Theobald Smith Society
The NJ Branch of the American Society for Microbiology
www.njmicrobe.org

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Fall 2023 Symposium Proceedings

27 October 2023

Bethany Hall

Seton Hall University, South Orange, NJ

Opening remarks by Gregory Wiedman, President, Theobald Smith Society Greg serves as the 82nd president of the Theobald Smith Society. He earned his PhD in Material Science at Johns Hopkins and completed post-doctoral research at Rutgers University Public Health Research Institute. Greg is Assistant Professor at Seton Hall University and advises the 3B Laboratory (Biochemistry, Biophysics, and Biomaterials). The lab studies drug synergy to develop new methods to treat infectious diseases. They use a range of techniques to achieve this goal from Analytical Chemistry (HPLC, Mass Spec, Impedance Spectroscopy, Circular Dichroism, and more!) The 3B Lab creates new therapeutic molecules using both combinatorial approaches (using Peptide Screens, SELEX) as well as rational design (using crystal structural analysis, NMR, modeling). Furthermore The 3B Lab is actively training students and members in cell culture techniques (fungal, bacteria, mammalian) to help facilitate interdisciplinary work.

Jennifer A. Leeds, PhD, American Society for Microbiology Distinguished Lecturer and Waksman Foundation Lecturer

"Challenges and Opportunities in Antibacterial Drug Discovery: a personal perspective."

Jennifer A. Leeds has spent most of her pharmaceutical career at Novartis, where she and her team discovered a potentially novel treatment for infections caused by *Clostridioides difficile* and a novel monobactam antibiotic. Leeds transitioned to corporate and business development, where she heads search and evaluation for Novartis on the West Coast of the U.S. and across Canada, leading in- and out-licensing deals and equity investments in biotech and serving on start-up boards. Leeds currently serves on the ASM Finance Committee and co-chairs the AAR/Drug Discovery Task

Force, working with ASM to re-engage applied microbiologists and the antimicrobial resistance community. She has served on NIH study sections, on several editorial boards and as an ASM Journals reviewer. Leeds is known for her service as a lecturer and mentor to dozens of students, postdoctoral fellows, faculty and industrial scientists. Leeds served on the Scientific Selection Board of the Novo Ventures REPAIR Fund, an all-antibiotic investment vehicle, and has advised several antibacterial start-up ventures. Leeds is a member of the Cornell College of Agriculture and Life Sciences Alumni Board of Directors, as well as a member of the President's Council of Cornell Women.

Dana Price, PhD, Young Investigator Award Recipient

"Genomic epidemiology and the New Jersey vector-borne disease landscape."

Dana Price is Associate Research Professor in the Department of Entomology, School of Environmental & Biological Sciences, Rutgers University. His research focuses on functional genomic analyses of vector arthropods and their holobiont - that is, the host and assemblage of commensal organisms (viruses, bacteria and eukaryotes) that live within and around them. His current research initiatives include: novel means of rapid and nondestructive vector-borne pathogen sampling and discovery, reverse-vaccine development targeting invasive arthropods, tick-borne disease dynamics and discovery in a rapidly changing landscape, and genotyping and spatiotemporal distribution of arthropod-borne viruses.

Invited Speakers

Truman Dunkley, Rutgers University, Camden, NJ "Playing with power: modulating ATP production in *E. coli*."

Liya Popova, Cooper Medical School of Rowan University, Camden, NJ "The regulation of gene expression by acetylated forms of HBsu during sporulation in *Bacillus subtilis*."

Bala Madduri, Rutgers New Jersey Medical School, Newark, NJ "Characterizing how *Mycobacterium tuberculosis* secreted effector proteins PE25 and PPE41 disrupt macrophage cell biology

Lylla Almosd, Rutgers University, New Brunswick, NJ "Microbial source tracking technologies to identify sources of fecal contamination in the Raritan River, NJ."

Adriana Machado, New Jersey City University, Jersey City, NJ "Antibiofilm and antimicrobial properties of *Schinus terebinthifolia* fruit extract."

POSTERS

J. Steen Hoyer[1], Anna E. Dye[2], Cyprian A. Rajabu[2,4], Charles Tunje Chiro[3], Alvin Crespo-Bellido[1], Divya Dubey[1], David O. Deppong[2], Evangelista Chiunga[2,4], Brenda Muga[5], Justin Koesterich[1], Paul E. Labadie[2], Paul Kuria[3], Ignazio Carbone[2], José Trinidad Ascencio-Ibáñez[2], Linda Hanley-Bowdoin[2], Joseph Ndunguru[4], Elijah M. Ateka[5], Siobain Duffy[1].

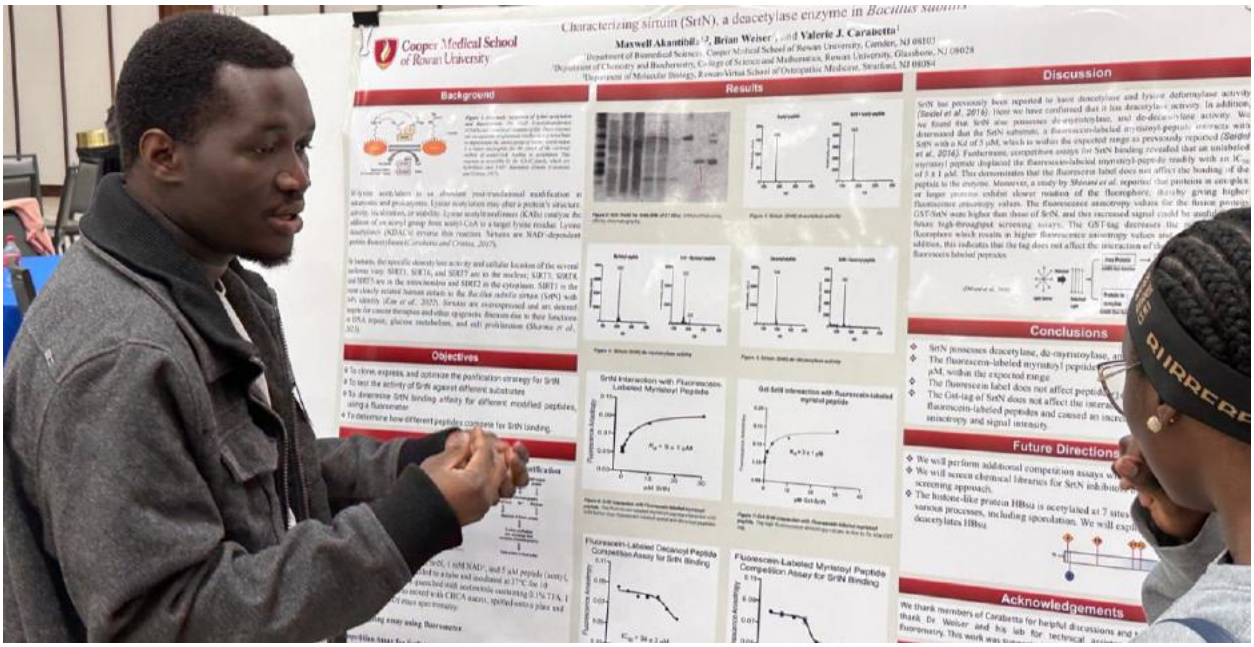
1. Rutgers University, New Brunswick NJ, USA
2. North Carolina State University, Raleigh NC, USA
3. Kenya Agricultural and Livestock Research Organisation, Mtwapa and Nairobi, Kenya
4. Tanzania Agricultural Research Institute, Dar es Salaam, Tanzania
5. Jomo Kenyatta University of Agriculture and Technology, Nairobi, Kenya

1. Characterization of the sirtuin SrtN in *Bacillus subtilis*.

M. Akantibila[1,2], V, J. Carabetta[2]

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

Nε-lysine acetylation is an abundant post-translational modification in eukaryotes and prokaryotes. Lysine acetylation may alter a protein's structure, activity, localization, or stability. Lysine acetyltransferases catalyze the addition of an acetyl group from acetyl-CoA to a target lysine residue. Deacetylases reverse this reaction. Sirtuins are NAD+-dependent protein deacetylases. In humans, the specific deacetylase activity and cellular location of the several isoforms vary. Sirtuins are desired targets for cancer therapies and other epigenetic diseases due to their functions in DNA repair, glucose metabolism, and cell proliferation. Here, we characterized a bacterial sirtuin (SrtN) in *Bacillus subtilis*. We successfully cloned, expressed, and purified SrtN for biochemical analysis. Next, we performed an enzyme assay to investigate its substrate specificity using MALDI-MS, and determined that is has deacetylase, demyristoylase, and dedecanoylase activity. Next, we used different labeled peptides in binding assays to determine their affinities with SrtN, to potentially develop a screening tool. We found that 75 nM of the fluorescently labeled peptide "FAM-PEG4- H4K16(myr)" binds with a Kd of 5 µM. Furthermore, we carried out competition assays with unlabeled myristoyl and decanoyl peptides and determined their IC50 values to be 5 µM and 34 µM, respectively. In conclusion, the decanoyl peptide have ~10-fold lower affinity for SrtN compared to the myristoyl peptide. Future work will be to carry out additional competition assays and screen chemical libraries for SrtN inhibitors using a high-throughput screening approach.

