Texas Branch
AMERICAN SOCIETY FOR MICROBIOLOGY

ABSTRACT BOOK

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UNDERGRADUATE ORAL PRESENTATIONS

UO1

Metabolic Supplementation of an Agmatinase Mutant in *Escherichia coli*: Implications on Motility and Pathogenicity

Akhil Nelapolu, Jacob Hogins, Larry Reitzer
The University of Texas at Dallas, Richardson, USA

Polyamines are organic polycationic alkylamines found in all living cells that play crucial roles in various biochemical pathways, and, as regulators, polyamines influence the progression of various diseases. Two polyamines, putrescine and spermidine, have been shown to affect *E. coli* motility, a function vital for disease progression in UTIs. Furthermore, the Reitzer lab has shown that putrescine biosynthesis is required for the growth and pili production of non-pathogenic E. coli. Here we sought to identify metabolites that can complement the motility phenotype of a putrescine depleted *E. coli* strain (W3110 ΔspeB). We hypothesize that putrescine, ornithine, and arginine would complement the gene expression and motility phenotypes of the mutant, but glutamate and spermidine would not. Motility was determined by the diameter cells grew to on motility plates after 36 hours. Gene expression of two putrescine regulated genes related to virulence: *entC* and soxS, was also measured by the relative fluorescence units generated by green fluorescent protein fused to the promoters of our genes of interest to assess the effect the supplemented metabolites had on stress gene regulation. Of the studied metabolites, putrescine supplementation restored the motility phenotype in the mutant to wild-type, while the other metabolites had no significant effect. The gene expression results showed that supplementation with spermidine, putrescine, and glutamate increased the expression of *soxS*, by 2-fold but had a limited effect on *entC*. We believe that this indicates that putrescine influences the spread of and progression of UTI's and other *E. coli* dominant infections in humans.

UO2

Comparison of the impact of phenazine-1-carboxylic acid (PCA) and phenazine-1-carboxamide (PCN) on quorum sensing inhibition

Sarah Hudson, Brianna Garret, Jennifer A. Hennigan
Abilene Christian University, Abilene, USA

Quorum sensing (QS) systems enable bacteria to modulate gene expression in response to accumulating chemical signals, such as N-acyl-homoserine lactones (AHLs), in dense populations. Gene pathways controlled by QS include biofilm formation, toxin and pigment production. Quorum sensing inhibitors (QSIs) disrupt communication in bacterial populations. This study compares QS inhibition of two molecules secreted by subspecies of *Pseudomonas chlororaphis*: phenazine-1-carboxylic acid (PCA) and phenazine-1-carboxamide (PCN). Environmentally isolated ***P. chloroaphis*** strains were identified as QSI-producers using *Chromobacterium violecum* as a QS biosensor. To measure violacein production, wild-type ***C. violaceum*** was cultured in triplicate with 5% *P. chlororaphis* extracts and violacein was measured at 585 nm using a UV-Vis spectrophotometer after extraction. Although violacein was reduced in cultures containing *P. chlororaphis* extracts, the reductions were not statistically significant. Biofilm formation was also reduced. PCA and PCN were identified in the extracts using thin layer chromatography. To confirm their QS impact, the violacein assay was repeated with purified PCA and PCN. Furthermore, we tested the effects of PCA and PCN using a *C. violaceum* mutant (CV026) deficient in AHL synthesis on soft agar containing exogenous N-hexanoyl-L-homoserine lactone (C6-HSL) or N-octanoyl-L-homoserine lactone (C8-HSL). PCA significantly reduced violacein, but PCN did not. Published structural modeling suggests PCA interacts with TraR, a receptor for N-3-oxo-octonanyl-L-homoserine lactones, in *E. coli*. We hypothesize PCA and PCN interact differently with a TraR homolog or similar AHL receptor in *C. violaceum,* suggesting their structural differences may elucidate molecular interactions with the QSI target.

UO3

Coevolution of *Pseudomonas aeruginosa* and *Drosophila melanogaster* via a Chronic Oral Infection Model

Antonio Rame, Noemi Del Campo, Anna Karki, William Cody
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Pseudomonas aeruginosa is a ubiquitous, opportunistic pathogen and frequent cause of chronic infection in the immunocompromised. Additionally, it is the leading cause of morbidity and mortality in people with the genetic disorder cystic fibrosis. In this study, we developed a modified Drosophila melanogaster oral feeding model to examine the Red Queen hypothesis and better understand host-pathogen interactions over multiple generations using three different treatment groups: host evolution, pathogen evolution, and coevolution. We monitored host survivability across generations and found that initial data indicate variation in host resistance and pathogen virulence in the coevolution treatment group within ten generations. Future transcriptional analysis at benchmark generations will aim to identify genes associated with increased pathogen virulence and host resistance, providing insights into the evolutionary dynamics of the chronic infection.

UO4

Elucidating the Genes Underlying Hydrocarbon Degradation in *Pseudomonas aeruginosa*

Theodore Nguyen, Aaliyah Ramirez, Jonathan Dannatt, William Cody
University of Dallas, Irving, USA

Each year, thousands of gallons of aviation fuel is spilled at airbases, airfields, and airports causing significant harm to the environment. Currently, there are no permanent, environmentally friendly solutions for cleaning up contaminated sites. Alternative methods include bioremediation, the use of microorganisms to remove hazardous pollutants. *Pseudomonas aeruginosa*, a metabolically robust and ubiquitous bacterium capable of degrading components of jet-fuel, has been found to be one of the most abundant microorganisms at contaminated sites. This capability is attributed to the Alkb1 and Alkb2 monooxygenases, enzymes that oxidize the hydrocarbons found in jet-fuel, enabling their breakdown. However, the upregulation of alkb1 and *alkb2* gene expression, which only occurs in the presence of hydrocarbons and absence of other carbon sources, is still poorly understood. Therefore, identifying the genes responsible for alkb1 and *alkb2* expression is essential for enhancing bioremediation strategies in environments with numerous carbon sources. Herein, insertional inactivated mutants were created in a hydrocarbon degrading strain of P. aeruginosa and a protocol was developed to analyze the terminal oxidation of n-C_8-C_{20} in memetic soil beds by gas chromatography-mass spectrometry (GC-MS). Additionally, these mutants were measured for hydrocarbon persistence with bacterial growth curves in liquid JetA media.

