenced using Next Generation Sequencing (NGS) at two time points. Using targeted sequencing methods (16S and ITS), we gained insights into the microbial community composition and diversity in SRE treated and control soils. These data allowed us to gain a deeper understanding of how SREs affect soil microbial composition and function. This understanding is crucial for soil management, in particular for efforts to revitalize poorly functioning soils.

7. Evaluating the antimicrobial activity of silver oxides against Gram-negative and -positive patient isolates.

Maxwell Akantibila[1,2], Gregory Caputo[2], Valerie Carabetta[1]

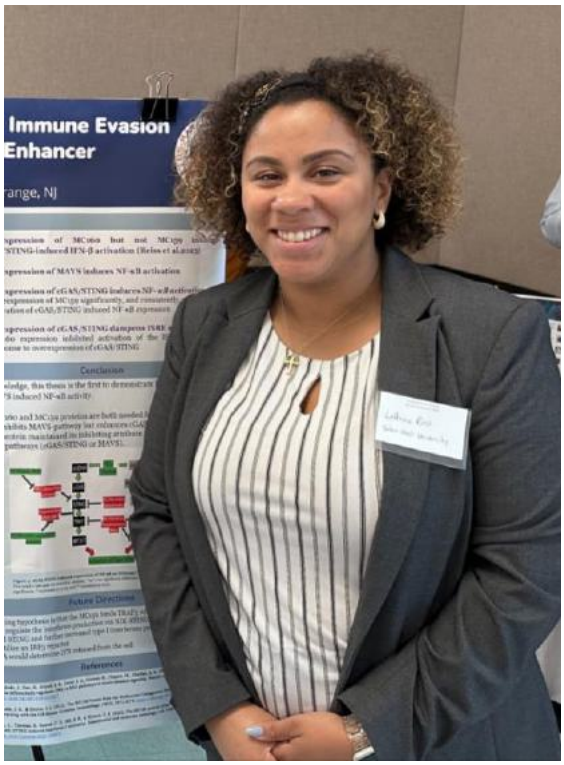
1. Department of Biomedical Sciences, Rowan University, Camden, NJ
2. Department of Chemistry and Biochemistry, Rowan University, Glassboro, NJ

Introducing a foreign material into the body during surgical implantation comes with a risk of antibiotic-resistant bacterial colonization and subsequent infection. Infections of surgically implanted devices often result to device failure, which leads to increased patient morbidity and mortality. In the United States, device-associated infections make up 25.6% of all infections related to healthcare. Out of all metals, silver has a wide range of antiviral, antifungal, and antibacterial properties. Silver needs to be oxidized to the Ag^+ ion to be used as a bactericidal agent and thus, silver salts, such as silver nitrate, have been used in medicine. The primary characteristic that makes silver efficient against bacteria is that it has the capacity to ionize in solution as it encounters tissues, body fluids, or water. Silver ions are bactericidal, because they can damage the cell wall, denature ribosomes, and interfere with DNA replication. They are deposited in the cell walls as granules, and disrupt the function of the cell envelope, which allows for damage to the intracellular contents. In addition, silver ions interact with nucleic acids, making it challenging for DNA polymerase and RNA polymerase to replicate DNA and transcribe RNA, respectively. Here, we evaluated the efficacy of silver (Ag), silver II oxide (AgO), and silver I oxide (Ag_2O) coated discs against clinical isolates of methicillin susceptible and resistant *Staphylococcus aureus* (MSSA and MRSA, respectively), Pan drug (PDR)-, extensively drug-resistant (XDR), and pan-susceptible (PS) *Acinetobacter baumannii*, *Pseudomonas aeruginosa*, and *Staphylococcus epidermidis*. We evaluated the efficacy of the silver-coated discs against both free-living bacteria and biofilms. We found out that all the multidrug resistant (MDR) bacteria were susceptible to AgO and Ag_2O, except *Pseudomonas aeruginosa*. AgO and Ag_2O reduced the biofilm masses of MRSA, *S. epidermidis*, XDR *A. baumannii*, and PS *A. baumannii*. Ag, AgO, and Ag_2O were also effective in reducing *P. aeruginosa* biofilm mass, but they were ineffective in reducing PDR *A. baumannii* biofilm mass. Our data provides support that silver-coated surgically implanted materials could be utilized to minimize or prevent microbial colonization and subsequent infections.

8. The molluscum contagiosum MC159 and MC160 immune evasion molecules: two great viral proteins better together.

LeAnna Ross, Daniel Brian Nichols

Department of Biological Science, Seton Hall University, South Orange, NJ



Molluscum contagiosum virus (MCV) is a human skin pathogen that causes persistent, benign lesions on the epidermis after infected skin to skin contact. The virus has dedicated 30% of its genome to immune evasion genes which dampen the host cell's response. The cytoplasm becomes an important area of replication for the virus, but also houses key immune pathogen recognition receptors such as cyclic GMP-AMP synthase (cGAS) that detect Pathogen Associated Molecular Patterns. MCV proteins MC160 and MC159 modulate both the cGAS/STING pathways for cytoplasmic dsDNA detection and the MAVS pathways for dsRNA detection. Both pathways ultimately end up with the production of type I interferons. Despite structural similarities, the MC160 and MC159 have noticeably different phenotypes. MC160 expression dampens both MAVS and cGAS/STING mediated activation of type I interferons. The MC159 protein on the other hand inhibits the MAVS pathway but surprisingly enhanced the cGAS/STING pathway. To better understand the mechanisms of MCV immune evasion proteins, this study characterized the expression of MC160 and MC159 alone on each pathway's mechanism to induce transcription factors that upregulate interferon expression. The overexpression of the MC159 protein alone upregulated activation of the type I interferon enhancer as well as the transcription factor NF-κB. The MC160 protein maintained its inhibiting attribute on both sides of the immune pathways (cGAS/STING or MAVS). Surprisingly, we also saw overexpression of the MC159 alone did not upregulate as it did previously with cGAS/STING, with cGAS/STING induced ISRE luciferase. This shows that both proteins may work better together in dampening immune responses versus working alone.

9. Identification and characterization of low Mg^{2+} stress-induced small proteins in *E. coli*

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

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

Many proteins derived from short open reading frames, consisting of less than 50 amino acids, have often been overlooked in early genome annotations due to minimum gene length cut-offs. Consequently, their prevalence and function remain largely unknown, hampering their identification and characterization. Recent advancements in computational and experimental methods have led to the discovery of approximately 150 small proteins in *E. coli* grown in nutrient-rich media. Notably, a significant proportion of these small proteins are hydrophobic and are predicted to be membrane-bound. Growing evidence indicates that small proteins play crucial roles in essential cellular processes such as cell division, growth, transport modulation, and signaling under specific conditions. Some well-studied small proteins have demonstrated their importance in specific growth conditions and their regulatory roles during stress responses. In light of these observations, small proteins accumulating under stress conditions likely play a regulatory role under that growth condition. To identify low Mg^{2+}-dependent expression of small proteins in *E. coli*, we utilized the translation initiation profiling method – RETapamulin enhanced Ribo-seq (Ribo-RET). Using Ribo-RET, we have identified a subset of 17 small proteins out of the >150 reported in *E. coli* to be upregulated under this condition. Remarkably, most of the 17 stress-induced small proteins, of which a staggering 14 had not been associated with this particular stress condition prior to this study. RNAseq data confirmed that most of these small proteins are transcriptionally upregulated under magnesium starvation, while transcriptional reporter assay revealed that at least eight are regulated by the PhoQ/PhoP two-component system that plays a vital role in regulating Mg^{2+} homeostasis. Furthermore, our analysis uncovered that nine small proteins are regulated as part of operons. Moreover, we confirmed the membrane localization of 9 small proteins using translation fusions. Ongoing investigations are focused on elucidating the loss-of-function and gain-of-function phenotypes of these small proteins, particularly their impact on growth and morphology under low magnesium stress. Future studies aim to identify potential binding partners of these small proteins and unravel their specific roles under low magnesium stress. Systematic characterization of these stress-induced small proteins will advance our understanding of their functions and contributions to bacterial survival and adaptation to stress responses, facilitating the development of novel antibiotics and therapeutics.

10. Human-derived *Lactobacillus* strains may deconjugate estrogen glucuronides.

Jeffrey Douyere[1,2], Ke Sui[1,2], Kainat Zafar[1,2], Amber Stone[1,2], Rhythm Chaudhary[1,2], Rocio Duran[1,2], and Diana E. Roopchand[1,2]

1. Department of Food Science and New Jersey Institute for Food, Nutrition and Health, Rutgers University, New Brunswick, NJ

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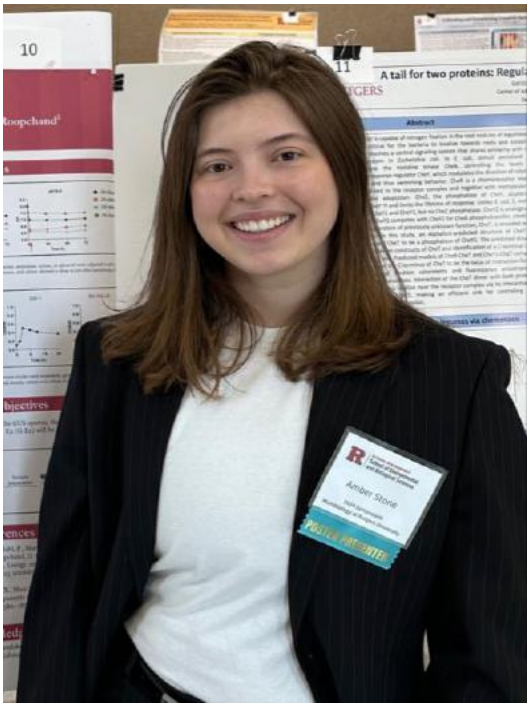

Estrogens are C18 steroid hormones biosynthesized from low-density lipoprotein (LDL) cholesterol. 17β-estradiol (E2) is the primary form of estrogen in reproductive age females and in males. Estrone (E1) is the main estrogen in postmenopausal females. Estrogen is important for both female and male reproduction as well as the proper function of many organ systems. Estrogen levels that are too high or low can result in various disorders. In the hypo-estrogenic state, females have increased risk of cardiovascular disease and osteoporosis while males may have decreased libido and sexual dysfunction (Chen et al., 2020). In hyper-estrogenic states, females are at high risk of breast and gynecological cancers and males may develop gynecomastia, prostate cancer, and erectile dysfunction (Johnson and Murad, 2009).