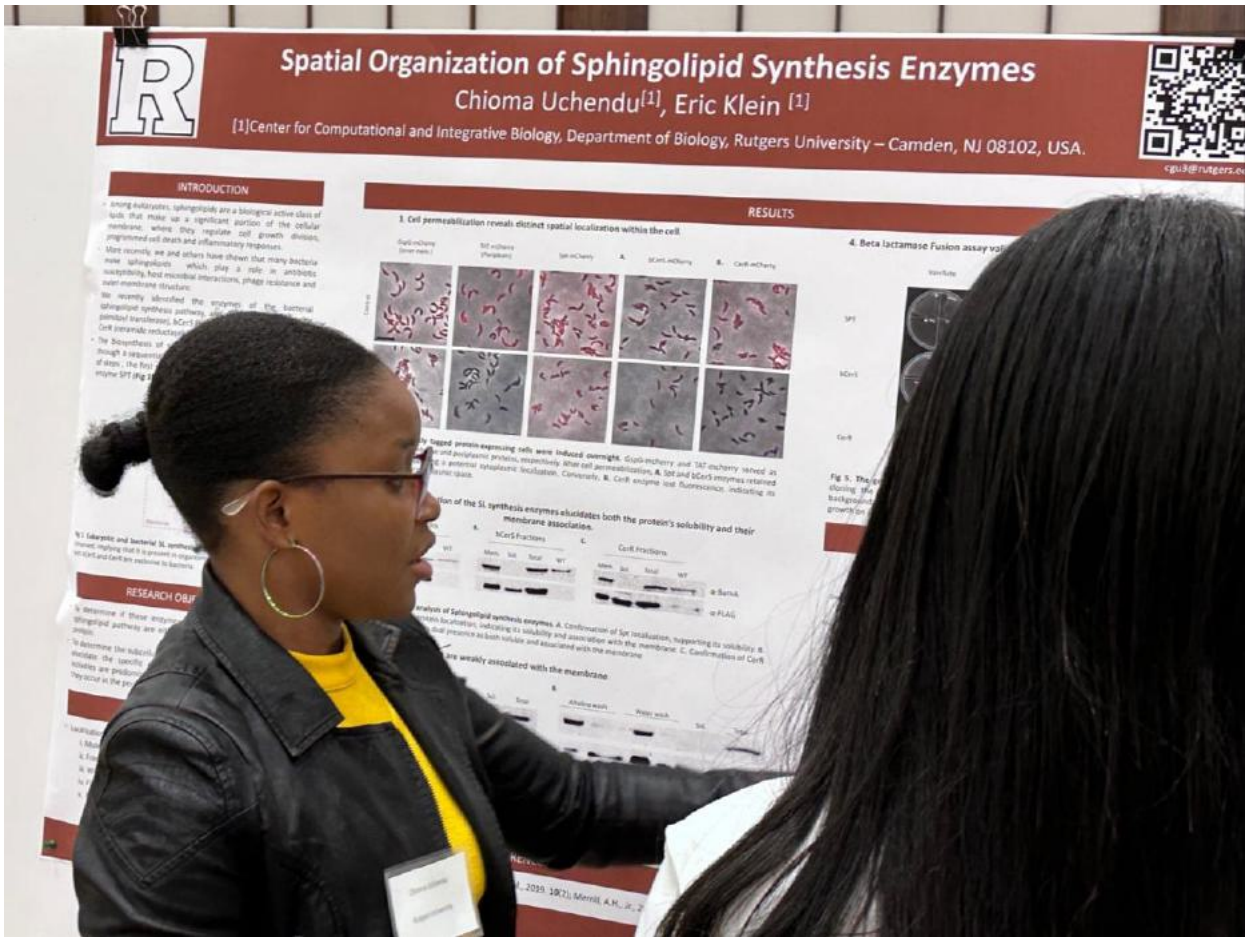


2. Spatial organization of sphingolipid synthesis enzymes.

Chioma Uchendu[1], Eric Klein[1,2]

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

Within the diversity of eukaryotes, sphingolipids make up a peculiar class of lipid dynamics that constitute a major portion of cellular membranes. Their significant role in cellular processes such as cell growth, division, programmed cell death, angiogenesis and inflammatory responses are prevalent. Intriguingly, recent study has unveiled the presence of sphingolipids in tons of bacterial species, introducing a novel context on their dynamism. These bacterial sphingolipids have been implicated in varied functions such as antibiotic susceptibility, host-microbial interplay, phage resistance, and modulation of outer membrane system. While our understanding of sphingolipid synthesis in eukaryotes is robust, the homologous pathway in bacteria remains ambiguous. A recent advance in our research has led to the identification of key enzymes that are involved in bacterial sphingolipid biosynthesis. Among these, the initial catalytic step is carried out by serine palmitoyl transferase, a conserved enzyme shared across both eukaryotic and prokaryotic domains. Notably distinct to bacteria, two additional enzymes namely bacterial ceramide synthase and ceramide reductase. Moreover, subcellular localization analysis indicates that these genes are spatially separated.

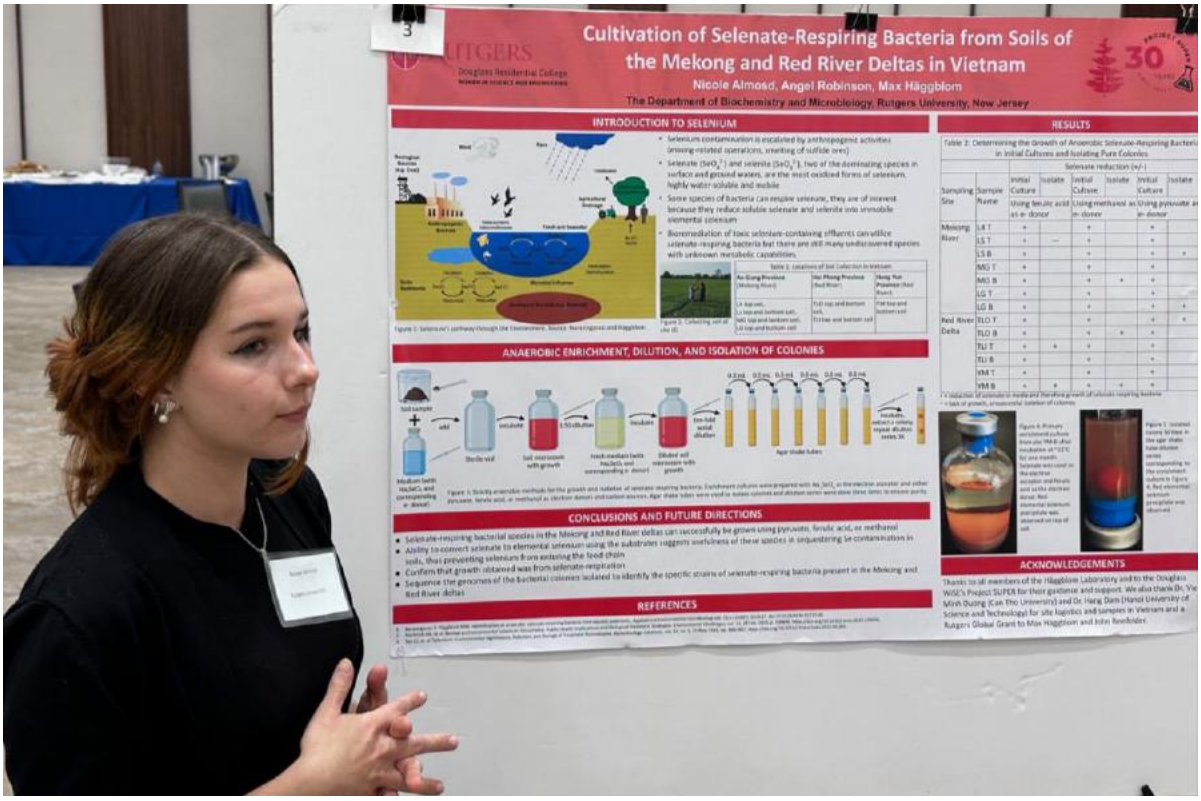


3. Cultivation of selenate-respiring bacteria from soils of the Mekong and Red River deltas in Vietnam.

Nicole Almosd, Angel Robinson, Max M. Häggblom

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

High concentrations of selenium in aquatic environments are toxic to plants and animals. Selenate-respiring bacteria can be used to precipitate and sequester selenium in soils for bioremediation. The objective of this study is to demonstrate the activity of anaerobic selenate-respiring bacteria from the Mekong and Red Rivers and isolate them for characterization. Selenate-respiring activity was observed in the original cultures due to the appearance of red elemental selenium precipitate. The cultures were enriched by transfer to fresh media and selenate-reducing bacteria were isolated with pyruvate, methanol, or ferulic acid as the electron donor and 10 mM selenate as the electron acceptor.