GRADUATE ORAL PRESENTATIONS

GO1

Understanding Molecular and Ultrastructural Mechanisms of Cadmium Detoxification in *Saccharomyces cerevisiae*

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Baylor University, Waco, USA

Cellular stress from cadmium toxicity affects gene expression and integrity, making it crucial to understand cellular adaptation to this heavy metal. This study investigates the role of the Nonsense-Mediated mRNA Decay (NMD) pathway in cadmium detoxification. NMD degrades aberrant mRNAs and regulates natural mRNAs involved in cellular processes. While *PCA1* and *CRS5* are known to mediate cadmium detoxification in *S. cerevisiae*, the specific involvement of the NMD in this process remains largely unexplored. We observed strain-specific *PCA1* regulation by NMD, and observed the *PCA1* mRNA levels to be unexpectedly higher in wild-type BY4741 than in NMD mutants under normal and cadmium stress conditions. Although the BY4741 strain lacks a functional pca1p transporter capable of exporting cadmium, it exhibited some cadmium tolerance. PCA1 deletion further improved tolerance, suggesting a compensatory mechanism beyond direct metal export. We investigated CRS5 a copper metallothionein potentially involved in cadmium binding, and found increased expression in NMD mutants both in wild-type and *pca1Δ* strains under cadmium stress, making it the potential compensating gene. To complement our molecular analyses, we used Transmission Electron Microscopy (TEM) with high-pressure freezing to preserve cellular structure and examine ultrastructural changes. TEM revealed vacuole enlargement, endoplasmic reticulum dilation, and cell wall distortions. STEM-EDX analysis showed cadmium accumulation primarily within vacuoles, suggesting that vacuolar sequestration plays a significant role in cadmium detoxification in *S. cerevisiae*. These findings provide insights into the interplay between gene regulation and structural adaptations in cadmium detoxification, contributing to our understanding of heavy metal stress responses in biological systems.

GO2

Differential regulation of the iron regulon by the nonsense-mediated mRNA decay pathway under iron scarcity

Jacqueline Carroll, Bessie Kebaara
Baylor University, Waco, USA

The nonsense-mediated mRNA decay (NMD) pathway is a highly conserved eukaryotic pathway that regulates mRNAs that prematurely terminate translation. Naturally occurring mRNA transcripts containing NMD-sensitive features such as atypically long 3' UTRs have been shown to experience regulation by NMD. This regulation can also be impacted by changes in the extracellular environment including bio-metal availability, resulting in differential regulation of transcripts. Iron plays a vital role in various metabolic processes including cellular respiration, DNA replication and repair, and translation. Prior research has shown that NMD is implicated in the maintenance of cellular iron homeostasis, though the extent is currently unknown. To study the link between iron homeostasis and NMD, we examined strains of *Saccharomyces cerevisiae* with functional (*UPF1*) and nonfunctional NMD (*upf1*Δ) in both iron-sufficient (CM) and iron-deficient contexts. Impaired growth was observed in the NMD mutant strain compared to the wild-type, though this disparity was exacerbated in iron-deficient conditions. The NMD mutant was also shown to accumulate higher levels of intracellular iron compared to the wild-type in CM and iron-deficient conditions. We also conducted transcriptome-wide RNAseq analysis of the wild-type and NMD mutant in CM and iron-deficient conditions, in which differential expression of the transcriptional activators *AFT1* and *AFT2* was observed. Subsequent Northern blotting validation indicates high likelihood that *AFT2* transcripts are subject to NMD-mediated regulation depending on iron availability. Downstream targets of *AFT1* and *AFT2*, such as membrane ferric-cupric reductases *FRE2/3/4*, encode multiple isoforms that are differentially regulated by NMD under normal growth conditions (CM) and low iron.

GO3

Lipopolysaccharide Remodeling Confers Resistance to Bam Complex Inhibitors

Teresa Sullivan[1,2], Anna Konovalova[1,2]

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Gram-negative bacteria present a significant problem for antimicrobial therapies due to the presence of an outer membrane, which restricts the entry of many compounds, including antibiotics. One strategy to overcome the outer membrane permeability barrier is to develop inhibitors that target essential proteins on the bacterial surface. One such protein is BamA, an essential component of the β-barrel assembly machinery (Bam) complex that folds and inserts all outer membrane proteins. BamA emerged as an attractive target, and its small molecule, peptide, and antibody inhibitors have been reported.

MRL-494 is a small molecule inhibitor of BamA, but mechanisms of action and resistance remain poorly understood. We isolated several novel MRL-494 resistant mutations, including those that target the PmrAB pathway. This pathway is well-studied for controlling enzymes that modify the lipid A portion of LPS, reducing its negative charge and conferring high resistance to polymyxin antibiotics. Through genetic analysis, I showed that the activation of PmrAB leads to resistance to MRL-494 in the manner dependent on lipid A modifications. Moreover, I demonstrated that this resistance is not MRL-494 specific. It applies more broadly to Bam complex inhibiting conditions and promotes resistance to another BamA inhibitor, darobactin.

LPS modifications, including those caused by *mcr-1* plasmid, are a major threat for clinical resistance to last-resort antibiotics like polymyxin B and colistin. The observation that the same LPS modifications confer cross-resistance to Bam complex inhibitors presents significant concerns and highlights the urgent need to address resistance mechanisms in order to develop effective antimicrobial therapies.