The estrobolome is the collection of gut bacteria that can metabolize estrogens and contributes to regulation of circulating estrogens. Many gut bacteria harbor gus (aka uidA), which encodes β-glucuronidase (GUS), a group of substrate specific enzymes responsible for removing the glucuronide moieties that make compounds more water soluble for subsequent excretion via bile into urine/stool. GUS-mediated deconjugation of glucuronidated E1 (G-E1) and E2 (G-E2) allows reabsorption of active E1 and E2 into enterohepatic circulation. We hypothesize that the composition of the estrobolome has the potential to remediate or worsen hypo-estrogenic or hyper-estrogenic states in males and females. Dietary compounds, medications, or supplements may influence the composition of the estrobolome and, subsequently, the levels of circulating estrogens. Basic knowledge of the bacterial strains with estrogen-specific GUS activity is limited, therefore we sought to identify strains of *Lactobacillus* that could deconjugate glucuronidated E1 and/or E2 in vivo.

11. A tail for two proteins: regulation of chemotaxis in nitrogen fixing bacterium *Sinorhizobium meliloti.*

Garima Verma[1], Rong Gao[1], Alfred Agbekudzi[2], Ti Wu[1], Birgit Scharf[2] and Ann Stock[1]

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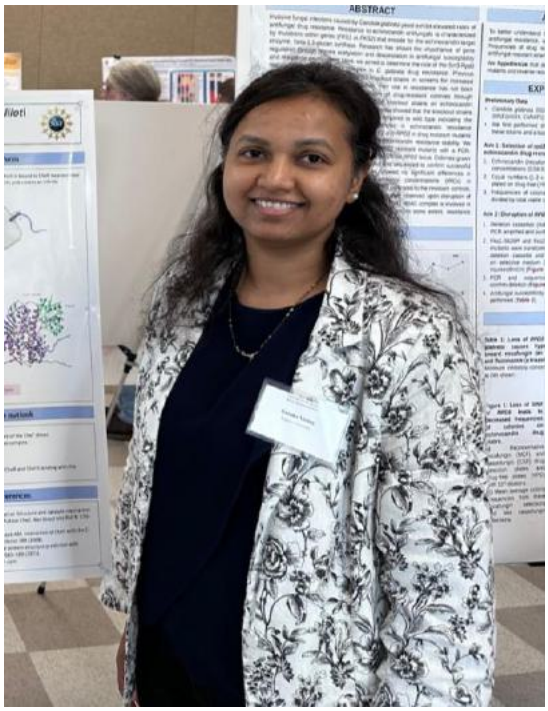
2. Department of Biological Sciences, Virginia Tech, Blacksburg, VA

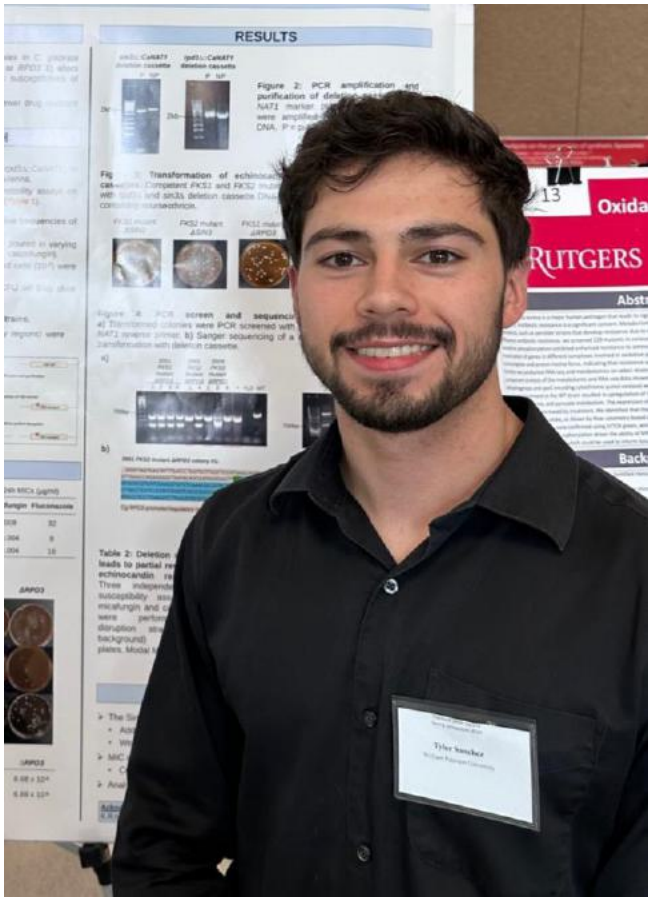


Sinorhizobium meliloti is capable of nitrogen fixation in the root nodules of leguminous plants. Chemotaxis is critical for the bacteria to localize towards roots and establish symbiosis. Chemotaxis involves a central signaling system that shares similarity with the extensively studied system in *Escherichia coli*. In *E. coli*, stimuli perceived by chemoreceptors regulate the histidine kinase CheA, controlling the levels of phosphorylation of the response regulator CheY, which modulates the direction of rotation of the flagellar motor and thus swimming behavior. CheR is a chemoreceptor methyl transferase that is localized to the receptor complex and together with methylesterase CheB mediates receptor adaptation. CheZ, the phosphatase of CheY, accelerates dephosphorylation of CheY~P and limits the lifetime of response. Unlike *E. coli*, *S. meliloti* has two CheY proteins, CheY1 and CheY2, but no CheZ phosphatase. CheY2 is analogous to CheY of *E. coli* whereas CheY1 competes with CheY2 for CheA phosphotransfer, creating a phosphoryl sink. A novel protein of previously unknown function, CheT, is encoded within the chemotaxis operon. In this study, an Alphafold-predicted structure of CheT and biochemical analyses reveal CheT to be a phosphatase of CheY1. The predicted structure guided engineering of deletion constructs of CheT and identification of a C-terminal tail of CheT that interacts with CheR. Predicted models of CheR-CheT and CheY1-CheT complexes indicated a pentapeptide at the C-terminus of CheT to be the locus of interaction for both proteins. Using isothermal titration calorimetry and fluorescence anisotropy, we confirmed the predicted interactions. Interaction of the CheT dimer with both proteins is hypothesized to allow CheT to localize near the receptor complex via its interaction with CheR and dephosphorylate CheY1, making an efficient sink for controlling CheY2 phosphorylation and motility of *S. meliloti*.

12. Assessing the role of the Sin3-Rpd3 HDAC complex on antifungal resistance in the fungal pathogen *Candida glabrata*.

Tyler Sanchez, Zubayeda Uddin, and Kelley R. Healey

Department of Biology, William Paterson University, Wayne NJ



Invasive fungal infections caused by *Candida glabrata* yeast exhibit elevated rates of antifungal drug resistance. Resistance to echinocandin antifungals is characterized by mutations within genes (FKS1 or FKS2) that encode for the echinocandin target enzyme, beta-1,3-glucan synthase. Research has shown the importance of gene regulation through histone acetylation and deacetylation in antifungal susceptibility and resistance development. Here, we aimed to determine the role of the Sin3-Rpd3 histone deacetylase (HDAC) complex in *C. glabrata* drug resistance. Previous studies identified both SIN3 and RPD3 knockout strains in screens for increased echinocandin drug susceptibility; however, their role in resistance has not been described. We first determined frequencies of drug-resistant colonies through selection of wild type and SIN3 and RPD3 knockout strains on echinocandin (micafungin and caspofungin) drug plates. Results showed that the knockout strains yielded decreased frequencies of colonies compared to wild type indicating the importance of the Sin3-Rpd3 HDAC complex in echinocandin resistance development. Another aim was to disrupt SIN3 and RPD3 in drug resistant mutants to determine if these genes are essential in echinocandin resistance stability. We transformed competent cells of FKS1 and FKS2 resistant mutants with a PCR-amplified deletion marker targeted to either the SIN3 or RPD3 locus. Colonies grown up on selective medium were PCR-screened and sequenced to confirm successful gene deletion. Drug susceptibility assays showed no significant differences in micafungin or caspofungin minimum inhibitory concentrations (MICs) in echinocandin resistant strains disrupted for SIN3 compared to the resistant controls; however, moderate decreases (4-fold) in MICs were observed upon disruption of RPD3. Overall, our work suggests that the Sin3-Rpd3 HDAC complex is involved in echinocandin tolerance, resistance development, and to some extent, resistance stability.

13. Oxidative phosphorylation potentiates antimicrobial sensitivity in *Staphylococcus aureus.*

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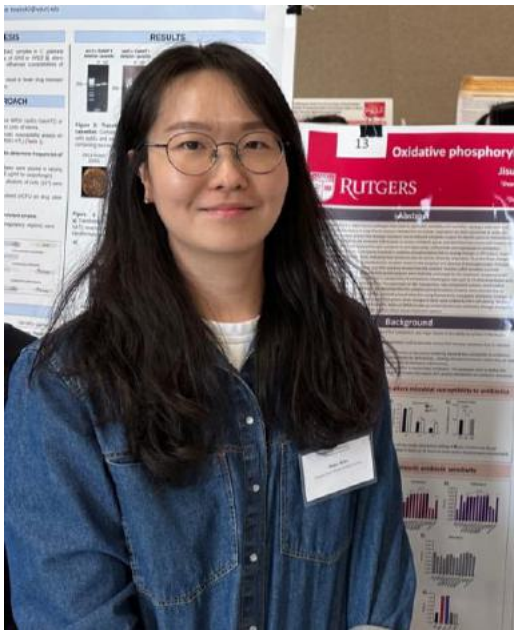
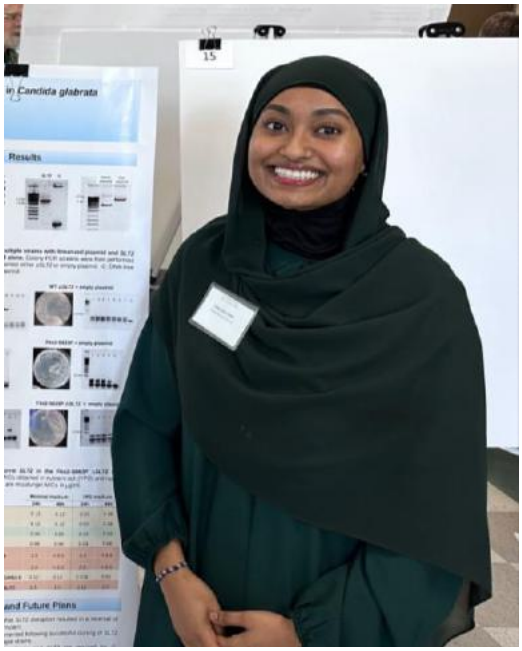