4. Antibiofilm and antimicrobial properties of *Schinus terebinthifolia* fruit extract.

Adriana Machado, Jerry Louis, Meriem Bendaoud

Dept. of Biology, New Jersey City University, Jersey City, NJ

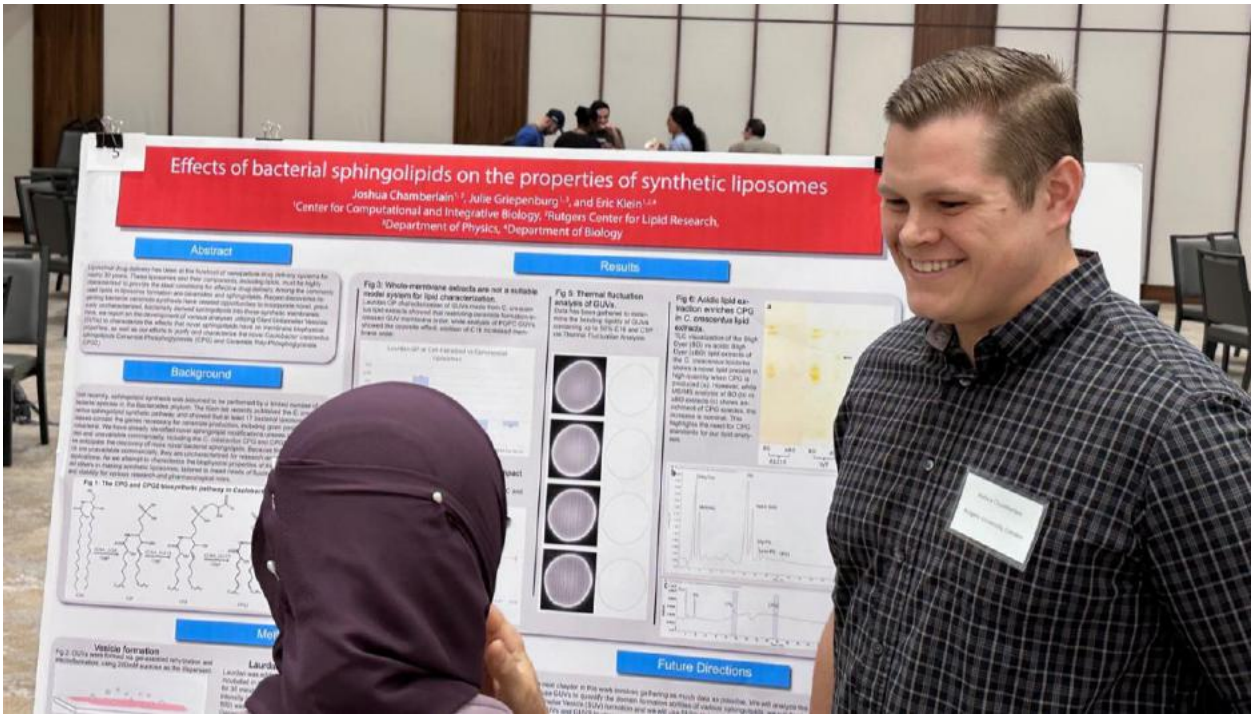
In the past few decades, the misuse and overuse of antimicrobials has led to an increase in the number of antimicrobial resistant infections, which according to the World Health Organization, has become a major worldwide concern and a threat to public health. The need to develop new therapeutic alternatives to address the ineffectiveness of conventional antimicrobial treatments is crucial. The rising field of phytotherapy offers an efficient approach to addressing this global health crisis. In this study, we investigate the *Schinus terebinthifolia* plant fruit extract as a potential low-cost alternative to antibiotics. This highly available plant is widely used in gastronomy and known in folk medicine for its wound healing and health-promoting properties. In this study, we evaluate the plant's fruit extract antimicrobial and antibiofilm properties against 22 different strains of bacteria and fungi using the broth microdilution, biofilm, and spot assays in microtiter plates. The results show that the fruit extract has a significant antibacterial effect on several gram-positive and gram-negative pathogenic bacteria including *Staphylococcus aureus, Enterococcus faecalis, Pseudomonas aeruginosa, Acinetobacter baumannii, Bacillus subtilis*, *Bacillus cereus, Escherichia coli*, and *Staphylococcus epidermidis*. In addition, the plant extract displays varying degrees of antibiofilm properties at different concentrations against bacteria and fungi. These findings suggest that the *S. terebinthifolia* fruit extract has the potential to be used as a novel antimicrobial alternative in the treatment of infectious diseases. Future studies will focus on further characterization of the fruit extract.

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

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

1. Center for Computational 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

The research field of drug delivery has been pursuing liposomal 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. Some key features include membrane permeability, fluidity, and stability. 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 characterization of the bacterial sphingolipids and ceramides native to the *Caulobacter crescentus* lipidome and quantify their effects on membrane permeability and fluidity.

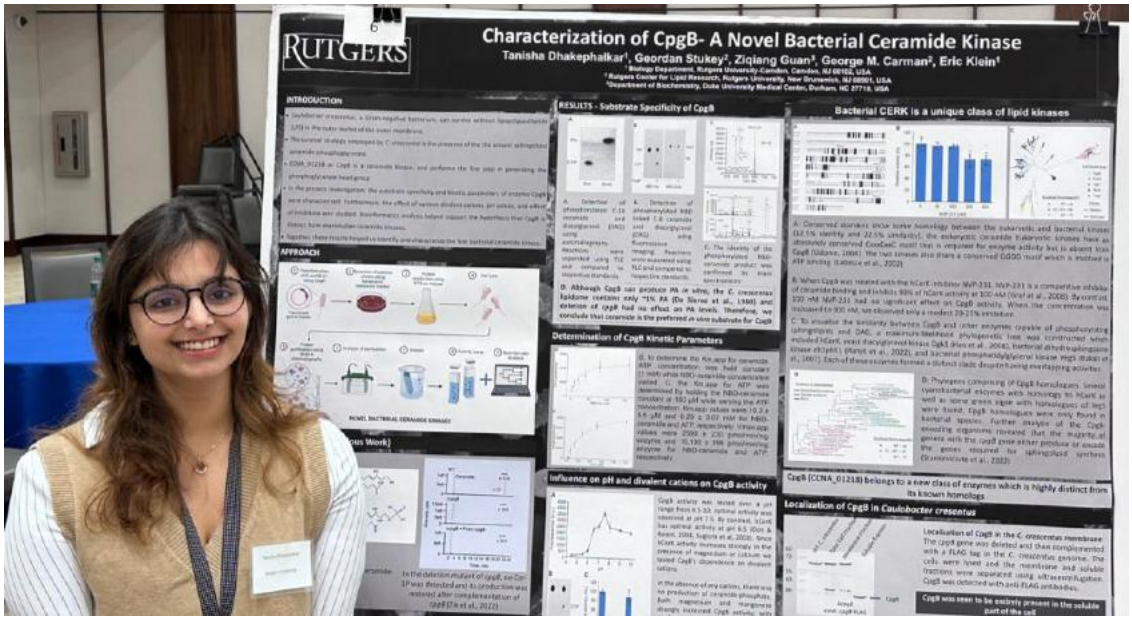


6. Characterization of an evolutionarily distinct bacterial ceramide kinase from Caulobacter crescentus.

Tanisha Dhakephalkar[1], Geordan J. Stukey[2,3], Ziqiang Guan[4] , George M. Carman[2,3], and Eric A. Klein[1,2,3,5]

1. Biology Department, Rutgers University-Camden, Camden, NJ
2. Department of Food Science, New Jersey Institute for Food Nutrition and Health, Rutgers University, New Brunswick, NJ
3. Rutgers Center for Lipid Research, New Jersey Institute for Food Nutrition and Health, Rutgers University, New Brunswick, NJ
4. Department of Biochemistry, Duke University Medical Center, Durham, NC
5. Center for Computational and Integrative Biology, Rutgers University-Camden, Camden, NJ

A common feature among nearly all gram-negative bacteria is the requirement for lipopolysaccharide (LPS) in the outer leaflet of the outer membrane. LPS provides structural integrity to the bacterial membrane, which aids bacteria in maintaining their shape and acts as a barrier from environmental stress and harmful substances such as detergents and antibiotics. Recent work has demonstrated that *Caulobacter crescentus* can survive without LPS due to the presence of the anionic sphingolipid ceramide-phosphoglycerate (CPG). Based on genetic evidence, we predicted that protein CpgB functions as a ceramide kinase and performs the first step in generating the phosphoglycerate head group. Here, we characterized the kinase activity of recombinantly expressed CpgB and demonstrated that it can phosphorylate ceramide to form ceramide 1-phosphate. The pH optimum for CpgB was 7.5, and the enzyme required Mg2+ as a cofactor. Mn2+, but no other divalent cations, could substitute for Mg2+. Under these conditions, the enzyme exhibited typical Michaelis–Menten kinetics with respect to NBD C6-ceramide (Km,app = 19.2 ± 5.5 μM; Vmax,app = 2590 ± 230 pmol/min/mg enzyme) and ATP (Km,app = 0.29 ± 0.07 mM; Vmax,app = 10,100 ± 996 pmol/min/mg enzyme). Phylogenetic analysis of CpgB revealed that CpgB belongs to a new class of ceramide kinases, which is distinct from its eukaryotic counterpart; furthermore, the pharmacological inhibitor of human ceramide kinase (NVP-231) had no effect on CpgB. The characterization of a new bacterial ceramide kinase opens avenues for understanding the structure and function of the various microbial phosphorylated sphingolipids.