GO4

Enterococcus faecalis Influences the Metabolism and Gene Expression of *Escherichia coli* When Grown in Coculture

Jose Resendiz[1], Shreya Mekal[1], Akhil Nelapolu[1], Jacob Hogins[1], Philippe Zimmern[2], Larry Reitzer[1]
[1]The University of Texas at Dallas, Richardson, USA. [2]The University of Texas Southwestern, Dallas, USA

Escherichia coli is the major causative agent for urinary tract infections (UTIs) but can be isolated in mono- or co-infections. Like *E. coli, Enterococcus faecalis*, is part of the healthy microbiota, most commonly found in the gastrointestinal tract. However, it can infect the host opportunistically by itself or enhance the virulence of other opportunistic pathogens in the local biome. Previous studies show that during a coinfection, *E. faecalis* significantly influences *E. coli's* growth serving as an "informant" during mineral scarcity producing metabolites that upregulate *E. coli* enterobactin synthesis. In this study, we investigated whether *E. faecalis* influences *E. coli* gene expression during co-culture by RNAseq analysis and follow-up genetic experiments. Three recently isolated uropathogenic *Escherichia coli* strains were cultured with and without three co-isolated *E. faecalis* strains as well as the model *E. faecalis* strain (OG1RF). Cells were grown for 3 hours before plating on BEA (to select for *E. faecalis*) and MacConkey (to select for *E. coli*) to determine the growth ratios of the two species. RNA was isolated from the remaining cells and the expression of stress related genes in *E. coli* grown in both conditions determined. We found that *E. faecalis* synergizes with *E. coli* creating a transcriptome that is often associated with virulence. Analysis of the *E. coli* grown with OG1RF mutants (Δ*pstG*, Δ*ldh*_1, Δ*arcA*) further identified candidate metabolites (glucose, lactate and ornithine) involved in the mechanism of synergism. Understanding the synergistic mechanisms will open better treatment avenues for UTIs and other *E. coli* coinfections.

GO5

Disrupt and Conquer: Mechanism of phage peptide membrane disruption

Michael Debrah[1,2], Luis Carrillo[1], Jolene Ramsey[1,2]
[1]Texas A&M University, College Station, USA. [2]Center for Phage Technology, College Station, USA

In 2024, the WHO updated the list of drug-resistant bacteria that are the highest threat to human health. Gram-negative bacteria comprise 82% of the critical and high-priority bacterial pathogens. As antibiotics become less effective in the clinic, scientists have become interested in using bacteriophages as an alternative therapy. Lysis allows phages to cross multiple layers of the cell envelope: the outer membrane, the peptidoglycan, and the inner membrane. The phage holin is responsible for initiating lysis by making holes in the inner membrane. Then, the phage endolysin degrades the peptidoglycan. Finally, spanins disrupt the outer membrane. We recently discovered a novel class of small cationic peptides that independently disrupt the outer membrane leading to cell death. Here, we explore properties of the peptide that are important for its development as a phage-derived therapeutic. We hypothesized this peptide, gp28, is effective because it destabilizes the outer membrane by binding to negatively charged phosphate groups in lipopolysaccharide. We tested this hypothesis from three angles, 1) studying gp28 interaction with LPS, 2) assaying gp28 specificity for bacterial membranes, and 3) leveraging host and phage genetics to identify cellular targets and determinants of resistance. Although our prior studies suggested gp28 permeabilized the inner membrane, here we show that localization to the inner membrane does not kill cells. We are characterizing gp28-resistant strains using whole genome sequencing. Additionally, we show gp28 has antibacterial activity against a panel of Gram-negative bacteria. These studies deciphering gp28 host killing lay the foundation for phage and phage peptide-mediated therapy.

EDUCATION ORAL SESSION

EO1

Relaunch of a paper reading course coupled with phage protein annotation using the Gene Ontology

Debby Siegele, Curtis Ross, Jolene Ramsey
Texas A&M University, College Station, USA

In authentic learning activities students experience what scientists encounter on a daily basis. One such activity is reading and interpreting research data published in the primary literature. When students are asked to interpret data in their courses, they may see the activity as merely an exercise without recognizing its relevance to the real world. In this presentation, we describe an approach that requires students to perform biocuration from primary research articles they read, peer review each other's conclusions, then contribute their results back to the biological research community. Students convert biological information about gene function described in the publications into standard Gene Ontology-based machine-readable annotations. Those annotations are submitted to the Gene Ontology Consortium databases used by biologists worldwide for bioinformatic research. Originally launched at Texas A&M University, we call this course Creating Annotations through Critical Analysis of Original research (CACAO). In our recent iterations of CACAO, A&M student teams have annotated primary articles focused on microbiology, specifically phage biology. With our 2.0 online platform for annotation at cacao.wiki, we invite participation from teams at other universities to promote friendly competition and capture more of the novel functions of phage gene annotation in ways that can benefit microbiology research. In this brief presentation, we will also discuss the practical aspects of managing such a course in terms of instructor workload.

EO2

Implementation of second-chance testing to enhance learning and improve grades in an entry-level college biology course

Rebecca Hunter, Jennifer Hennigan, Darby Ice, Joshua Brokaw
Abilene Christian University, Abilene, USA

In order to address the persistent problem of underperformance and low retention in the first of a two-sequence majors introductory biology course, we implemented second-chance testing as an alternative grading practice in Fall 2023. A second-chance testing strategy allows students to retest on content that they have not yet mastered by the time of the summative assessment. Although there is little published research on second-chance testing, the use of this testing strategy has been shown to increase grades and reduce test anxiety in college-level STEM courses. We divided the Introductory Biology I course content into 17 learning objectives (LOs). Students tested over 3-4 LOs at a time in class and had the opportunity to retest on up to two of the LO exams during subsequent in-class retake days. During Fall 2023, we assessed the impact of exam retakes on student grades and on their perceptions of the course. Preliminary results show an average grade improvement of 3.9% on retake exams compared to first exams. Additionally, students had an overall positive perception of the retake testing system, reporting that it increased their confidence and motivation. We increased the weight of the first LO exam attempt in Fall 2024 and will report preliminary results comparing average grade improvement and frequency of retesting between Fall 2023 and Fall 2024.