Staphylococcus aureus is a major human pathogen that leads to significant morbidity and mortality, causing a wide variety of infections. Antibiotic resistance is a significant concern. Metabolism via cellular respiration has been connected to antibiotic resistance, such as persister strains that develop resistance due to reduced metabolic output. To identify genes in metabolism that influence antibiotic resistance, we screened 229 mutants in various metabolic genes and identified that strains with mutations in oxidative phosphorylation exhibited enhanced resistance to aminoglycoside, sulfonamide and cephalosporin antibiotics. Inactivation of genes in different complexes involved in oxidative phosphorylation led to varying changes in ATP output, oxygen consumption and proton motive force, indicating that resistance was not purely driven by respiration. To probe these changes further we conducted RNA-seq and metabolomics on select strains in the presence and absence of aminoglycosides. Principal component analysis of the metabolomic and RNA-seq data showed that the resistant mutants (sdhA encoding succinate dehydrogenase and qoxC encoding cytochrome quinol oxidase) were relatively unchanged by the treatment compared to WT. Antibiotic treatment in the WT strain resulted in upregulation of metabolism-related gene sets such as C5-Branched dibasic acid, TCA cycle, galactose, and pyruvate metabolism. The expression of ABC transporters, two-component systems, and histidine metabolism were decreased by treatment. We identified that the oxidative phosphorylation mutants were resistant due to decreased antibiotic uptake, as shown by flow cytometry-based detection using fluorescently conjugated antibiotics. Changes in membrane permeability were confirmed using SYTOX green, while changes in lipid were evident by FM 4-64 staining. We have identified that oxidative phosphorylation drives the ability of MRSA to take up certain bactericidal antibiotics through changes in the membrane permeability, which could be used to inform future treatment options.

14. The cell wall integrity pathway is required for FKS2-mediated echinocandin drug resistance in *Candida glabrata*.

Zubayeda Uddin, Saira Tahsin, Gabrielle Popencuk, and Kelley R. Healey

Department of Biology, William Paterson University, Wayne, NJ



Candida glabrata is a fungal pathogen that can cause invasive infections in immunocompromised patients. These infections are treated with echinocandin antifungal drugs that inhibit fungal cell wall synthesis by targeting beta-1,3-glucan synthase. Mutations in FKS1 or FKS2 genes that encode for this target enzyme lead to resistance; however, multiple fungal tolerance pathways, including the cell wall integrity (CWI) pathway, are activated upon drug exposure prior to FKS mutation and yield hypersusceptibility to echinocandins when targeted. To determine the role of the CWI pathway in echinocandin-resistant cells, we first disrupted a CWI pathway gene (SLT2) in a drug-resistant FKS2 mutant (S663P). Subsequent drug susceptibility assays performed showed that disruption of SLT2 surprisingly led to reversal of echinocandin resistance. Our next aim was to complement this phenotype with reintroduction of SLT2 on a plasmid. We cloned SLT2 onto *C. glabrata*-specific plasmids which were expressed in our SLT2 knockout strains using a gap-repair cloning technique. More recent work generated additional plasmid-bearing control strains required for comparison. Drug susceptibility assays revealed successful complementation of the FKS2 mutant that was disrupted for SLT2. With SLT2 reintroduced, this strain demonstrated elevated MICs compared to wild type and empty-plasmid controls, as expected. In addition, the added presence of plasmid-borne SLT2 did not alter susceptibility profiles of wild type or resistant cells that contained intact chromosomal copies of SLT2. These studies reveal that SLT2 influences echinocandin resistance in *C. glabrata* and provide additional insight into how targeting the CWI pathway may increase echinocandin efficacy and how FKS2 gene expression is controlled.

16. Queuosine biosynthetic enzyme (QueE) moonlights as a cell division regulator.

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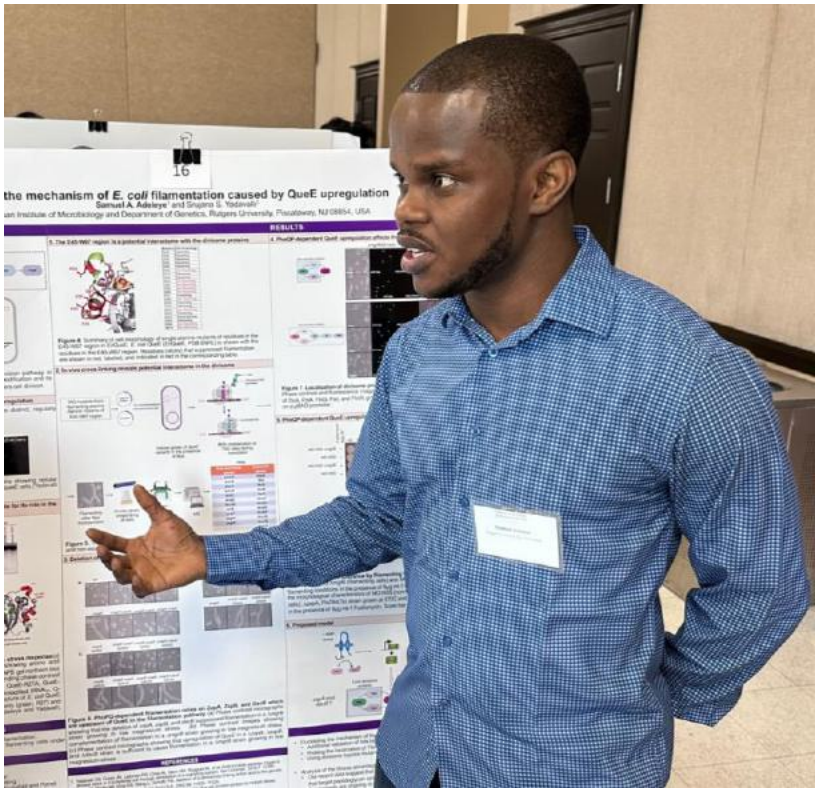
Department of Genetics and Waksman Institute of Microbiology, Rutgers University, Piscataway, NJ



A biosynthetic enzyme called QueE, which catalyzes a step in the formation of queuosine (Q) tRNA modification, is upregulated when cells are exposed to sub-MIC levels of cationic antimicrobial peptides (cAMPs). This is caused by a strong activation of the PhoQP signaling system, a system that senses cAMPs, log magnesium, and low pH in gamma proteobacteria. When cellular QueE levels are high, it co-localizes with the central cell division protein FtsZ at the septal site, blocking division and resulting in filamentous growth. In this study, we show that QueE affects cell size in a dose-dependent manner. Using alanine scanning mutagenesis of amino acids in the catalytic active site of QueE important for Q formation, we pinpoint particular residues in QueE that contribute distinctly to each of its functions – Q biosynthesis or regulation of cell division, establishing QueE as a moonlighting protein. We further show that QueE orthologs from enterobacteria like *Salmonella typhimurium* and *Klebsiella pneumoniae* also cause filamentation in these organisms, but the more distant counterparts from *Pseudomonas aeruginosa* and *Bacillus subtilis* lack this ability. Finally, using cellular localization of divisome proteins, we show that QueE upregulation causes the inhibition of septation by affecting the recruitment of late divisome proteins.

17. Exploring the influence of manganese oxide nanomaterial on biofilm growth on drinking water pipes.

Lylla Almosd[1], Travis Santana[2], Rouzbeh Tehrani[2], N.L. Fahrenfeld[1]

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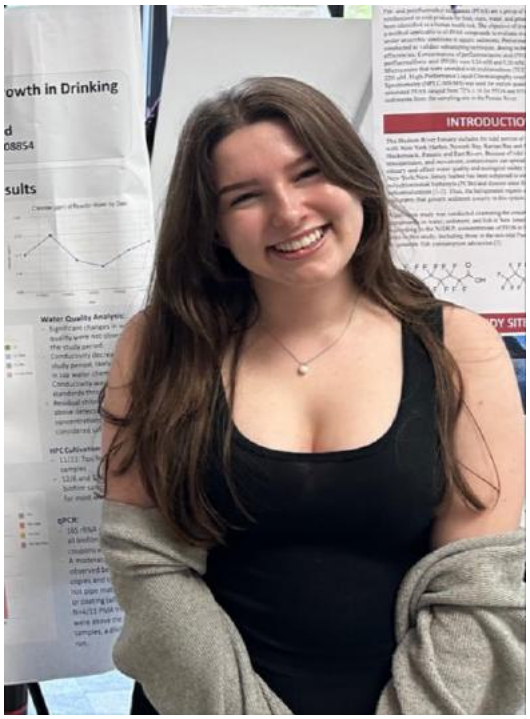

The number of nontuberculous mycobacterium-related deaths is rising significantly each year as the population of immunocompromised individuals increases. Nontuberculous mycobacteria (NTM) are opportunistic pathogens that can infiltrate household plumbing systems and cause disease in those with compromised immune systems. These opportunistic pathogens seem to persist despite the implementation of various processes within water treatment plants and disinfection treatments that attempt to rid drinking water of harmful pathogens to make it safe for human consumption. These pathogens are found in water and soil environments and they tend to accumulate via biofilm formation on the surface of pipes within household plumbing systems. The purpose of this study was to test the ability of nanomaterial pipe coatings provided by Dr. Tehrani's lab at Temple University for their ability to prevent the formation of biofilm on household plumbing systems and/or lyse bacterial cells within biofilms that form. A biofilm annular reactor was used to stimulate the environment of a household plumbing system as it was filled with tap water and run with nanomaterial and control coupons rotating inside it. The reactor was run continuously. Water quality analysis was performed weekly, and biofilm samples were collected, plated, and processed on day 7, day 21, and day 35. Filtered water samples and PMA-treated and untreated biofilm samples were subject to DNA extraction and 16s rRNA qPCR analysis. Water quality analysis revealed that the conductivity of the reactor water decreased each week, the chlorine levels fluctuated, the temperature remained constant, and the pH steadily increased. The nanomaterial adhered more strongly to the PVC surface than the copper surface. Heterotrophic plate count data was inconclusive. qPCR analysis revealed that 16s rRNA was above detection in all the biofilm samples, but no conclusions concerning the antimicrobial capacity of the nanomaterial treatment can be drawn. It is important to test potential point-of-use treatments to make progress in tackling the issue of opportunistic pathogens in drinking water that put many lives in danger.