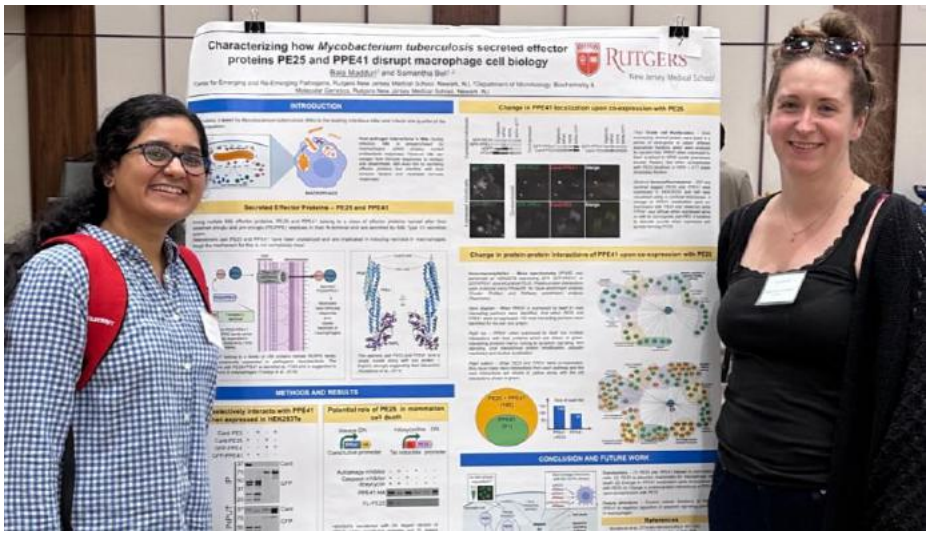


7. Characterizing how *Mycobacterium tuberculosis* secreted effector proteins PE25 and PPE41 disrupt macrophage cell biology.

Bala Madduri[1], Samantha Bell[1,2]

1. Center for Emerging and Re-Emerging Pathogens, Rutgers New Jersey Medical School, Newark, NJ,

2. Department of Microbiology, Biochemistry & Molecular Genetics, Rutgers New Jersey Medical School, Newark, NJ



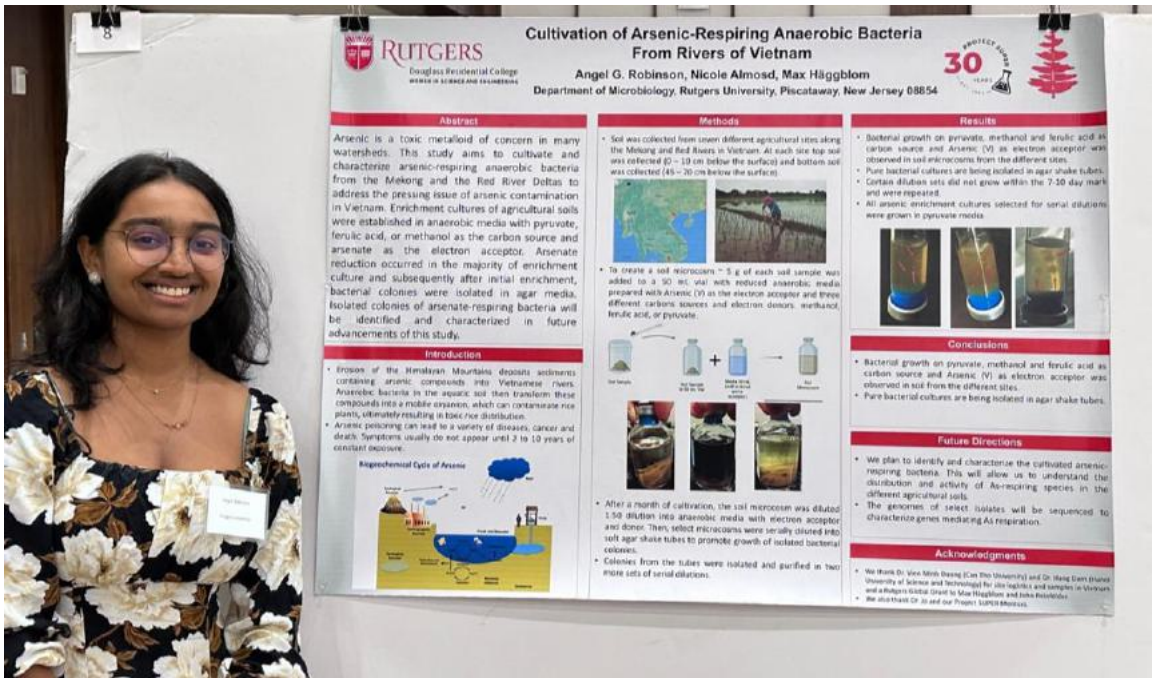
Tuberculosis (TB), caused by *Mycobacterium tuberculosis* (Mtb), is the leading infectious killer and infects one quarter of the global population. During infection, Mtb can evade host immune responses to multiply and disseminate via secreted effector proteins that interfere with, modulate, and protect from potent antibacterial responses. One such class of mycobacterial effector proteins are the PE/PPE proteins, which are encoded by an impressive 10% of the Mtb genome. Because this gene family is so large specifically in pathogenic species of mycobacteria and because some PE/PPEs have demonstrated roles in immune regulation and host cell interaction, they are believed to be critical for Mtb virulence and pathogenesis. However, the cellular functions of the 168 PE/PPE proteins have yet to be comprehensively characterized, at least in part because of their high GC content and large regions of extremely repetitive sequences. Nevertheless, one heterodimeric pair of PE/PPE proteins, PE25 and PPE41, has been crystalized and is implicated in inducing necrosis in macrophages, though the mechanism for this is not completely clear. To mechanistically characterize the cellular and molecular function of PE25/PPE41 in macrophages, we ectopically expressed PE25 and PPE41 in various cell types. We then performed immunoprecipitations and confirmed the predicted interaction between PE25 and PPE41 in host cells. To study the cellular localization of PE25 and PPE41, we performed cell fractionation and immunofluorescence microscopy, and with both approaches, found a reorganization of PPE41 upon co-expression with PE25: while PPE41 was diffuse when expressed alone, it localized to discrete puncta when co-expressed with the puncta-forming PE25. To contextualize this change in localization and to understand the biological function of the PE25/PPE41 heterodimer, we performed unbiased immunoprecipitation with mass spectrometry (IP-MS) to identify host binding partners for the pair. Consistent with previous reports, IP-MS hits of interest included BAX, NSDHL, ARL and vesicle associated membrane proteins. Our ongoing work seeks to connect these protein-protein interactions with mechanistic functions of PE25/PPE41 during Mtb infection, including modulation of cell death pathways and inhibition of phagosome-lysosome fusion. Through these studies, we aim to identify novel host-pathogen interactions between Mtb and macrophages and further unravel how the largely uncharacterized PE/PPE proteins contribute to Mtb virulence.

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

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

Department 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 culture 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.