EO3

From Lectures to Learning: AI Tools Enhancing Science Education

Greg Frederick
AUC School of Medicine, Cupecoy, Sint Maarten (Dutch part)

This presentation explores the transformative potential of Google's NotebookLM AI tools in enhancing science education. NotebookLM's advanced capabilities in data organization, real-time collaboration, and personalized learning pathways significantly improve the efficiency and effectiveness of science students' study routines. By integrating AI-driven insights, students can better understand complex scientific concepts, engage in interactive learning experiences, and receive instant feedback, fostering a deeper comprehension and retention of knowledge. Furthermore, the ability to streamline research, facilitate group projects, and provide tailored educational resources makes it an invaluable tool for students in various scientific disciplines. This talk will highlight case studies and empirical data demonstrating the positive impact of NotebookLM on science faculty impressions and utilization of AI tools in education.

EO4

AI Tools and the Potential Impact on Teaching

Lee Hughes
University of North Texas, Denton, USA

Purpose: This project aimed to improve medical students' test-taking skills and confidence during the General Principles Block (GPX) at Texas Tech University Health Sciences Center through USMLE-style formative assessments and rationales focused on challenging topics. Six optional, in-person sessions were held outside lecture hours to enhance exam performance and confidence.

Background/Rationale: The GPX block bridges anatomical sciences and organ system blocks, providing a critical period to solidify clinical principles and test-taking abilities. Topics were selected based on a needs-analysis poll to address gaps in student performance and confidence.

Methods: A needs analysis pre-survey identified key areas of difficulty, guiding the selection of topics for review. Each session included timed quizzes with clinical vignettes, rationales, and explanations from TTUHSC lectures and USMLE resources. Topics covered were Unit 1 (Glucose, Amino Acid Metabolism), Unit 2 (Protein Trafficking, Medical Genetics, Drug Metabolism), and Unit 3 (Antibiotics). Exam performance and student feedback assessed effectiveness.

Results: Exam performance showed no significant differences across all units between attendees (Group A) and non-attendees (Group B). However, attendees performed better on topics in Units 1 (86% n=110 vs 81% n=72, $p<0.0001$) and 2 (91% n=97 vs 87% n=86, $p<0.0001$). Unit 3 did not show significance likely due to heavy institutional use of 3rd-party resources such as Sketchy. Feedback indicated 92% of attendees felt sessions improved understanding, and 87% reported increased confidence.

Conclusions: USMLE-style formative assessments enhanced understanding and performance on targeted topics. Positive feedback supports broader implementation to improve medical education at TTUHSC.

CAREER SESSION
Goldschmidt Graduate Student Awardee Alumna

2D and 3D in Vitro Immunization Platforms for Immune Response Assessment

Tetiana Bowley, Thomas Vigil, Daniel Jacobsen, Elizabeth Clarke, Apoorv Shanker, Katie Davis-Anderson, Jessica Kubicek-Sutherland
LANL, Los Alamos, USA

Vaccines are critical for combating emerging and pandemic outbreaks. A successful vaccine against a human pathogen enables long-term protective immunity, including both innate and adaptive immune responses. Optimization of a successful vaccine candidate takes approximately ten years and has 94% failure rate. The challenges of vaccine development include individual approach to each pathogen for immunological response evaluation, choice of the right vaccine platform for a specific pathogen, lengthy experimental design of testing studies, and high costs. The COVID-19 pandemic has emphasized the urgent need for rapid development of effective countermeasures that can combat new pathogens. RAPTER (Rapid Assessment Of Platform Technologies To Expedite Response) project involves development of an *in vitro* immunization (IVI) pipeline to generate immunological data sets for machine learning predictions. Here, we have established 2D and 3D IVI platforms to evaluate immune responses to any given pathogen. We compared innate immune responses using cytokine profiling following *E.coli* lipopolysaccharide (LPS) or Polyinosinic:polycytidylic acid (Poly I:C) treatment of dendritic cells in 2D versus 3D IVI systems. Our findings indicate that both 2D and 3D cultures provide results that complement each other. For future studies, we will evaluate adaptive immune response to different pathogens by co-culturing treated dendritic cells with T cells and B cells. All the experimental findings will be analyzed by the ML tool to improve prediction of choosing the right vaccine platform for any viral or bacterial pathogen. These studies will aid in developing a successful vaccine candidate in a fast and efficient manner.

EDUCATION POSTER PRESENTATIONS

EP1

Improving Medical Student Confidence and Exam Performance on Particular Topics: The Impact of Short USMLE-styled Formative Assessments in a Pre-Clinical Block

Grady Edens, Gurvinder Kaur, Cassie Kruczek
Texas Tech University Health Sciences Center, Lubbock, USA

Purpose: This project aimed to improve medical students' test-taking skills and confidence during the General Principles Block (GPX) at Texas Tech University Health Sciences Center through USMLE-style formative assessments and rationales focused on challenging topics. Six optional, in-person sessions were held outside lecture hours to enhance exam performance and confidence.

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Conclusions: USMLE-style formative assessments enhanced understanding and performance on targeted topics. Positive feedback supports broader implementation to improve medical education at TTUHSC.