18. Method development for per- and polyfluoroalkyl substances (PFAS) biotransformation studies.

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1. Department of Environmental Sciences, Rutgers University, New Brunswick, NJ
2. Tetra Tech, Austin, TX

Per- and polyfluoroalkyl substances (PFAS) are a group of chemicals that are synthesized to coat products for heat, stain, water, and grease resistance, and have been identified as a human health risk. The objective of this research is to develop a method applicable to all PFAS compounds to evaluate microbial transformations under anaerobic conditions in aquatic sediments. Preliminary experiments were conducted to validate subsampling techniques, dosing techniques, and extraction efficiencies. High-Performance Liquid Chromatography coupled to tandem Mass-Spectrometry (HPLC-MS/MS) was used for analyte quantification.

19. Understanding the distribution of target microbial pathogens in adult blow fly (*Calliphoridae*) tissue at three sites in New Jersey, USA

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2. Ernest Mario School of Pharmacy, Rutgers University, Piscataway, NJ
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5. Forensic Entomology & Wildlife Laboratory (FEWL), School of Interdisciplinary Forensics (SIF), Arizona State University-West Valley Campus, Glendale, AZ
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This is an exploration study investigating the role of target fly taxa as reservoirs of pathogenic microbes in coastal United States. The goal of this ongoing study is to document the occurrence and abundance of specific pathogens in the gut tissue of adult non-biting "filth flies" (Diptera) collected at three sites in New Jersey. Adult flies like blow flies (*Calliphoridae*) and house flies (*Muscidae*) have been shown to harbor and spread microbes that cause disease such as *E. coli*, dysentery, and anthrax. Our hypothesis is that we will be able to see patterns in the distribution of pathogens in blow fly guts when analyzing pathogen occurrence compared to fly trapping location. Trapping sites for this study included a coastal barrier island (4 traps), an inland urban-rural area (2 traps), and the campus of a municipal WWTP (2 traps). Water samples were vacuum filtered. The filters and adult fly gut tissue are molecularly tested for signatures of target microbes. Microbial pathogens of interest in this study are SARS-CoV-2 and *Vibrio* spp. Controls include human associated Bacteroides (HF183), 16S rRNA gene for bacteria, and Pepper Mild Mottle Virus (PMMoV) for RNA viruses. Analysis from a preliminary trial in 2022 demonstrated detection of both PMMoV and SARS-CoV-2 N1 gene in wastewater, with N1 being observed 1 – 4 orders of magnitude lower than PMMoV. In flies, PMMoV was observed but we did not detect the N1 gene in flies collected in June 2022 despite detection of N1 in the wastewater influent collected on that day. Investigators in Iran and Kansas, USA reported detection of SARS-CoV-2 in *Musca domestica* (L) (Diptera, *Muscidae*) via testing a wild-caught fly for COVID-19 and controlled feeding experiments respectively. Our study aims to build on this foundation by demonstrating that the gut microbiota of adult flies are impacted by the pathogens found at different biogeographical locations. Further, this project seeks to address the need of aquaculture workers to understand how pathogens like *Vibrio* spp. persist in the environment. These pathogens are often associated with warming waters and present serious challenges for human health and economic stability.

20. Exploring Fur and IsrR genetic regulation in *Staphylococcus epidermidis*

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1. Department of Biochemistry and Microbiology, Rutgers University, New Brunswick, NJ
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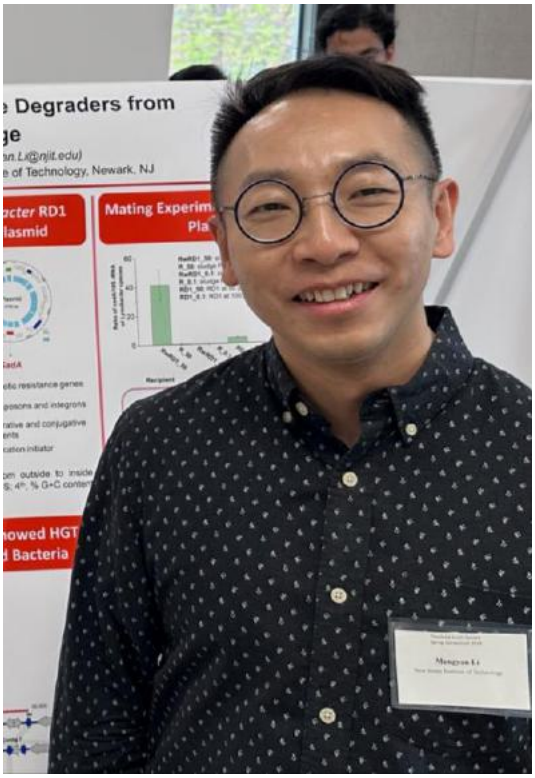
Staphylococcus epidermidis is a skin commensal. This opportunistic pathogen can cause infections ranging from minor skin irritations to life-threatening bloodstream infections, especially in those with weakened immune systems or those who utilize medical equipment like catheters or implants. Iron is an essential micronutrient that is necessary for bacterial growth and pathogenicity. Because iron overload is toxic, Fe homeostasis is tightly regulated by various regulatory genes, including ferric uptake regulator (Fur) and IsrR. Fur is a Fe-binding transcriptional regulator that suppresses transcription of genes responsible for iron absorption. Fur is demetallated during growth in iron-repleted conditions, and repression is released. Fur also represses the transcription of isrR, which codes a small non-coding RNA. IsrR interacts with mRNA transcripts to modulate expression in *Staphylococcus aureus*. The interaction between these iron regulatory genes and the bacterial response to iron availability in *S. epidermidis* have not been investigated. Herein, we tested the hypothesis that Fur and IsrR regulate Fe uptake and usage in *S. epidermidis*. To this end, we created Δfur and ΔisrR mutants in *S. epidermidis*. Using these mutants, we demonstrate roles for Fur and IsrR in regulating extracellular protease expression, hemolysin activity, and copper ion homeostasis.

21. Uncovering the molecular and microecological basis for the biotransformation of sulfonamides.

Mengyan Li, Dung Ngoc Pham

Department of Chemistry and Environmental Science, New Jersey Institute of Technology, Newark, NJ



The spread of antimicrobial resistance in the environments, especially its transfer into human pathogens, has emerged as a global concern. A culprit for the rise of antimicrobial resistance issues is misuse and/or unrestricted disposal of antibiotics, conducive to the prevalence of these persistent compounds that render the dissemination of antimicrobial resistance genes (ARGs). This is worsened at municipal wastewater treatment plants (WWTPs) where antibiotics are frequently detected with relatively high concentrations. In this present study, we focus on characterizing sludge microorganisms that are capable of inactivating antibiotics and investigating their molecular foundations. Sulfamethoxazole (SMX) was selected as a representative for sulfonamides (SAs), a class of synthetic antibiotics that have been extensively used and frequently detected in surface and wastewater. We successfully isolated two SMX degraders, *Lysobacter* sp. RD1 and *Xanthobacter* sp. LD2, from the enrichment of activated sludge samples from two different WWTPs located in northern New Jersey. In both strains, along with the degradation of SMX, accumulation of 3-amino-5-methylisoxazole (3A5MI) was observed by HPLC, indicating both strains can cleave the S-N bond and subsequently dismiss the antimicrobial effort of SMX. Using long-read Nanopore Minion Technology, complete genomes of these two isolates was sequenced, revealing the existence of sadA genes that encode the class D FMNH2-dependent monooxygenases, known for their ability of catalyzing the breakdown of S-N linkage in SAs. This is the first report revealing the occurrence of sadA genes in gram-negative bacteria prevailing in the diverse environments. We also investigated the phyletic patterns of these antibiotic inactivation genes and surrounding mobile elements to elucidate molecular evidence for horizontal gene transfer (HGT) processes and predict donors, recipients, and co-acquired genes. Mating assays were conducted to confirm the HGT of sadA from RD1 to other gram-negative sludge bacteria, revealing their spreading potential under the pressure of SMX. The identification of sadA in gram-negative bacteria and the associated molecular processes also provide an opportunity to design efficient methods for eliminate SAs and thus decelerate the dissemination of antibiotic resistance in our environment.

22. Exploring hospital disinfectant efficacy against extensively drug-resistant (XDR) *Pseudomonas aeruginosa*.

Elena Sierra, Valerie J. Carabetta

Dept of Biomedical Sciences, Cooper Medical School of Rowan University, Camden, NJ

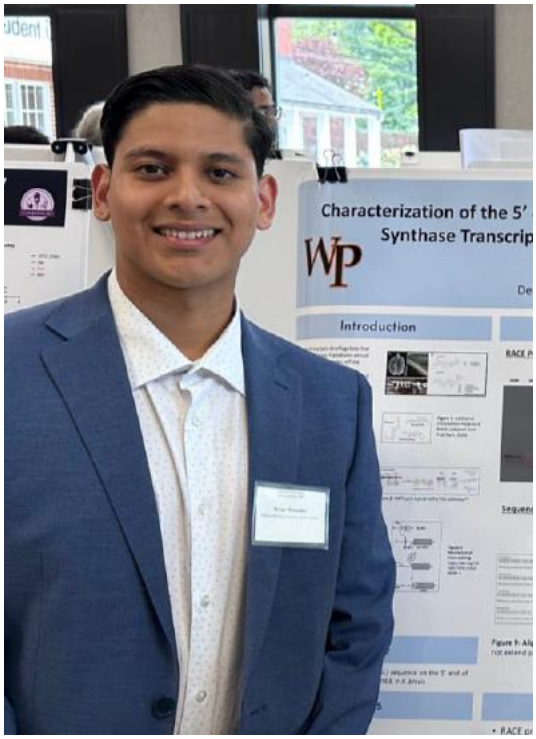
Pseudomonas aeruginosa, a prevalent Gram-negative bacterium and a significant contributor to hospital-acquired infections, presents a pressing public health challenge due to its rapid development of resistance to various antimicrobials. *P. aeruginosa*, an opportunistic pathogen, rarely causes illness in healthy individuals, and it is a leading cause of life-threatening infections in those with compromised immune systems, particularly cystic fibrosis patients. It is most commonly associated with ventilator-associated pneumonia, urinary tract infections, and otitis externa. Additionally, its ability to form biofilms complicates eradication efforts and contributes to the establishment of chronic infections. The biofilm is a complex assembly of bacteria within a matrix of extracellular polymeric substances (EPS). This structure serves as a vital survival mechanism for bacteria confronted with unforeseen environmental shifts, such as variations in temperature and nutrient availability. Bacteria within a biofilm can evade host immune defenses and exhibit resistance to antimicrobial therapies that can be up to 1000 times greater than their free-floating counterparts.