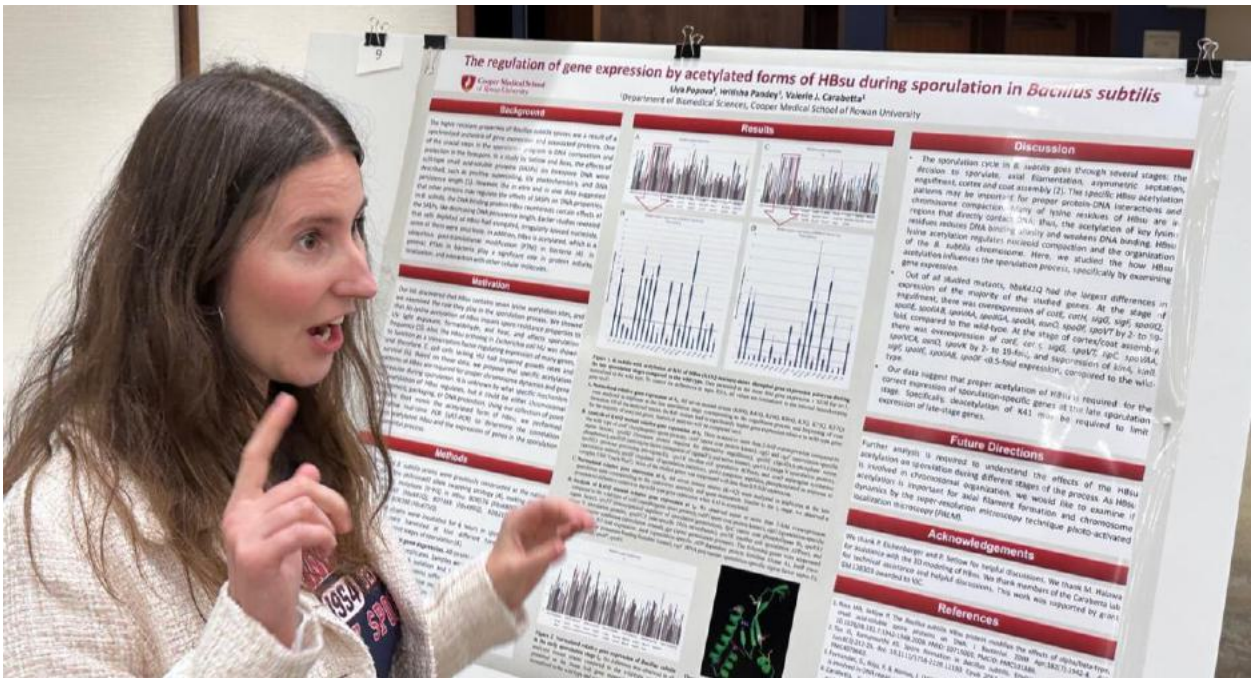


9. The regulation of gene expression by acetylated forms of HBsu during sporulation in *Bacillus subtilis*.

Liya Popova, Hritisha Pandey, Valerie J. Carabetta

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

The highly resistant properties of *Bacillus subtilis* spores are a result of a synchronized orchestra of gene expression and associated proteins. One of the crucial steps in the sporulation program is DNA compaction and protection in the forespore. In a study by Setlow and Ross, the effects of α/β-type small acid-soluble proteins (SASPs) on forespore DNA were described, such as positive supercoiling, UV photochemistry, and DNA persistence length. However, the in vitro and in vivo data suggested that other proteins may regulate the effects of SASPs on DNA properties. In *B. subtilis*, the DNA-binding protein HBsu counteracts certain effects of the SASPs, like decreasing DNA persistence length. Earlier studies revealed that cells depleted of HBsu had elongated, irregularly spaced nucleoids, or were anucleate. In addition, HBsu is acetylated, which is a ubiquitous posttranslational modification (PTM) in bacteria. In general, PTMx in bacteria play a significant role in activity, localization, and interaction with other cellular molecules. Our lab discovered that HBsu contains seven lysine acetylation sites, and we examined the role they play in the sporulation process. We showed that Nε-lysine acetylation of HBsu impairs spore resistance properties to UV light exposure, formaldehyde, and heat, as well as affects the frequency of sporulation. Using our collection of point mutations that mimic acetylated form of HBsu, we performed quantitative real-time PCR (qRT-PCR) to determine the correlation between acetylated HBsu and expression of genes in the early and late sporulation phases. Analysis of gene expression relative to the wild-type strain showed overexpression of cotE , cotH, sigG, spoIVCA, spoVAA, lipC, spoVT, gpr, spoVK, asnO, rsfA during late sporulation phases, suggesting that deacetylation of K41 HBsu is required for proper late gene expression. Further analysis of the relative gene expression data is required to determine the contributions of the other acetylation sites on the expression of examined genes.

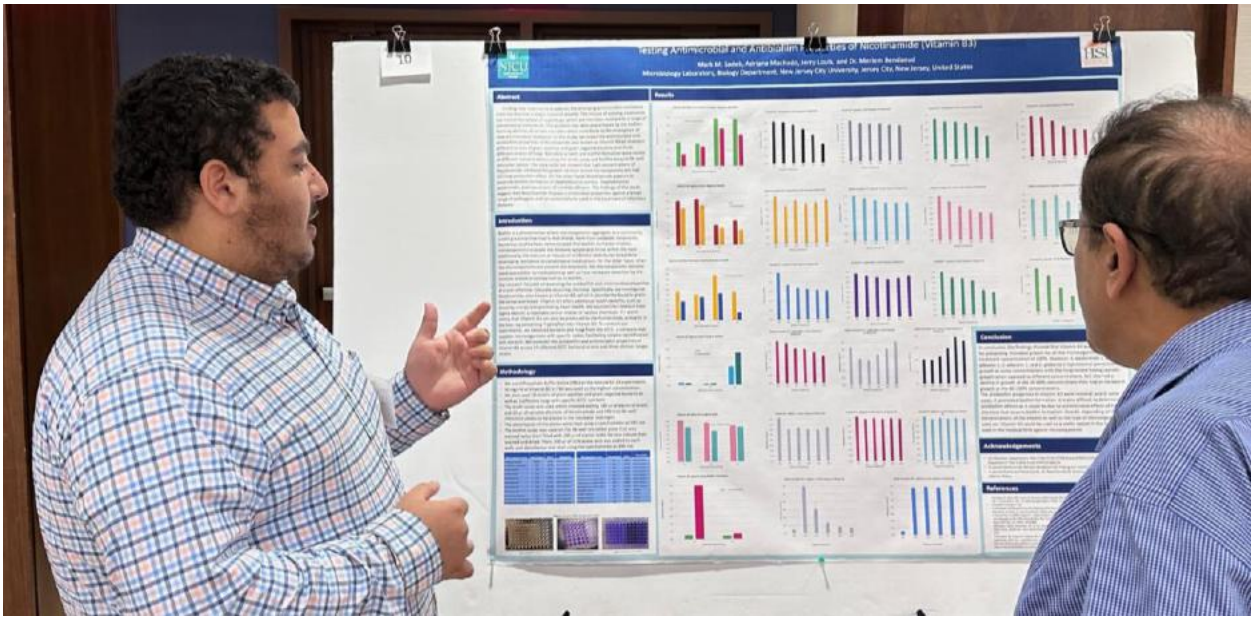


10. Testing antimicrobial and antibiofilm properties of nicotinamide (Vitamin B3).

Mark M. Sadek, Adriana Machado, Meriem Bendaoud

Dept. of Biology, New Jersey City University, Jersey City, NJ

Finding new treatments to address the emerging antimicrobial resistance crisis has become a major research priority. The misuse of existing treatments has fueled the spread of superbugs, which are microbes resistant to a range of conventional treatments. This problem has been exacerbated by the biofilm-forming abilities of certain microbes which contribute to the emergence of new antimicrobial resistance. In this study, we tested the antimicrobial and antibiofilm properties of nicotinamide also known as Vitamin B3 on nineteen different strains of gram-positive and gram-negative bacteria and three different strains of fungi. Microbial growth and biofilm formation were tested at different concentrations using the broth assay and biofilm assay in 96-well microtiter plates. The data collected showed that high concentrations of nicotinamide inhibited the growth of most tested microorganisms but had minimal antibiofilm effect. On the other hand, nicotinamide appears to promote biofilm formation of *Staphylococcus aureus*, *Staphylococcus epidermidis*, and two strains of *Candida albicans*. The findings of this study suggest that nicotinamide displays antimicrobial properties against a broad range of pathogens and can potentially be used in the treatment of infectious diseases.

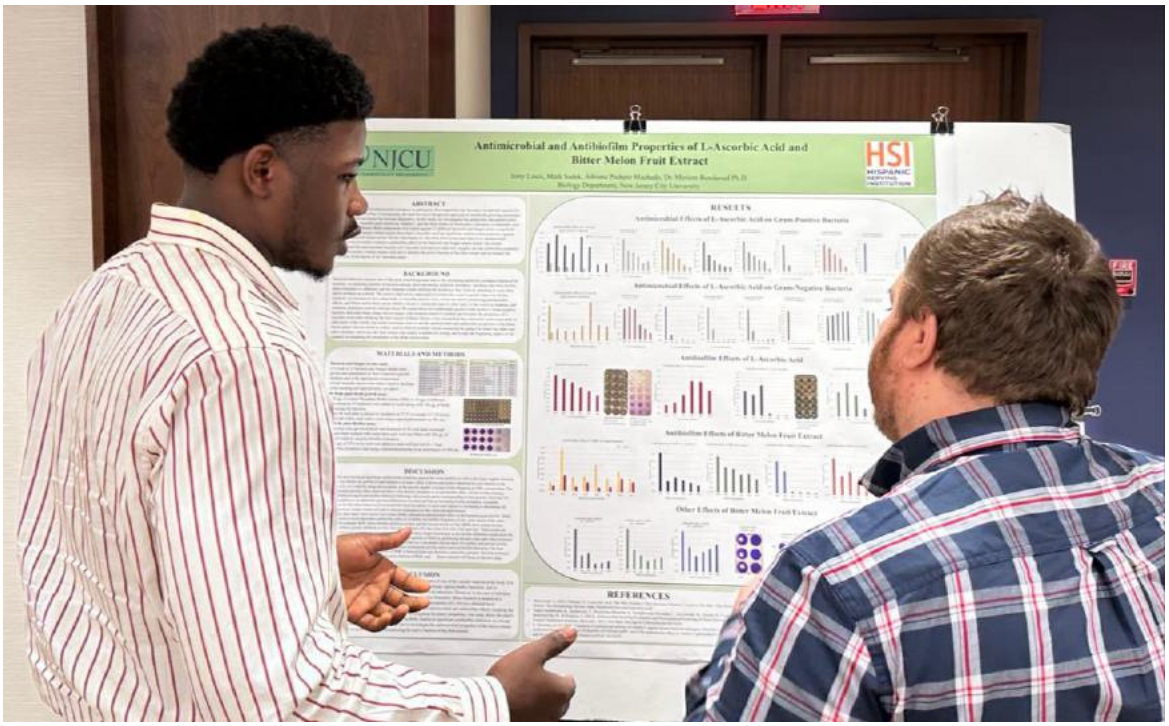


11. Antimicrobial and antibiofilm properties of L-ascorbic acid and bitter melon fruit extract.

Jerry Louis, Adriana Pinheiro Machado, Meriem Bendaoud,

Dept. of Biology, New Jersey City University, Jersey City, NJ

The growing number of emerging infectious diseases and antimicrobial resistance have become a worldwide concern for human health and welfare as available treatments are becoming inefficient. Biofilm formation, a multi-layer of microorganisms attached to each other and to surfaces, plays an important role in the development of antimicrobial resistance, and makes it difficult to eradicate the infection with conventional treatment. The need for novel antibiofilm and antimicrobial compounds is crucial to combat the increasing occurrence of antimicrobial resistance. In this study, we investigate the antibiofilm and antimicrobial properties of the widely known vitamin L-ascorbic acid, and the fruit extract of *Momordica charantia*, a commonly used plant in the Caribbeans. Both compounds were tested against 22 different bacterial and fungal strains using a broth assay and a biofilm assay. Initial results show that L-ascorbic acid has significant antimicrobial properties against almost all bacterial strains tested but no effect on the fungal strains. On the other hand, *M. charantia* fruit extract had little to no antimicrobial properties but revealed a significant selective antibiofilm effect on bacterial strains including *Staphylococcus aureus*, *Staphylococcus epidermis*, *Bacillus subtilus*, *Escherichia coli*, *Pseudomonas aeruginosa*, and fungal strains including *Candida glabrata* and *Candida albicans*. Our results substantiated the antimicrobial benefits of L-ascorbic acid and provided new insight into the antibiofilm properties of the fruit extract against a broad range of microorganisms. Further studies will be conducted to identify the active fraction of the fruit extract and investigate the antimicrobial and antibiofilm properties of the leaves of *M. charantia* plant.