EP2

Microbial March Madness: A Course-based Undergraduate Research Experience for Exploring the Environmental Fitness of Microbes

Jacqueline Miralles Salazar, Joshua Reid, Catherine Wakeman
TTU, Lubbock, USA

The "Microbial March Madness" Course-based Undergraduate Research Experience (CURE) leverages the excitement of NCAA basketball to engage students in microbiological research. This innovative course allows students to explore the environmental adaptations and fitness of various microbes by developing and testing hypotheses in a competitive, bracket-style format. Embracing Texas Tech University's (TTU) enthusiasm for March Madness, students prepare and experimentally test an NCAA-style bracket where different microbes are pitted against each other based on their environmental fitness. Throughout the course, students will design a bracket using eight bacteria from a pool of 18 possible “players” commonly used BSL-1 Bacteria in the classes of Microbiology. This bracket represents their hypothesis that they will then design experiments to assess using various stress conditions chosen by the students. This hands-on approach fosters critical thinking, collaboration, and scientific communication skills. Additionally, students will gain awareness of the work involved in being a researcher and enhance their science identity as they experience the process of scientific discovery firsthand. By integrating sports-themed competition with scientific inquiry, the "Microbial March Madness" CURE aims to enhance student engagement and deepen their understanding of microbial ecology and environmental adaptation.

UNDERGRADUATE POSTER PRESENTATIONS
Antimicrobial Microbiology

UP1

Antimicrobial Potential of South American Plant Extracts Against *S. aureus*

Karmyn Aguilar, Max Heimlich, Patricia Baynham
St. Edward's University, Austin, USA

Antimicrobial resistance (AMR) is a growing problem causing 1.27 million infections worldwide and contributing to 4.95 million deaths during 2019. These numbers are projected to increase to over 10 million lives each year and at a cost of 100 trillion USD by 2050 unless solutions are found.
This project focuses on identifying additional antimicrobial agents within plant extracts.

We examined 82 plant extracts for their potential antimicrobial activity against *Staphylococcus aureus* through the Kirby-Bauer disk diffusion. Blank disks were impregnated with an ethanol-based plant extract or ethanol control and allowed to dry. They were then placed onto Muller Hinton agar plates inoculated with a 0.5 McFarland standard of *S. aureus*. After incubation at 37° C for twenty-four hours, any zones of inhibition were measured to assess antimicrobial activity.

This study found that eleven plant extracts displayed zones of inhibition against *S. aureus* ranging from 10mm to 22mm. Further research into these eleven plant extracts, such as bacterial cytological profiling, could be helpful in determining the mechanisms that prevent bacterial growth. Research testing these extracts in combination with various antibiotics could also be helpful in creating potential therapies for AMR infections.

UP2

Multi-drug-resistant plasmid pUTAK-22-1 of Shiga Toxin-Producing *Escherichia coli* serotype O111:H8

Jacob Alford[1], Irvin Rivera[1,2], Mariana Sainz Garcia[1], Sara Koenig[1,2], Felix Borregio[1], Joseph Bosilevac[3], Mark Eppinger[1,2]
[1]The University of Texas at San Antonio, San Antonio, USA. [2]South Texas Center for Emerging Infectious Diseases, San Antonio, USA. [3]Agricultural Research Service, U.S. Meat Animal Research Center, Clay Center, USA

Background: The production of phage-borne Shiga toxin (Stx) is a virulence hallmark of Stx-producing *E. coli* (STEC). This pathovar is responsible for food-borne outbreaks causing severe human disease. The carriage of antibiotic resistance poses challenges in both clinical and agricultural settings. For this study, we collaborated with USDA to sequence the genomes of multi-drug resistant STEC isolates to closure. Here, we present the complete genome of MDR-plasmid pUTAK-22-1. **Materials and Methods:** The plasmid was profiled *in silico* using a developed Python pipeline, packaging the following tools: The Incompatibility group was determined with PlasmidFinder. Antibiotic resistance (AR) and virulence genes were cataloged with CARD and VFDB. Plasmid-borne mobile genome loci were identified using mobileOG-db, IslandViewer, TnCentral, ISEScan, and IntegronFinder. Phylogenetically related plasmids were identified with PLSDB, and serotypes of respective host strains were identified with ECtyper. Plasmids and cataloged features were visualized in BRIG. **Results:** The presented 129 kb MDR-plasmid carries eight resistance genes, conferring resistance to aminoglycosides, sulfonamides, macrolides, and diaminopyrimidines. The *sul1* and *qacE* genes are found in duplicate, suggesting potential dosage effects. Plasmid pUTAK-22-1 further harbors a conjugal transfer machinery, mediating its own transmission, and belongs to the high-copy number IncB/O/K/Z-subtype. The identified plasmid characteristics are of high epidemiological concern, facilitating a rapid dissemination of multidrug resistance into larger bacterial host populations.

UP3

Antibiotic synergy with a phage-encoded peptide to reveal host interactions

Luis Carrillo, Michael Debrah, Jolene Ramsey
Texas A&M University, College Station, USA

Antimicrobial resistance was estimated to be responsible for 2.7 million global deaths in 2019 and contributed to 4.95 million deaths. As the “age of antibiotics” wanes we have seen some success in using bacteriophage, a natural bacterial predator to treat bacterial infections. Most dsDNA phages kill bacteria using a multigene lysis cassette for progeny release. They encode a holin and endolysin to target the inner membrane and break down the meshwork of peptidoglycan. Phages of Gram-negative hosts require additional proteins, the spanins, to remove the outer membrane barrier. Bioinformatic analysis revealed that ~15% of dsDNA phages lack a spanin-encoding reading frame, suggesting these phages have additional mechanisms to disrupt the outer membrane. Our group previously demonstrated that the small cationic protein gp28 of phage phiKT disrupts the outer membrane by an unknown mechanism. Given that the outer membrane barrier is a major determinant of intrinsic antibiotic resistance, in this project we probed the synergistic lethality of antibiotics with the expression of gp28 in E. coli strain MG1655. Broad-range susceptibility testing revealed specific β-lactams and the quinolone nalidixic acid exhibit a greater-fold of lethality in the presence of gp28. In ongoing work, we will screen for host factors that mitigate or promote E. coli lethality in the presence of gp28 to identify its cellular targets. Our data will then provide a framework for future development of gp28 or other cationic phage peptides as a potential antimicrobial agent in the current antibiotic clinical crisis.