Our study aims to evaluate the susceptibility of three extensively drug-resistant (XDR) *P. aeruginosa* isolates from Cooper University Hospital (CUH) to bleach, ethanol, and four commonly used hospital disinfectants, both in their free-living planktonic state and under biofilm conditions. First, we determined the minimum inhibitory concentration (MICs) of our strains to standard-of-care antibiotics, and we determined that they were extensively drug-resistant (XDR). MIC and minimum bactericidal concentrations (MBCs) were determined by broth microdilution and time-kill assays performed to assess *P. aeruginosa's* susceptibility to the disinfectants. The MIC and MBC of the active ingredients for the disinfectant wipes were below the concentration used in the wipes used by CUH. Disinfectant wipes were recreated in solution for the time-kill assays. Time-kill assays revealed that a small subpopulation of free-living planktonic P. aeruginosa remained viable when exposed to 0.63% bleach, while 55% and 70% of ethanol, Super Sani Cloth®, Sani Cloth AF3®, Sani Cloth Prime®, and a skin antiseptic of 2% chlorhexidine gluconate killed 99.9% of the bacteria. Bacteria in biofilms, however, exhibited 40% survival after 4 minutes of exposure to 0.63% bleach. Less than 9% of survival was demonstrated when exposed to the hospital wipes and the skin disinfectant at 1 min, 2 min, and 4 min. When comparing the XDR strains to the control strains, we observed resistance against bleach in all strains tested when biofilms were present. Selecting a proper disinfectant is crucial in the healthcare setting to prevent the spread of more pathogenic bacteria. This emphasizes the importance of our study in evaluating disinfectant efficacy against extensively drug-resistant *Pseudomonas aeruginosa* strains, aiding in the selection of appropriate disinfectants for infection control protocols.

23. Characterization of the 5′ ends of hybrid nonribosomal peptide synthetase/polyketide synthase transcripts in the harmful algal bloom species, *Karenia brevis*.

Bryan Menendez, Emily A. Monroe

Department of Biology, William Paterson University of New Jersey, Wayne, NJ



Karenia brevis causes harmful algal blooms that lead to marine animal death and health complications in humans through the production of neurotoxins called brevetoxins. Brevetoxins are synthesized by polyketide synthase (PKS) enzymes, but *K. brevis* also contains nonribosomal peptide synthetases (NRPS) and hybrid NRPS/PKS enzymes that synthesize different secondary metabolites. Characterization of these pathways will provide information on the full metabolome of K. brevis and their regulation. PKS transcripts previously studied contain a trans-splicing signal on their 5′end called the spliced leader (SL), suggesting they are regulated post-transcriptionally. The objective of this study is to identify if the spliced leader (SL) is present on the 5′ end of hybrid NRPS/PKS transcripts in K. brevis. To examine the 5′end, 5′ RACE was performed on two hybrid transcripts, contigs 1930 and 10563. Based on the current sequence data, the PCR products for both contigs would be at least 250 bp. RACE PCR products were 350 bp for Contig 1930 and 150 bp, 200 bp, and 300 bp for Contig 10563 suggesting RACE produced cDNAs longer than the original sequence. RACE PCR products were cloned using the TOPO cloning kit and transformed into TOP10 competent *E. coli* cells. Plasmids were isolated from transformed *E. coli* cells and sequenced. Sequence alignments between RACE sequences and the original contig 10563 showed no additional sequence at the 5′ end of the 10563 contig. Sequence alignments showed a short extension of the 1930 contig, and a partial SL sequence was identified. This is the first report of the presence of the SL sequence on the newly identified hybrid NRPS/PKS transcripts in *K. brevis*. This work improves our understanding of the gene regulation of these NRPS/PKS transcripts and regulation of secondary metabolism in this harmful algal bloom species.

24. Cloning and expression of a caspase-3 candidate from the harmful algal bloom species, *Karenia brevis*.

Grace Bustamante, Emily A. Monroe

Department of Biology, William Paterson University of New Jersey, Wayne, NJ

Karenia brevis is the dinoflagellate responsible for harmful algal blooms off the coast of Florida. Blooms of *K. brevis* lead to fish kills, marine mammal mortalities, and negative human health impacts including Neurotoxic Shellfish Poisoning (NSP) and acute respiratory disease. Blooms can persist for months at a time until they eventually terminate; however, the molecular mechanisms involved in bloom termination remain poorly understood. There is evidence that *K. brevis* exhibits hallmarks of programmed cell death (PCD) including accumulation of reactive oxygen species (ROS), DNA fragmentation, and caspase activity, that may be involved in bloom termination. A caspase 3 candidate (KB-cas 3) has been identified and characterized from two *K. brevis* transcriptomes. The objectives of this study were to clone KB-cas 3 into an expression vector and overexpress KB-cas 3 in *E. coli* to measure caspase activity. The KB-cas 3 gene from the newest transcriptome was PCR amplified from *K. brevis* cDNA, cloned into a pET46 EK/LIC vector, and transformed into BL21 pLysS *E. coli* competent cells. KB-cas 3 protein expression was induced using IPTG, and total protein lysates were examined by SDS-PAGE to determine if KB-cas 3 was overexpressed. Preliminary analysis suggested an induced protein around 80 kDa consistent with the caspase protein size. To confirm the results, the experiments were repeated with an untransformed control. Analysis of total protein from transformed and untransformed samples revealed the protein of interest was not unique to the transformed sample. Western blots using an anti-His antibody confirmed the result that there are no unique proteins of interest expressed in the transformed sample suggesting the KB-cas 3 was not overexpressed. Future work will focus on troubleshooting the overexpression of KB-cas in *E. coli* and determining caspase activity of this caspase candidate. Characterization of a caspase protein in a dinoflagellate will increase our understanding of PCD processes in single-celled eukaryotes and may be useful in mitigation of *K. brevis* bloom events.

25. Reticulate evolution of begomoviruses.

J. Steen Hoyer[1], Alvin Crespo-Bellido[1], Divya Dubey[1], Yeissette Burgos-Amengual[1,2], Siobain Duffy[1]

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2. Department of Biology, University of Puerto Rico at Mayagüez

Begomoviruses, named for bean golden mosaic virus, have one or two genome segments, DNA-A and -B (single-stranded, 2.7 kb each). Multiple distantly related begomoviruses cause major losses in crops such as cassava (mosaic disease) and tomato (leaf curl and yellow leaf curl diseases). Mosaic disease of cassava caused a severe famine in regions of East Africa in the late 1990s and has recently spread to East Asia.

We have explored the phylogenetic history of the longest coding genes on the virion-packaged (V) and complementary (C) strand for both DNA-A (Crespo-Bellido *et al.* doi.org/MJGC) and DNA-B (Dubey et al. 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, a cluster of eleven monopartite sweetpotato-infecting viruses found across the world, and a recombinant, tomato latent virus. Analysis of the coat protein suggests possible co-evolution with whitefly vectors, particularly for a cluster found primarily in Africa. These results provide a framework for interpreting the likely spread of begomoviruses as climate change advances.

26. Structural and biophysical insights into echinocandin action in *Candida glabrata*.

Jennifer Jiang[1,2], Mikhail V. Keniya[3], Anusha Puri[1,2], Xueying Zhan[4], Jeff Cheng[1,2], Huan Wang[5], Yun-Kyung Lee[1,2], Nora Jaber[1,2,6], Zheng Shi[5], Sang-Hyuk Lee[7], Min Xu[4], David S. Perlin[3], Wei Dai[1,2]

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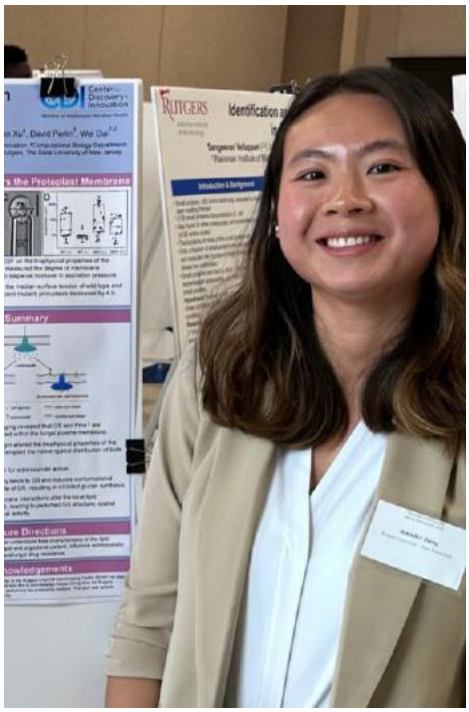
2. Institute for Quantitative Biomedicine, Rutgers, The State University of New Jersey, Piscataway, NJ
3. Hackensack Meridian Health-Center for Discovery and Innovation, Nutley, NJ
4. Computational Biology Department, Carnegie Mellon University, Pittsburgh, PA
5. Department of Chemistry and Chemical Biology, Rutgers, The State University of New Jersey, Piscataway, NJ
6. Graduate School of Biochemistry, Rutgers, The State University of New Jersey, Piscataway, NJ
7. Department of Physics and Astronomy, Rutgers, The State University of New Jersey, Piscataway, NJ

Fungal plasma membrane proteins are key therapeutic candidates for antifungal agents, yet they remain poorly characterized in their native environment. Here, we employ a multimodal integrative approach to elucidate the structure and functional organization of plasma membrane protein complexes in *Candida glabrata*, focusing on the polysaccharide synthase β-(1,3)-glucan synthase (GS) and the H^+-ATPase Pma1. Cryo-electron tomography (cryo-ET) and live cell imaging reveal that GS and Pma1 are heterogeneously distributed into distinct plasma membrane microdomains. Treatment with caspofungin, an echinocandin antifungal that targets GS, modifies the biophysical properties of the plasma membrane and disrupts the native disruption of both GS and Pma1. Based on these findings, we propose a model that considers how echinocandins modify the lipid microenvironment, which in turn disrupts the native spatial distribution of embedded membrane proteins. Our work emphasizes the importance of interrogating the structural and dynamics of fungal plasma membrane proteins in situ to understand their spatial organization and inform the rational development of novel antifungal therapies.



27. Enriching biofilms for effective biodegradation of commingled emerging contaminants: optimal inoculation strategy and microbial community analysis.