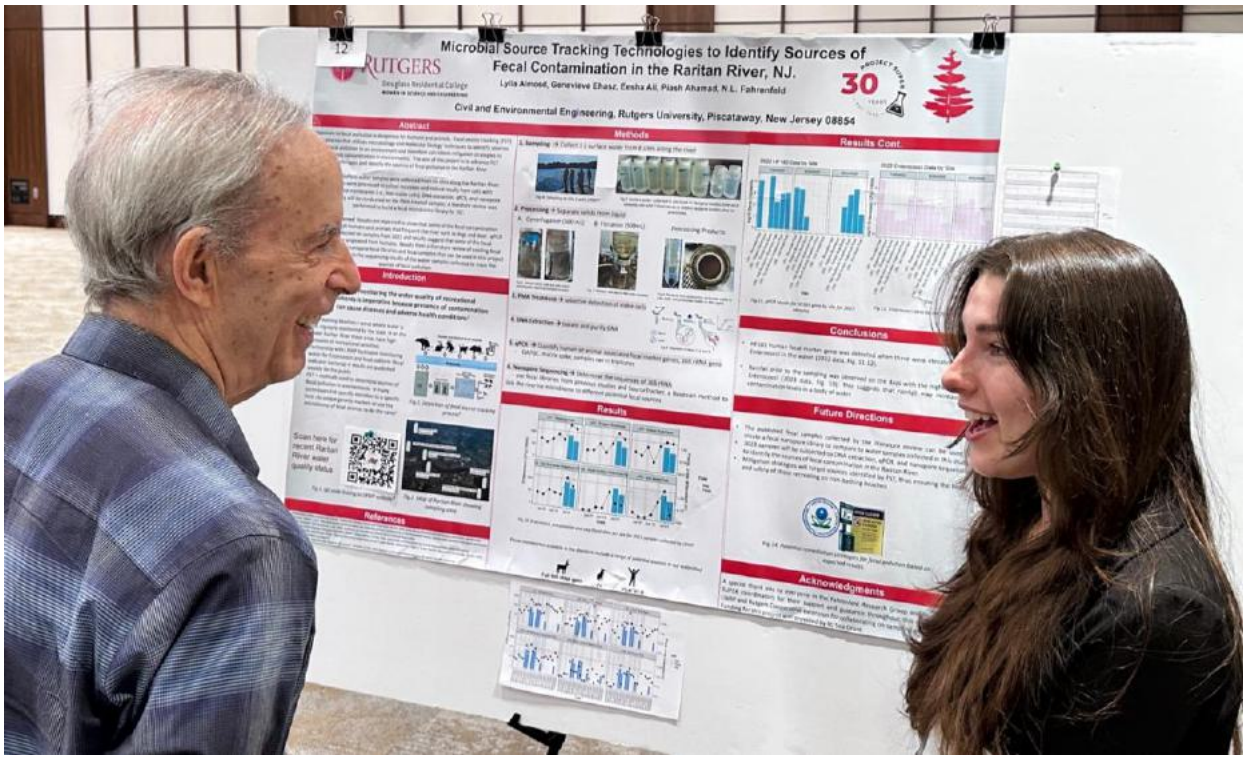


12. Microbial source tracking technologies to identify sources of fecal contamination in the Raritan River, NJ.

Lylla Almosd, Genevieve Ehasz, Piash Ahamad, N.L. Fahrenfeld

Dept. of Civil and Environmental Engineering, Rutgers University, New Brunswick, NJ

Fecal contamination above water quality standards has been detected in the Lower Raritan River for years. Determining the sources of fecal contamination (FC) using fecal source tracking techniques can inform mitigation strategies. In this study, fecal source tracking techniques were applied to the Raritan River. Water samples were collected from six non-bathing sites along the river for DNA-based analysis for fecal marker genes using viability-based techniques. Subsequently, nanopore sequencing will be conducted. Based on results from 2022, it is expected that humans are one source of FC. The ultimate aim of this work is to advance fecal source tracking techniques and use the results to minimize contamination, ensuring the health and safety of those who recreate in the Raritan River.

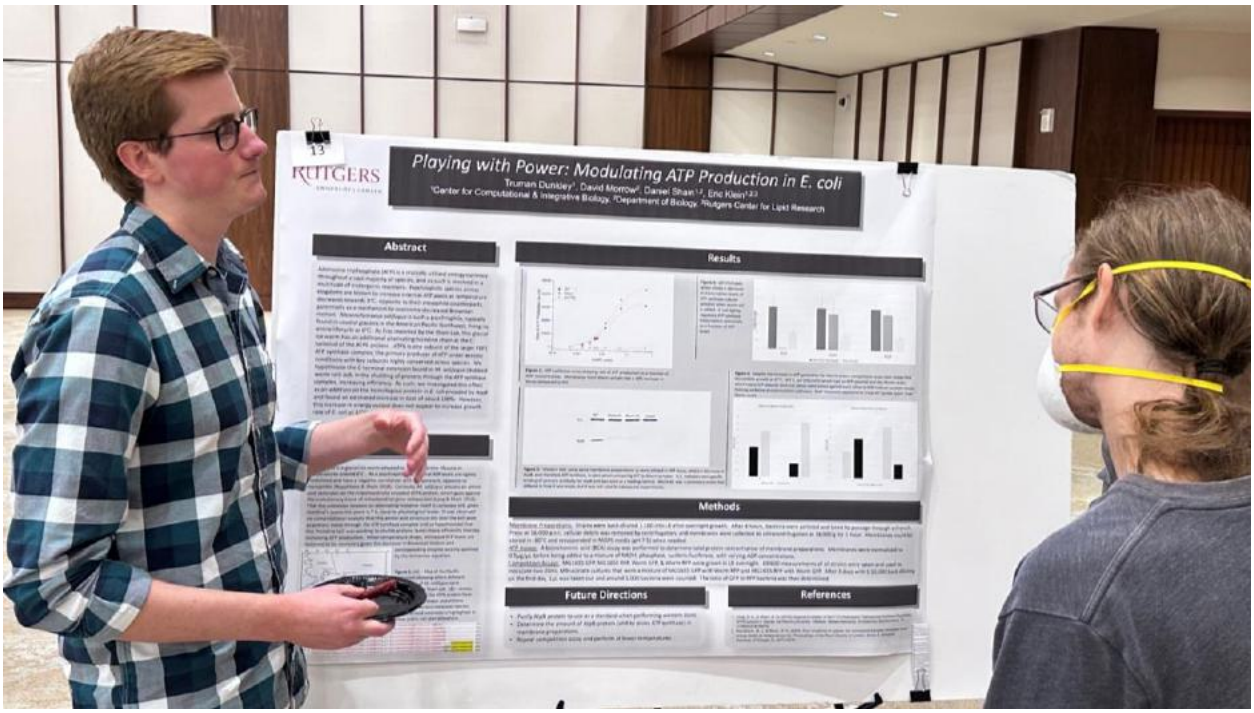


13. Playing with power: modulating ATP production in *E. coli*.

Truman Dunkley[1], Eric Klein[1,2,3]

1. Center for Computational and Integrative Biology, Rutgers University, Camden, NJ
2. Department of Biology, Rutgers University, Camden, NJ
3. Rutgers Center for Lipid Research, Rutgers University, New Brunswick