UP4

Antibiotic susceptibility profiles of local foodborne Enterococcus isolates

Dale Staggemeier, Jesus Alvarez, Anand Karki, Todd Primm
Sam Houston State University, Huntsville, USA

Enterococcus faecalis are facultative Gram-positive cocci that commonly reside in the human intestinal tract. It is typically regarded as part of the normal gut microbiota and is generally harmless in healthy individuals. *E. faecalis* has gained significant attention due to its ability to quickly acquire resistance to various antibiotics, primarily by horizontal gene transfer. It is classified as one of the high priority ESKAPE pathogens by the Infectious Diseases Society of America. Our lab has surveyed local retail chicken samples for *Enterococcus*, *Campylobacter*, and *Salmonella*. We are currently focusing our exploration of *Enterococcus* on 12 strains of *E. faecalis*. The complete genomes of these strains are being sequenced. The susceptibility profile of these strains against 12 representative antibiotics is being determined *in vitro* using the microbroth method. Based on the minimal inhibitory concentrations, they are being classified as clinically resistant, intermediate, or sensitive using breakpoints from the Clinical and Laboratory Standards Institute. By comparing experimental susceptibility with genomic sequences we hope to define genetic resistance determinants and possibly if those genes were transferred recently.

UP5

Antibiotic Resistance Characterization of Soil-Isolated *Serratia marcescens*

Chukwuemeka Nnamani, Katelyn Porter, Claire Edwards
St. Edwards University, Austin, USA

Antibiotic resistance has established itself as a major concern amongst the scientific community. Antibiotic resistant (ABR) microbes are a health concern, especially when it comes to multidrug resistance. ABR microbes reduce the effect of common antibiotics, rendering them ineffective and prolonging illnesses. This study was intended to develop and validate experimental approaches for identifying and studying ABR microbes. Soil acts as a reservoir for ABR bacteria, so samples were collected and cell density was measured using serial dilution and spread plating on LB (Luria Bertani), MAC (MacConkey) agar. To quantify the ABR bacterial population, the same protocols were performed with the addition of 20 ug/mL tetracycline (tet). Two ABR bacterial colonies were cultured on MAC+tet agar to ensure that the isolates were gram negative and resistant to tet. DNA was isolated using a Zymogen kit for further analysis, and 16S PCR was performed to amplify the 16S ribosomal DNA sequence from each isolate. Amplified 16S DNA samples were sequenced by Eton Bioscience Inc. Finch TV and NCBI BLAST were used to identify both isolates as *Serratia marcescens.* Given the existence of multiple tetracycline resistance strains, Tet A and Tet B PCR were performed, with Tet A yielding inconclusive results and Tet B testing negative. The Serratia marcescens isolate was then analyzed with Kirby Bauer tests to determine if it had multidrug resistance, and it showed resistance to erythromycin, streptomycin, penicillin and tetracycline.

UP6

Identification of Two Multi-drug Resistant Isolates Obtained from Soil Samples

Katelyn Porter, Emeka Nnamani
St. Edwards University, Austin, USA

Antibiotic resistant (ABR) bacteria have become a globalized health concern in recent years. Antibiotic resistant microbes are more difficult to treat than non-resistant microbes, because they contain resistance genes to certain antibiotics. Because bacteria can release antibiotics as a natural defense mechanism, soil can harbor large populations of ABR bacteria. This research was intended to establish lab approaches for studying ABR bacterial populations in soil samples as well as further characterize two ABR isolates. Total and gram negative tetracycline-resistant (tetR) bacterial populations from soil samples obtained around the St. Edwards retaining pond were quantified using cell density calculations from colonies grown on luria bertani agar with 20 ug/mL added tetracycline (LB+tet) and MacConkey agar with 20 ug/mL added tetracycline (MAC+tet), respectively. One tetR isolate cultured in the previous experiment, as well as one tetR isolate cultured from a farm soil sample were further characterized using Kirby Bauer disk diffusion, 16S PCR, and tetR PCR protocols. Both isolates displayed multidrug resistance. 16S PCR products were sequenced by Eton Bioscience Inc., and both Finch TV and NCBI BLAST were used to identify the isolates as *Providencia alcalifaciens* and Achromobacter sp. The results of tetB PCR indicated that neither isolate has the tetB resistance gene. The results from tetA PCR were inconclusive. This result could have been caused by primer offsite binding during the extension step of PCR due to the temperature in the thermal cycler being too low.

UP7

Tiny Earth

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Historically most of our antibiotics have come from soil, and we need new antibiotics to respond to the threat of antimicrobial resistance. Here at Texas A&M University of San Antonio, we work together to find novel yet unidentified antibiotics in soil around our campus. We have been working with the Bacteriology 2025 class, who have collected soil samples from across the campus, from areas with low and high human activity, and where the soil is damaged or healthy. Using protocols from Tiny Earth, we have extracted and isolated the bacteria from the samples, growing them on L agar and patching representative colonies onto bacteria that represent antibiotic resistant strains, to see if they can inhibit growth, thus possibly producing antibiotics. We have already screened against 4 bacteria and have identified 5 isolates with potential. Our work over the coming weeks will involve PCR to identify these bacteria and further characterization of the antimicrobial extracts. This research is done in parallel with the Bacteriology students who are studying the microbiome of the samples, and we aim to determine which sites on campus have the greatest potential for antimicrobial discovery. In the future, we will educate our peers about the need for new antibiotics and the importance of protecting our soils.