Chao Li, Mengyan Li

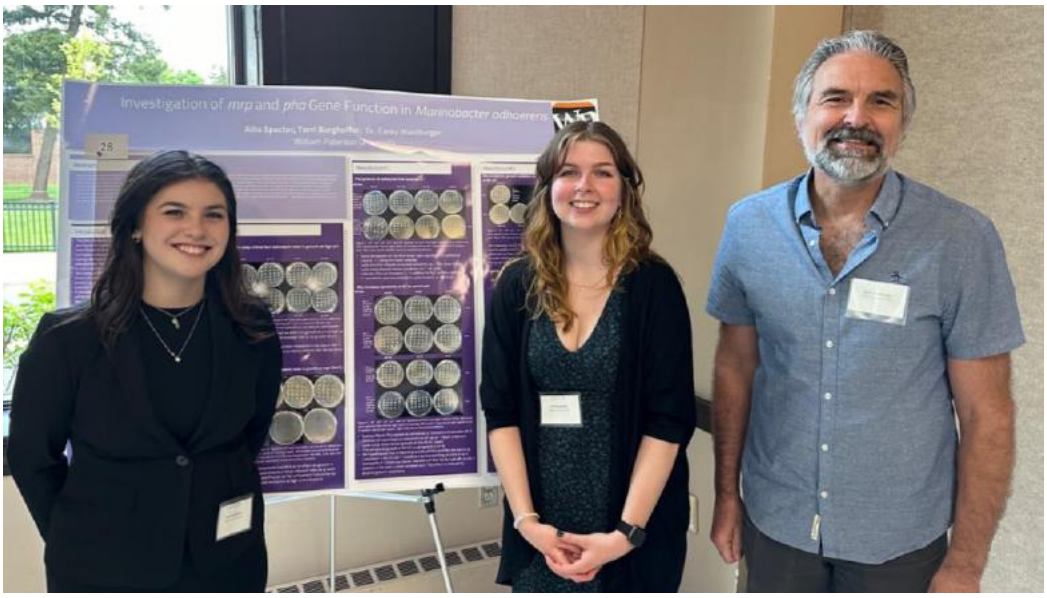
Department of Chemistry and Environmental Science, New Jersey Institute of Technology, Newark, NJ

Contaminants of emerging concern (CECs) have aroused growing public attentions due to widespread occurrence, high recalcitrance, and potential health effects. In this study, biofilms were enriched by different activated sludge inoculums, to assess the impacts of inoculating sludges and carbon supplementation on select CECs, spanning from pesticides (aminotriazole [AMT], atrazine [ATZ] and N, N-diethyl-meta-toluamide [DEET]), antibiotics (sulfamethoxazole [SMX] and trimethoprim [TMP]), to pharmaceutical and personal care products (carbamazepine [CBZ] and lidocaine [LDC]). Average removal of 87% DEET, 71% SMX, and 60% TMP were achieved in Phase I by consortia of suspended biomass and biofilms, and with removal of 50% CBZ, 58% DEET, and 59% SMX by biofilms in Phase II. In contrast, minimal removal of ATZ was observed throughout the operation, indicating its high recalcitrance. Inoculum combinations of L, PL, and RL showed better removal as the consortia containing suspended biomass and biofilm, whereas biofilms enriched from individual R and P are the best on comprehensive CEC removal. Multi-dimensional correlation consensus revealed potential SMX degraders (*Nitrospira* and *Kouleothrix*), TMP degrader (*Caulobacter*), DEET degraders (*Alcanivorax*, *Bacillus*, *Reyranella* and *Caulobacter*), AMT degraders (*Paenibacillus*, *Sphingomonas*, A4b, *Ferruginibacter* and *Mucilaginibacter*) and CBZ degraders (*Labrys* and *Methylophilaceae*). Members of these genera have been reported with relevant CEC degradation abilities or contain genes involved in CEC biodegradation. Abundances of potential degraders found in biofilms explain compounds removal in Phase II and further support the statistical results, also suggesting R benefits accumulation of biofilm degraders of AMT, CBZ, SMX and DEET. L promotes enrichment of DEET, TMP and SMX degraders. And P is conducive for enriching biofilms degraders of SMX and DEET.

28. Examination of the roles of the mrp and pha genes in the marine bacterium *Marinobacteria adhaerens*.

Allie Spector, Torri Burghoffer, Carey Waldburger

Dept. of Biology, William Paterson University, Wayne, NJ



Karenia brevis is a toxic dinoflagellate that forms blooms in the Gulf of Mexico, harming the surrounding ecosystem. *Marinobacter adhaerens* are gram negative bacterial marine community members with *K. brevis* and were isolated from laboratory cultures of *K. brevis*. A bioinformatic analysis revealed a high content of sodium transport clusters of orthologous groups (COGs) and additional evaluation of the sodium transport-related COGs led to the identification of genes that encode for mrp (multi resistance and pH) and pha genes (pH adaptation) that occur in neighboring loci in the bacterial chromosome. The mrp and pha genes belong to a family of antiporters that have been shown to play roles in pH homeostasis, resistance to elevated levels of various cations, pathogenesis, and biofilm formation in a variety of bacteria. The tandem arrangement is uncommon and published studies of genes in this arrangement are lacking, giving us an opportunity to carry out novel research to explore the roles of these antiporters in *M. adhaerens*. We have made knockout variants for both the mrp and pha genes individually as well as a double deletion through the use of suicide vectors constructed for this purpose. Studies to be presented here show that there is significant redundancy of function with respect to pH homeostasis. However, specificity of the monovalent cation that is pumped varies as Mrp transports Na^+ and K^+ while Pha transports Na^+ and Li^+. We will also present RT-qPCR results of the effects of high pH and salt concentration on the expression of the mrp and pha genes.

29. Determining how itaconic acid modulates *Staphylococcus aureus* physiology.

Nupur Tyagi[1], Tania Wong Fok Lung[2], Jeffrey M. Boyd[1]

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

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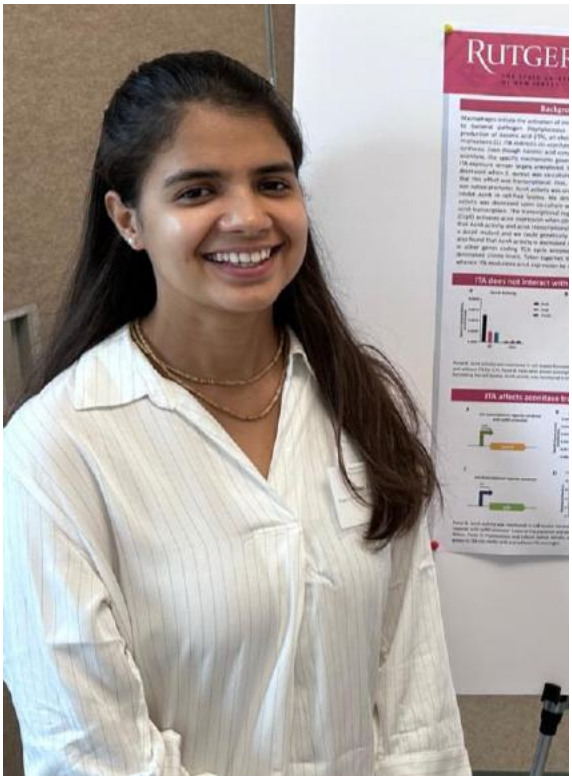


Macrophages initiate the activation of immune responsive gene 1 in response to bacterial pathogen *Staphylococcus aureus* infection, leading to the production of itaconic acid (ITA), an electrophilic metabolite with significant implications. ITA redirects cis-aconitate from the TCA cycle to fuel its own synthesis. Even though itaconic acid competes with aconitase substrate, cis-aconitate, the specific mechanisms governing aconitase (AcnA) response to ITA exposure remain largely unexplored. We observed that AcnA activity was decreased when *S. aureus* was co-cultured with ITA. Two results suggested that this effect was transcriptional. First, when acnA was expressed using a non-native promoter, AcnA activity was unaffected by ITA. Second, ITA did not inhibit AcnA in cell-free lysates. We determined that acnA transcriptional activity was decreased upon co-culture with ITA suggesting that ITA effects acnA transcription. The transcriptional regulator catabolite control protein E (CcpE) activates acnA expression when citrate is present. We determined that AcnA activity and acnA transcriptional activity was not affected by ITA in a ΔccpE mutant and we could genetically complement the phenotypes. We also found that AcnA activity is decreased in *S. aureus* that contain mutations in other genes coding TCA cycle enzymes, which might be the result of diminished citrate levels. Taken together, these findings have led to a model wherein ITA modulates acnA expression by interfering with CcpE regulation.

30. Zebrafish together with CRISPR / Cas9 and reporter vectors, act as efficient genome editing tool, for knocking in and knocking out of simple sequences repeats.

Alshymaa Yusef Hassan

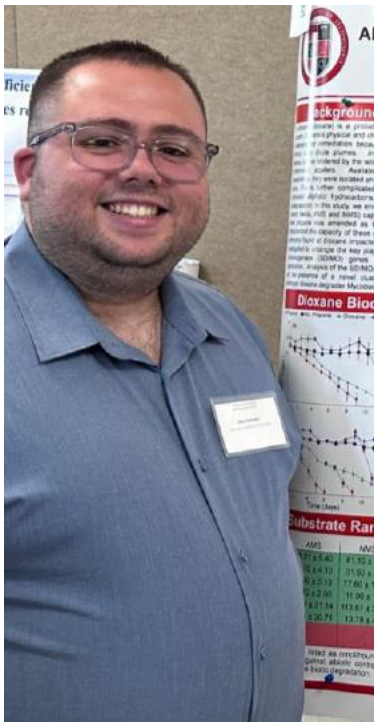
Molecular Biology Program, Seton Hall University, South Orange, NJ

The zebrafish, *Danio rerio*, serves as a model for gene function studies since humans share 70% of the zebrafish's genes. More than 80% of the genes linked to human disease were found. What takes a human embryo a month to develop, a zebrafish does in a single day. In fish, genes can also be directly turned on or off. Zebrafish are used in a variety of studies. Gene engineering technology known as CRISPR/Cas9, or clustered regularly interspaced short palindromic repeats, holds significant promise for the production of plants, cell lines for cancer treatment, and the repair of tragically damaged genes in human embryos. Gene editing capabilities of CRISPR Cas9 can be extended to complex cells and species, including eukaryotes. According to genome sequence research, non-coding DNA contains microsatellite repeats (SSRs), which are repeating DNA segments that repeat specific DNA motifs five to fifty times on average. The polymorphism/variation in the SSRS resulted from uneven recombination events in the chromosome and/or DNA polymerase slippage. They exhibit greater genetic variety because of their higher mutation rate compared to other regions of DNA. Simple sequence repeats are becoming a popular choice for markers in fish genetic studies because of their high polymorphism, co-dominant nature, abundance in the genome, and capacity for reproduction. In fish genetic research, these markers have been used to reveal genetic variability, strain and species identification, the creation of genetic linkage maps, and parentage assignment.