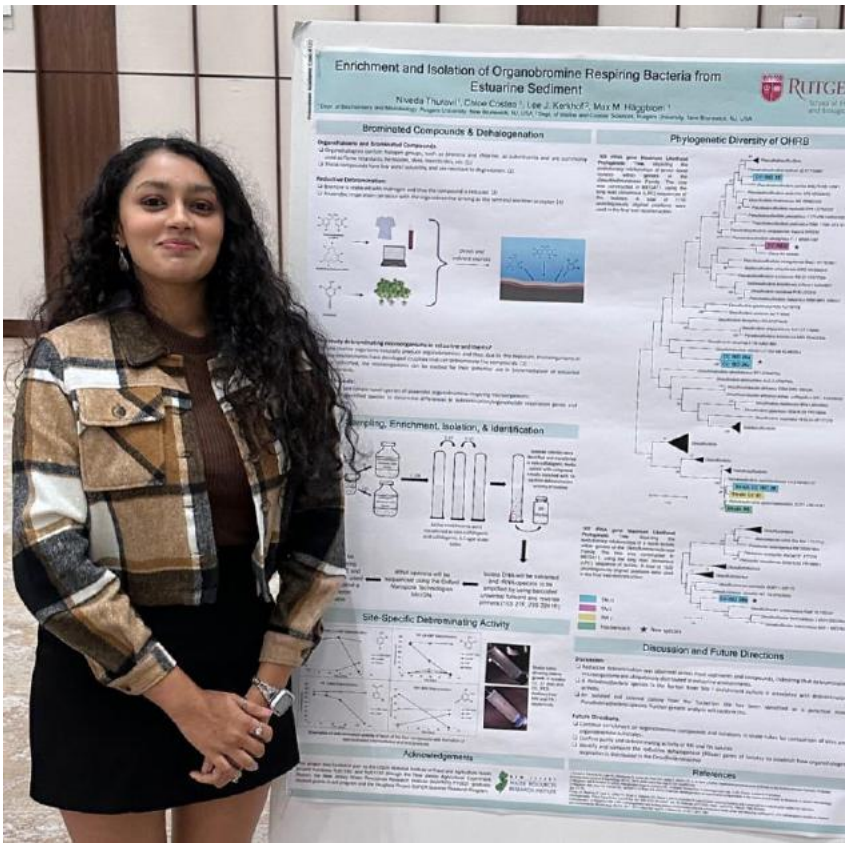
Adenosine triphosphate (ATP) is a crucially utilized energy currency throughout a vast majority of species, and as such is involved in a multitude of endergonic reactions. Psychrophilic species across kingdoms are known to increase internal ATP pools as temperature decreases towards 0℃, opposite to their mesophile counterparts, potentially as a mechanism to overcome decreased Brownian motion. *Mesenchytraeus solifugus* is such a psychrophile, typically found in coastal glaciers in the American Pacific Northwest, living its entire lifecycle at 0℃. As first reported by the Shain Lab, this glacial ice worm has an additional alternating histidine chain at the C-terminal of the ATP6 protein. ATP6 is one subunit of the larger F0F1 ATP synthase complex; the primary producer of ATP under aerobic conditions with key subunits highly conserved across species. We hypothesize the C-terminal extension found in *M. solifugus* (dubbed worm tail) aids in the shuttling of protons through the ATP synthase complex, increasing efficiency. As such, we investigated this effect as an addition on the homologous AtpB protein in *E. coli*, and found an increase in Kcat of about 100%.



14. Enrichment and isolation of organobromine respiring bacteria from estuarine sediment.

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

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



Organohalogens are among the most prevalent chemical pollutants in the environment. Microbial reductive debromination, allows for the removal of their halogen substituents, making them less toxic and more bioavailable for further breakdown. The main focus of our study was to enrich and isolate organobromine respiring bacteria from estuarine sediment, utilizing 2,4-dibromophenol (2,4-BP), 2,6-dibromophenol (2,6-DBP), 3-bromobenzoic acid (3-BBA), and 3,5-dibromo-4-hydroxybenzonitrile (bromoxynil) amended anaerobic cultures. Sediments from various New Jersey sites (Tuckerton Bay, Raritan River, Passaic River and Mullica River) as well as an international site (Cyprus), were used to set up anaerobic microcosms which were monitored for debrominating activity through high performance liquid chromatography (HPLC). All sites, regardless of salinity and sulfate levels, and geographic location, showed debromination of 2,4-DBP, 2,6-DBP, 3-BBA, and bromoxynil. This activity was maintained through multiple transfers into enrichment media. Colonies of various morphologies were isolated using subsequent dilutions into semi-solid agar shake tubes, and were then tested for debrominating activity. Genetic analysis of a colony isolated from a Raritan river enrichment with confirmed debrominating activity, revealed the most abundant organism to be a member of the *Halodesulfovibrio* genus and was seen to debrominate within six days of incubation. Further genetic analysis of all isolated colonies will allow for the phenotypic and genotypic comparison of the organobromine respiring microbial communities found in these sites under various conditions.

15. Cassava ssDNA viruses in coastal Kenya: high within-plant and within-field diversity.

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Mosaic disease of cassava is a major threat to food security in Sub-Saharan Africa and is caused by bipartite single-stranded DNA viruses (family *Geminiviridae*, genus *Begomovirus*) with small genomes and high nucleotide-substitution rates. These viruses are transmitted by whiteflies (*Bemisia tabaci*) and can be spread over long distances by the transport of infected cuttings. Genome sequencing data from across Africa over the past 40 years is relatively sparse and uneven, and diversity at the local scale has received little attention. In this work we measured mutational diversity and co-infection in individual plants in farmer fields.

We sampled plants in 8 fields in coastal Kenya (a major cassava-producing region) in November 2018, intentionally sampling plants with strong mosaic symptoms (leaf curling and chlorosis). We deeply sequenced DNA from 2 to 4 plants from each field using the Illumina NovaSeq 6000 platform. We inferred consensus sequences, analyzed maximum-likelihood phylogenetic trees, and measured subconsensus variation with VarScan.

Consensus genome sequences were most similar to East African cassava mosaic virus (EACMV) isolates from Kenya or Comoros. DNA-A sequences from each individual field did not cluster together, indicating that uncontrolled spread has maintained a high level of diversity, in contrast to surveys of tomato yellow leaf curl begomoviruses in Kenya and elsewhere. Diversity within fields was lower for DNA-B: different DNA-B haplotypes were dominant in the north, center, and south of the sampling range. Within-plant (subconsensus) diversity was high and two plants showed evidence of coinfection with two distinct EACMV haplotypes each.

17. Evaluation of antibacterial activity of *Lactobacillus* spp. in commercial yogurts.

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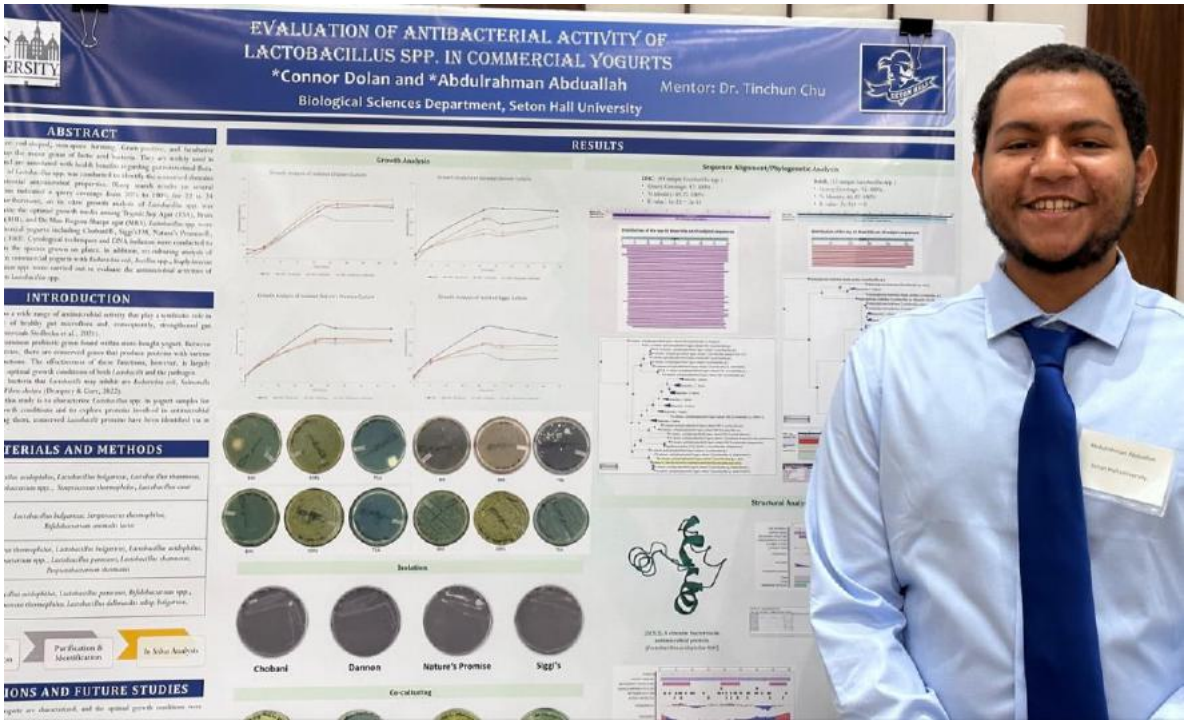
Lactobacillus spp. are rod-shaped, non-spore forming, Gram-positive, and facultative anaerobes making up the major genus of lactic acid bacteria. They are widely used in yogurt production and are associated with health benefits regarding gastrointestinal flora. An in-silico analysis of *Lactobacillus* spp. was conducted to identify the conserved domains associated with potential antimicrobial properties. Blastp search results on several antimicrobial proteins indicated a query coverage from 70% to 100% for 22 to 24 *Lactobacillus* spp. Furthermore, an in vitro growth analysis of *Lactobacillus* spp. was evaluated to determine the optimal growth media among Tryptic Soy Agar (TSA), Brain Heart Infusion agar (BHI), and De Man-Rogosa-Sharpe agar (MRS). *Lactobacillus* spp. were isolated from commercial yogurts including Chobani®, Siggi'sTM, Nature's Promise®, and Dannon Light & Fit®. Cytological techniques and DNA isolation were conducted to identify and confirm the species grown on plates. In addition, co-culturing analysis of *Lactobacillus* spp. from commercial yogurts with *Escherichia coli*, *Bacillus* spp., *Staphylococcus* spp., and *Mycobacterium* spp. were carried out to evaluate the antimicrobial activities of isolated colonies from *Lactobacillus* spp.