UP8

Antimicrobial characterization of novel *Paenibacillus fortis 79R4*

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Methane is the second most produced greenhouse gas, and is extremely harmful to the environment due to its ability to trap heat. A significant amount of methane emissions come from cows. Recent research has demonstrated that the addition of nitrate to cow feed can help decrease the amount of methane produced, and the addition of a nitrite-metabolizing bacteria can prevent nitrite toxicity. *Paenibacillus fortis 79R4* is a rumen-derived novel bacterium that has an exceptional ability to metabolize nitrite. Previously noted strength from a co-culture showed hope for antimicrobial activity against pathogens, which would elevate the use of 79R4. Disk diffusion assays and BLAST analyses showed no production of antibiotics, but the presence of an antibiotic gene. In a combined study, the interaction between 79R4, *Salmonella*, tungstate, and nitrate was studied. The addition of nitrate provides an additional electron acceptor for anaerobic respiration or fermentation and can enrich pathogens in the gut. It was found that tungstate was a significant predictor of *Salmonella* proliferation. Additionally, when 79R4 and *Salmonella* were combined with tungstate, they suppressed each other's growth. The activity showed a competitive relationship between 79R4 and *Salmonella* for tungstate, but confirmation requires further study. These results emphasize the potential of 79R4 as a methane reducing supplement and the protection of the gut against pathogens.

UP9

Prevalence of tetracycline- and cefotaxime- resistant bacteria near water sources at a cow-calf operation in Texas

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Antibiotic-resistant bacterial strains originating from agricultural sites are of significant public health concern. While antimicrobial resistance (AMR) has been more associated with feedlot operations, some herds at cow-calf operations have been documented to have high AMR rates despite comparatively little prior antibiotic exposure. The purpose of this longitudinal project is to survey resistance to tetracycline and third-generation cephalosporins in Enterobacterales isolated from different grazing pastures at the Rhoden Field Laboratory in Abilene, Texas. Soil samples were taken near water sources in six pastures prior to and following the rotation of cattle from February 2024-October 2024. The water sources included two natural sources, six tanks with effluent water, and one tank with municipal water. Following enrichment, samples were serially plated on MacConkey agar with and without tetracycline (16 µg/mL) or cefotaxime (4 µg/mL) and colonies enumerated. The average tetracycline resistance in soil samples was over 100 times greater than that calculated for local non-agricultural environments. Cefotaxime-resistant bacteria were present at varying concentrations in samples from all sites and at a greater prevalence than tetracycline-resistant bacteria. Furthermore, fecal samples were also analyzed for cefotaxime-resistant and tetracycline-resistant bacteria following grazing in different pastures. To date, all fecal samples, except one, were positive for cefotaxime-resistant bacteria. In contrast, tetracycline-resistant bacteria were in much lower abundance in fecal samples, with the exception of one mature cow that was consistently colonized by a tetracycline-resistant coliform. Additional work is necessary to determine if isolated cefotaxime- or tetracycline- resistant bacteria carry clinically relevant antibiotic resistance genes.

UP10

Biodegradation of Triclosan by Crop-Associated Plant Growth-Promoting Bacteria

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Triclosan (TCS), an antimicrobial in commercial products, has been detected in South Texas commercial farm irrigation water, leading to its accumulation in agricultural soils and potential disruption of beneficial microbial communities. Its persistence raises concerns about long-term soil health and the stability of plant-microbe interactions. This study aims to assess the ability of antimicrobial-resistant plant growth-promoting bacteria (AM-PGPBs), to increase phosphate and iron availability and to degrade TCS. Bacterial isolates were obtained from commercial crops (onions, cilantro, and Swiss chard) grown in the Rio Grande Valley after serial dilution of the phyllosphere and rhizosphere on R2A agar amended with antimicrobials (ampicillin, tetracycline and triclosan). Standardized cultures incubated on PVK and CAS agar plates were observed for clear halos or orange halos to identify phosphate solubilizers and siderophore producers, respectively. Growth patterns of isolates were screened on minimal salts medium with triclosan (MSMT, 2 g L^{-1} triclosan) to determine their ability to utilize TCS as a carbon source and minimal salts medium with glucose (MSMG, 2 g L^{-1} glucose) as a growth comparison. Of 24 isolates, 62.5% showed phosphate solubilization, and 79.16% produced siderophores. TCS-resistant isolates showed the highest triclosan degradation potential (60% high growth), while AMP-resistant isolates had mixed results (13.3% high growth), and TET-resistant isolates exhibited the least degradation. Onion-associated bacteria had the highest number of high-growth isolates, suggesting plant-specific microbial interactions influence degradation. Further bacterial identification and soil microcosm studies are needed to confirm their role in triclosan reduction and bioremediation potential.

UP11

Efficacy of Repurposed ClpXP Proteolytic Complex Inhibitors in *Bacillus anthracis* Sterne Strain

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As increasing antimicrobial resistance continues to limit treatment options for bacterial infections, several new approaches have sought to avoid the challenges faced by traditional antibiotics. One such approach is targeting virulence factors, which are necessary for pathogens to evade host defenses and establish infection but not for survival outside the host. This strategy could provide an effective form of treatment while reducing selective pressures for bacteria to evolve resistance mechanisms. Studies have shown that the ClpXP proteolytic complex is essential for virulence in *Bacillus anthracis* and that deletion of the ClpX subunit increases sensitivity to the cell-envelope-targeting antibiotics penicillin and daptomycin as well as the human antimicrobial peptide LL-37. Previously, we used computational modeling to identify commercially available inhibitors of the ClpXP complex and demonstrated that one, ritanserin, mimics the phenotype of a *B. anthracis* Δ*clpX* knockout mutant in antimicrobial susceptibility assays. In this study, we evaluated ritanserin in comparison to four other inhibitors identified during the same screen—siramesine, xaliproden, fluspirilene, and R59022—by determining the fractional inhibitory concentration (FIC) index of each when used in combination with penicillin. Notably, all inhibitors used except R59022 have undergone at least phase II clinical trials for other purposes. We found that two out of the three inhibitors with the highest predicted binding affinity, ritanserin and siramesine, exhibited synergistic interaction with penicillin, while the remainder of the interactions were indifferent. Our results further demonstrate the potential of structural biology techniques to identify and repurpose existing drugs for use as novel antibiotics.