31. Cometabolic 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.

Jose Manuel Diaz Antunes, Mengyan Li

Dept. of Chemistry and Environmental Science, New Jersey Institute of Technology, Newark, NJ



Soluble di-iron monooxygenases (SDIMO) are central to bioremediation of many organic pollutants. Bioprospecting efforts combined with advancing molecular techniques have led to the discovery of various SDIMOs. As more SDIMOs have been discovered, their potential uses have also expanded. 1,4-Dioxane (dioxane) is of particular interest as water contaminant of emerging concern given that it is a probable human carcinogen, easily spreads in the environment, and is impervious to classic remediation methods. With the increasing regulation of dioxane levels in local water supplies in New Jersey and many other states, there exists an increasing need to cope with dioxane contamination effectively and cost-efficiently. Current chemical and physical treatment methods are both expensive and invasive as they require harsh conditions to oxidize dioxane or concentrate dioxane with synthetic adsorbents. Biological methods provide an effective and cost-efficient solution, but bioaugmentation efforts are hindered by the temperature incompatibility of current lab isolates >25^{o}C with ground water temperatures (≤14^{o}C).

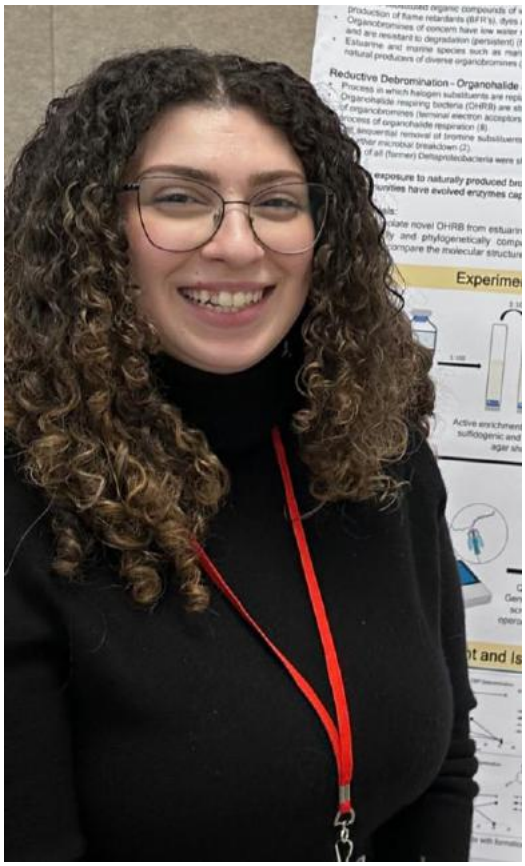
Group-6, propane monooxygenase (prm) PRM are critical dioxane degrading enzymes, capable of more rapid dioxane degradation with less chlorinated solvent inhibition, a broader substrate range and higher affinity for dioxane than the archetypic Group-5 tetrahydrofuran monooxygenase (thm). These Group-6 SDIMOs, however, are fairly new and broadly understudied. Group-6 prm have recently been identified in microbes with highly favorable characteristics. Particularly, in microbes capable of overcoming most chlorinated aliphatic hydrocarbon (CAH) inhibition of dioxane degradation and in those capable of growing at cold temperatures that are aquifer relevant (4~14^{o}C). Further analysis of group-6 SDIMOs reveals divergence within the group, showing three distinct clusters. These clusters exhibit different physiological characteristics. Furthermore, we have enriched two consortia capable of degrading dioxane when amended with propane at 14^{o}C which contain a high abundance of SDIMOs of high identity to Group-6. Additionally, recent studies have highlighted the potential for propane biosparaging for the promotion of dioxane degradation.

In this study, our overarching goal is to further characterize and understand propane monooxygenase (prm) from Group-6 SDIMOs, particularly in the context of environmental remediation of water pollutants, specifically dioxane. Combining conventional enrichment techniques with amplicon-based sequencing and SDIMO biomarkers, we will be able to uncover the SDIMOs involved in dioxane degradation at aquifer relevant temperatures of 14 ^{o}C. Resting-cell assays were used to uncover the capacity of these consortia to tackle cooccurring CAHs, while bioinformatics and data mining were employed to uncover key distinctions between the novel clustering within group-6. Overall, this work will assess the characteristics of Group-6 prm, particularly, as key tools in dioxane biodegradation.

32. Unraveling the diversity of organobromine-respiring bacteria: enrichment and isolation of novel *Desulfovibrionaceae* and *Desulfuromonadaceae* species from estuarine sediment.

Chloe Costea[1], Niveda Thuravil[1], Lee J. Kerkhof[2], Max M. Häggblom[1]

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



Organobromines are a significant group of chemical pollutants in the environment due to their known toxicity and persistent nature. Due to the size of the bromine substituent and high degree of halogenation, these contaminants can be resistant to microbial degradation. Microbial reductive debromination offers a promising approach for the detoxification of these compounds by removing their halogen substituents, thereby exposing their carbon skeleton for further breakdown. This process can be coupled with the oxidation of electron donors in an energy yielding process known as organohalide respiration. This study explored the diversity of organohalide-respiring bacteria (OHRB) by targeting different reductive dehalogenases by using brominated phenol and benzoate compounds to enrich and isolate novel OHRB from estuarine sediments. In addition, the impact of environmental conditions, such as sulfate and salinity levels, historical contaminant exposure, and geographic location, were investigated by including sediment samples from multiple sites in New Jersey and the Mediterranean island of Cyprus. All sediment cultures showed debromination activity on all compounds tested, which was maintained in subsequent enrichment cultures with the organobromines as the sole electron acceptor. While sulfate has been found to be a strong inhibitor of reductive dichlorination, in contrast, reductive debromination was not affected when cultures were provided sulfate as an alternative electron acceptor. Novel debrominating species were isolated from different sites after enrichment on 2,4-dibromphenol, 2,6-dibromophenol, and bromoxynil and identified as members of the *Desulfovibrionaceae* and *Desulfuromonadaceae* families. The wide variety of sulfate reducing bacterial species identified as capable of reductive debromination, suggests that the capability for reductive debromination is widely distributed. Further genetic analysis of novel debrominating bacterial species, will provide insights into the phenotypic and genotypic diversity of organobromine respiration, enhancing our ability to implement bioremediation techniques for cleanup of organohalogen contaminated sites.

33. Novel cryophiles from Arctic and Antarctic soils.

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

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

Cryophiles are well-adapted natives of the planet's Arctic and Antarctic biomes. Microorganisms that live in permafrost or seasonally frozen environments have adapted all aspects of their biological functions in order to survive and maintain metabolic activity at sub-zero temperatures. While there has been significant progress in the study of cryophilic microbes, the impact of these under-studied microorganisms on carbon and nitrogen cycling is not well understood and presents a substantial knowledge gap for accurate climate modeling. Studying microbial life that exists and thrives at temperatures below the freezing point of water, in subzero conditions, presents a significant challenge. This project aims to partner cultivation of representative cryo-active species with ribosomal rRNA amplicon sequencing of the community in order to gain a deeper understanding of polar soil microbiomes and how the communities will respond to a changing climate. The subzero active community members are targeted using novel culturing techniques through the use of osmotic pressure, to emulate the water activity at subzero temperatures, selective enrichment and isolation of potential subzero active bacteria. One major benefit of this approach is culturing at temperatures above 0°C allowing for higher growth rates and cultivation. Enrichment and isolation of cryophilic organisms has been achieved using these techniques with soil from Arctic tundra and the Antarctic. Enrichment cultures and isolates growing at 4 °C have been screened for novel strains of interest by rRNA operon sequencing. Several potentially new species having been identified, with full genome sequencing of representative strains for detailed characterization of cry-adaptation. Two novel *Pseudomonas* strains, most closely related to the known ice-nucleating *Pseudomonas borealis*, were enriched from Finnish tundra soil. An Antarctic soil isolate was obtained from high osmotic soil enrichments, with a remarkably rapid growth rate at 4°C. This study underscores the importance of combining targeted enrichment/cultivation with molecular community analysis for a deeper understanding of the sub-zero active polar microbiome.

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

Joshua Chamberlain[1,2,5], Julie Griepenburg[1,3], and Eric Klein[1,2,4,5]

1. Center for Computation and Integrative Biology, Rutgers University, Camden, NJ
2. Rutgers Center for Lipid Research, Rutgers University, New Brunswick, NJ
3. Department of Physics, Rutgers University, Camden, NJ
4. Department of Biology, Rutgers University, Camden, NJ
5. Theobald Smith Society, https://njmicrobe.org

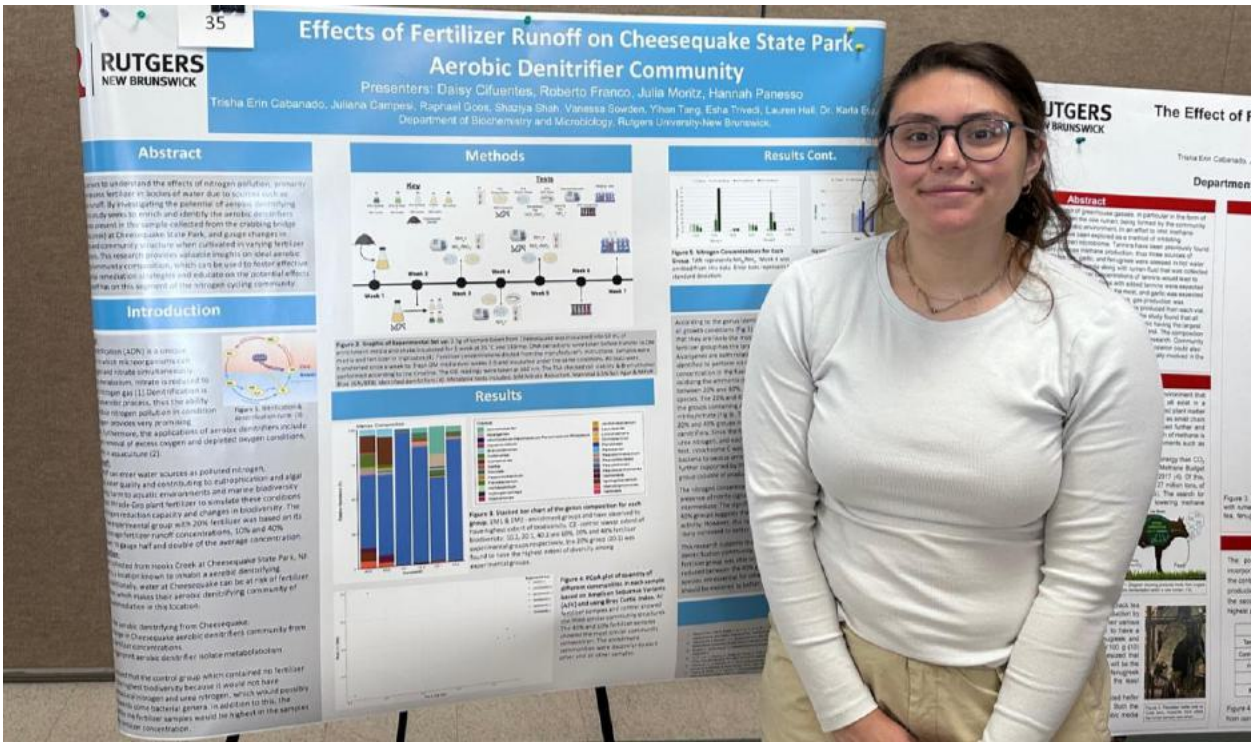
Liposomal drug delivery has been at the forefront of nanoparticle drug delivery systems for nearly 30 years. These liposomes and their components, including lipids, must be highly characterized to provide the ideal conditions for effective drug delivery. Among the commonly used lipids in liposome formation 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).