18. Exploring sporicidal efficacy of novel formulations and their anti-spore mechanisms.

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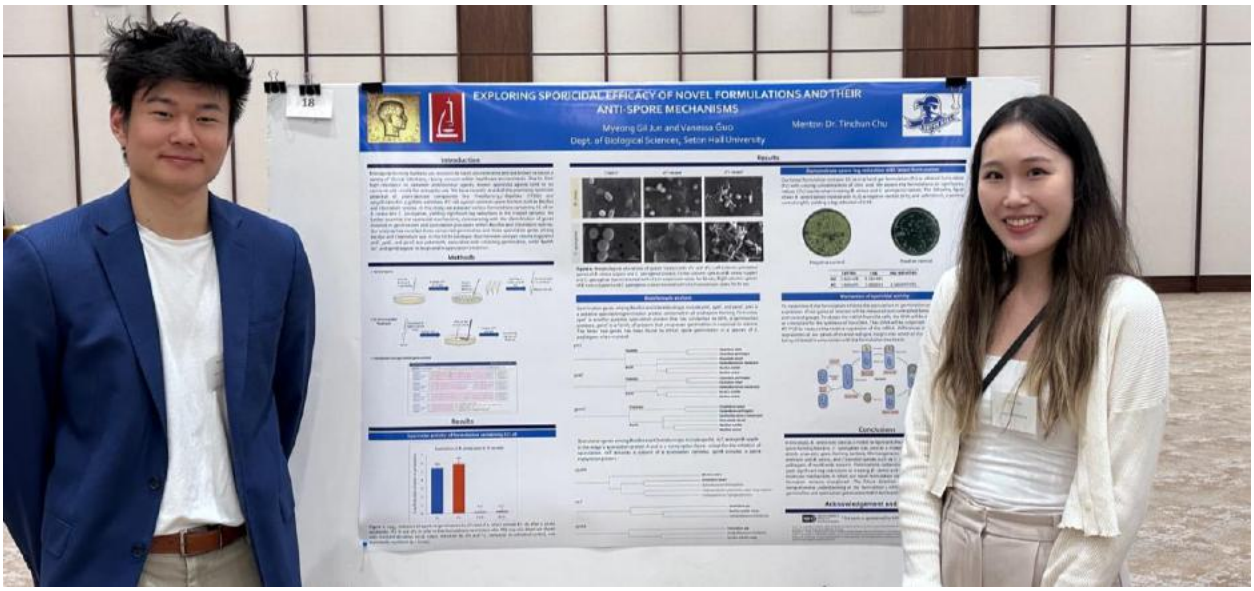
Endospore-forming bacteria are resistant to harsh environments and are known to cause a variety of clinical infections, raising concern within healthcare environments. Due to their high resistance to common antimicrobial agents, known sporicidal agents tend to be corrosive and unsafe for antiseptic use. We have recently revealed the promising sporicidal potential of plant-derived compounds like Theaflavin-3,3'-digallate (TFDG) and epigallocatechin-3-gallate-palmitate (EC-16) against common spore-formers such as *Bacillus* and *Clostridium* species. In this study, we assessed various formulations containing EC-16 on *B. cereus* and *C. sporogenes*, yielding significant log reductions in the treated samples. We further examine the sporicidal mechanisms, commencing with the identification of genes involved in germination and sporulation processes within *Bacillus* and *Clostridium* species. Our analysis has unveiled three conserved germination and three sporulation genes among *Bacillus* and *Clostridium* spp. in the NCBI database. Bioinformatic analysis results suggested ytxC, yyaC, and gerC are potentially associated with inhibiting germination, while spoOA, ricT, and spmB appear to be pivotal in sporulation inhibition.



19. Macrophages with depleted mtDNA have depressed immune response to intracellular bacteria.

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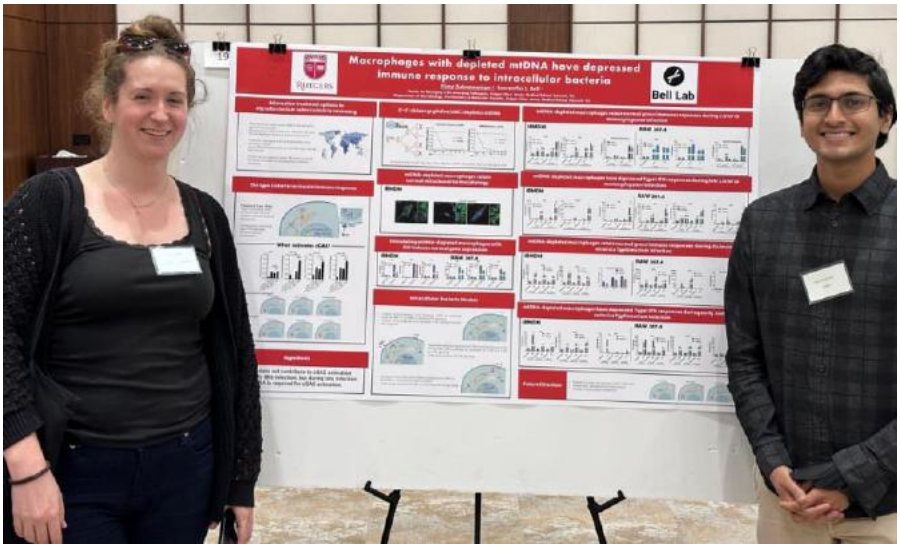

Mycobacterium tuberculosis (Mtb), the intracellular bacterial pathogen that causes tuberculosis (TB), remains one of the deadliest infectious diseases worldwide and killed 1.6 million people in 2021. Mtb evades killing in the macrophage by secreting bacterial proteins into the host cytosol that promote its own survival and replication. However, in doing so, Mtb also releases DNA into the cytosol, which is sensed by the macrophage via the cytosolic DNA sensor cyclic GMP-AMP synthase (cGAS). Upon binding DNA, cGAS activates the STING/TBK1/IRF3 signaling axis, which causes an immune response marked by increased expression of interferon-β (IFN-β). IFN-β is a type I interferon (IFN) traditionally associated with antiviral defenses, but this antiviral response is often detrimental during bacterial infections. Previous work has shown that in addition to detecting cytosolic bacterial DNA, cGAS can detect other types of host DNA such as cytosolic mitochondrial DNA (mtDNA); therefore, the precise origin of cGAS-bound DNA during Mtb infection remains unclear. To better understand the respective contribution of host and bacterial DNA, we are assessing how the depletion of mtDNA in macrophages (RAW 264.7, immortalized bone marrow-derived macrophages (iBMDM), and U937 macrophage cell lines) affects activation of the cGAS/STING/TBK1 signaling axis during Mtb infection. We depleted macrophage mtDNA by growing cells in the presence of 2′-3′-dideoxycytidine (ddC), which preferentially inhibits mitochondrial DNA replication, thus diluting mtDNA as the cells grow and divide. To investigate the type I IFN expression of mtDNA depleted macrophages in the context of intracellular bacterial infection, we infected these macrophages with *Listeria monocytogenes* and *Salmonella enterica typhimurium.* Depleting mtDNA reduces type I IFN induction during early infection in *Salmonella* and late infection in *Listeria monocytogenes*. We confirmed that mtDNA depletion doesn't alter cell-intrinsic bacterial control mechanisms since CFUs aren't dramatically different and mtDNA depletion doesn't alter mitochondrial morphology. Ultimately, we will use these studies to define the source of the cGAS-activating DNA during Mtb infection, where we predict that both bacterial DNA and mtDNA contribute to the type I IFN response with differing dynamics and magnitude. Together these results will inform the mechanism by which the STING/TBK1/IRF3 axis is activated during Mtb infection and reveal novel host targets for future therapies for Mtb infection.

20. Development of *Cryptococcus* inspired antimicrobial peptide

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Invasive fungal infections are serious diseases that are often difficult to treat with very limited drug options. Our recent studies revealed that lipid translocase (flippase) function in *Cryptococcus neoformans* (*Cn*) is important for fungal virulence and may be developed as a potential novel drug target. We have developed a series of peptides (Cryptomysyn) based on the sequence of *C. neoformans* Cdc50, a regulatory subunit of lipid flippase. One of our peptides (KKOO) possesses low minimum inhibitory concentration (MIC) of 8 μg/mL against *Cn*, broad spectrum activity against multiple important fungal pathogens, and synergy with the standard lineup of antifungal drugs. One example of a promising drug combination against *Cn* was KKOO at a concentration of 1 μg/mL and itraconazole at a concentration of 1/16 μg/mL. This mixture possessed a fractional inhibitory concentration index (FICI) of 0.375, indicating drug synergy. Annexin binding assays and fluorescent microscopy provided evidence to support the hypothesis that these peptides act as phospholipid flippase inhibitors. This preliminary work will enable future studies on the precise mechanism of action of the peptide and provides a starting point for exploration of other membrane proteins as antimicrobial peptide scaffolds.