35. Effects of fertilizer runoff on Cheesequake State Park aerobic denitrifier community.

Hannah Panesso, Daisy Cifuentes, Roberto Franco, Julia Moritz, Trisha Erin Cabanado, Juliana Campesi, Raphael Goos, Shaziya Shah, Vanessa Sowden, Yihan Tang, Esha Trivedi, Lauren Hall, Karla Esquilín-Lebrón

Department of Biochemistry and Microbiology, Rutgers University

This study strives to understand the effects of nitrogen pollution, primarily focusing on excess fertilizer in bodies of water due to sources such as agricultural runoff. By investigating the potential of aerobic denitrifying bacteria, this study seeks to enrich and identify the aerobic denitrifiers (ADN) species present in the sample collected from the crabbing bridge area (Hooks creek) at Cheesequake State Park, and gauge changes in biodiversity and community structure when cultivated in varying fertilizer concentrations. This research provides valuable insights on ideal aerobic denitrifying community composition, which can be used to foster effective environmental remediation strategies and educate on the potential effects fertilizer runoff has on this segment of the nitrogen cycling community.

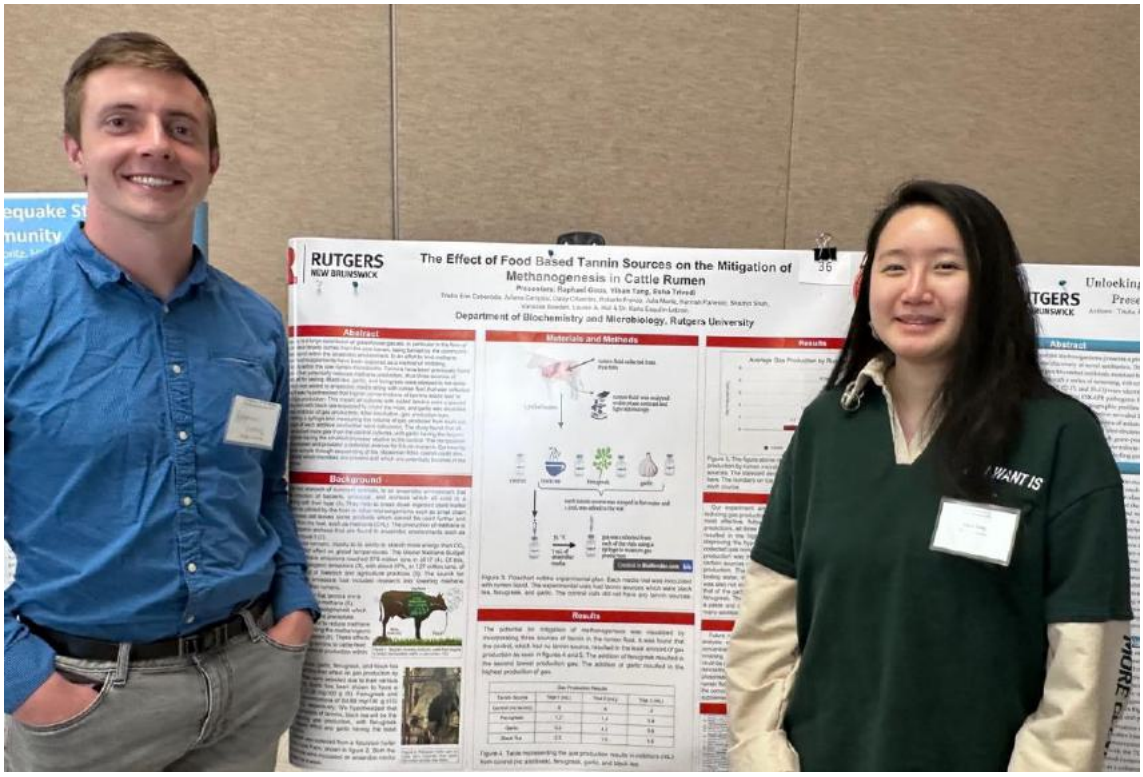


36. Unlocking nature's antibiotic potential: isolation and characterization of soil microbes.

Juliana Campesi, Vanessa Sowden, Shaziya Shah, Trisha Erin Cabanado, Daisy Cifuentes, Roberto Franco, Raphael Goos, Julia Moritz, Hannah Panesso, Yihan Tang, Esha Trivedi, Lauren A. Hall, Karla Esquilin-Lebrón

Department of Biochemistry and Microbiology, Rutgers University

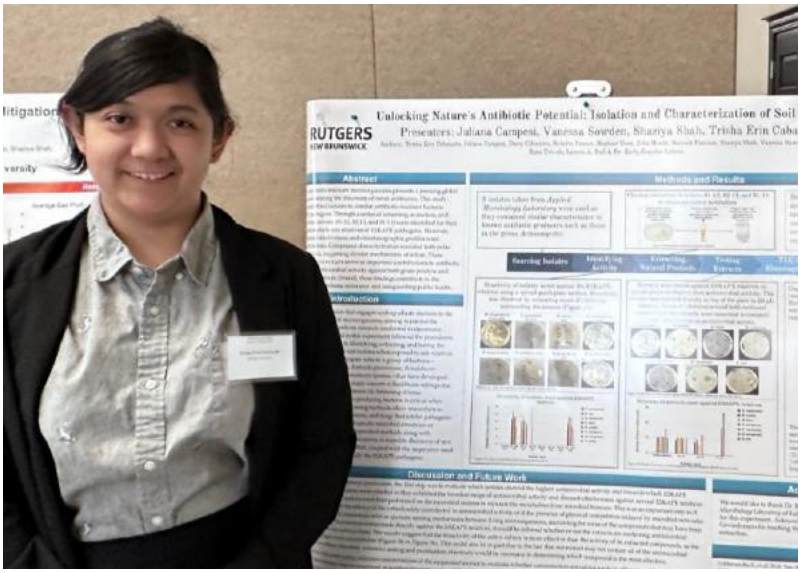
The emergence of antibiotic-resistant microorganisms presents a pressing global health challenge, necessitating the discovery of novel antibiotics. This study explores the potential of soil isolates to combat antibiotic-resistant bacteria known as ESKAPE pathogens. Through a series of screening, extraction, and testing processes, three isolates (01-12, 02-13, and 01-11) were identified for their effectiveness against multiple safe relatives of ESKAPE pathogens. However, variations in compound effectiveness and chromatographic profiles were observed among the isolates. Compound characterization revealed both polar and nonpolar compounds, suggesting diverse mechanisms of action. These isolates and their respective extracts serve as important contributions to antibiotic research as they exhibit antimicrobial activity against both gram-positive and gram-negative ESKAPE relatives. Overall, these findings contribute to the broader goal of combating antibiotic resistance and safeguarding public health.



37. The effect of food based tannin sources on the mitigation of methanogenesis in cattle rumen.

Raphael Goos, Yihan Tang, Esha Trivedi, Trisha Erin Cabanado, Juliana Campesi, Daisy Cifuentes, Roberto Franco, Julia Moritz, Hannah Panesso, Shaziya Shah, Vanessa Sowden, Lauren A. Hall, Karla Esquilín-Lebron

Department of Biochemistry and Microbiology, Rutgers University



The dairy industry is a large contributor of greenhouse gasses, in particular in the form of methane. This methane largely comes from the cow rumen, being formed by the community of microorganisms found within the anaerobic environment. In an effort to limit methane production, natural food supplements have been explored as a method of inhibiting methanogenic activity within the cow rumen microbiome. Tannins have been previously found to be a feed additive that potentially reduces methane production; thus three sources of tannins were selected for testing. Black tea, garlic, and fenugreek were steeped in hot water to make a "tea", which was added to anaerobic media along with rumen fluid that was collected from a heifer cow. It was hypothesized that higher concentrations of tannins would lead to larger inhibition of gas production. This meant all cultures with added tannins were expected to inhibit gas production with black tea expected to inhibit the most, and garlic was expected to cause the lowest inhibition of gas production. After incubation, gas production was determined by inserting a syringe and measuring the volume of gas produced from each vial, and then the average of each additive production was calculated. The study found that all variable cultures produced more gas than the control cultures, with garlic having the largest increase and fenugreek having the smallest increase relative to the control. The composition of the gasses was unknown and provided a potential avenue for future research. Community analysis of the rumen sample through sequencing of the ribosomal rRNA operon could also be performed to identify which microbes are present and which are potentially involved in the production of methane.

Society President-Elect Jennifer Sun, Treasurer and Past President Valerie Carabetta, Branch Councilor and Past President (2-terms) Jeff Boyd, invited speaker Napur Tyagi (Boyd Lab) and poster presenter Navitri Naidu (Boyd Lab) at the symposium.

2016 Waksman Lecture Awardee Bradley Hillman and 2023 Young Investigator Awardee Dana Price enjoying the symposium.

Adrianna Borgia and Joe Schwartz from Big ASM were on-hand to support the symposium providing advice to budding authors, as well as prospective reviewers and editors of world-class ASM journals.