UP12

Identification of Fungal Enophytes of *Euphorbia dentata* and *Euphorbia bicolor* through Phenotyping and Genotyping Methods

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Two Texas native spurges, Euphorbia dentata and Euphorbia bicolor (Euphorbiaceae), known to synthesize bioactive chemicals, were found by our lab to have endosymbiotic fungi in the intercellular spaces of their organs. Plant extracts from these two species have shown anticancer and anti-pain activities. Previously, we morphologically identified nine fungal species. Our main research goal is to identify the fungal secondary metabolites, since pharmacological studies are finding plant endophytic fungi as potential sources for bioactive chemicals. Cultures of endophytic fungi from E. dentata and E. bicolor were maintained through subcultures on V8 agar medium. Fungal endospores and hyphae were phenotyped using Lactophenol Cotton Blue stain on wet mount microscope slides. Fungal genera were identified using spore morphology and hyphae branching characteristics. Multiple species of Alternaria were identified. One fungus could possibly be Calvatia or Bovista. Individual fungal species were freeze-dried, ground to powder, and DNA extracted for genotyping in order to definitely identify the fungal species. However, the yield of DNA after extraction was extremely low. Multiple tests would have to be conducted to achieve a good DNA yield. Optimization of fungal growth using agar and especially liquid media will improve the isolation and chemically characterization of fungal secondary metabolites that could lead to thediscovery of new pharmacologically activeanticancer and anti-pain chemicals.

Pathogenic Microbiology

UP13

Coevolution of *Pseudomonas aeruginosa* and *Drosophila melanogaster* in a Chronic Infection Model and investigation of the Red Queen Hypothesis

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Pseudomonas aeruginosa is a ubiquitous, opportunistic bacterium that provides for both chronic and acute infections in a number of hosts. Acute infection with *P. aeruginosa* is common in healthcare environments, and chronic infection is the leading cause of morbidity and mortality in those with the genetic disorder cystic fibrosis. *Drosophila melanogaster* has the ability to model both chronic and acute bacterial infections, with a comparable innate immune system to that of humans. Using a modified chronic oral infection model, the dynamics of host-pathogen coevolution proposed by the Red Queen Hypothesis can be better understood. Here, host survival is observed in three treatment groups, host evolution, pathogen evolution, and host-pathogen coevolution. Variation in host resistance and virulence have been observed as early as the fifth generation of each treatment group. Additional generations and further study will allow for characterization of host resistance and virulence mechanisms.

UP14

Tight Junction Proteins Redistribution in Calu-3 Epithelial Cells Following Pneumolysin-Induced Damage

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Streptococcus pneumoniae is a pulmonary pathogen that disrupts the lung epithelial lining, traveling to distant anatomical sites, including the central nervous system. Pneumolysin (Ply), a pore-forming virulence factor, causes cellular death at lytic concentrations, while low concentrations allow for recovery. Tight junctions (TJ) help maintain membrane integrity, emphasizing their importance for epithelial barrier stability. While Ply's detrimental effects on TJs are well documented, the mechanisms underlying TJ protein expression and distribution remain unclear. We sought to characterize the dynamics of TJ proteins at the injury and recovery phases. We hypothesized that Ply causes intracellular retraction of the transmembrane protein occludin before translocating back to cellular junctions during recovery. We used the Calu-3 cell line, isolated from lung adenocarcinoma patient (ATCC). Confluent monolayers grown on Transwell® Inserts were treated with 5 µg/mL Ply for 5 hours, followed by toxin-free complete media to allow cell recovery and TJ reassembly. Immunofluorescence was employed to locate junctional proteins occludin and zonula occludens (ZO-1), at 5-, 10-, and 24- hours post-treatment. Our preliminary data show occludin localization both intracellularly and at the cell membrane at 5 and 10 hours post-treatment, while intercellular ZO-1 remains junction-bound. By 24- hours, both proteins colocalized at the cell junctions. Transepithelial electrical resistance (TEER) data reflected tight junction recovery within hours of Ply removal, highlighting a critical window for repair. Understanding TJ proteins dynamics during recovery may reveal the key proteins and signaling pathways involved in barrier restoration, with potential implications for therapeutic strategies targeting pneumococcal infections.

UP15

Assessing the Urinary Tract Infection Potential of Post Menopausal Women Throughout the Day: An Assessment of the Urinary Microbiome and *Escherichia coli* Growth Potential

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Urinary tract infections (UTIs) are the most common bacterial infection. While traditionally considered sterile, the bladder has a few resident microbes which may protect the host from infection. The bacterium *Escherichia coli* is the leading cause of UTIs. Due to the wide genetic diversity in *E. coli* strains, *E. coli* is classified into four relevant phylogenetic groups (A, B1, B2, D). Strains from groups A and D (AD) are relatively less common compared to the more frequently isolated B2 strains which account for 50-67% of *E. coli*-caused UTIs.

How the urine environment, including the microbiome and nutrient levels, fluctuates throughout the day and how *E. coli* responds to these factors are unknown. We hypothesize that one reason why B2 strains are more commonly isolated is because they are better equipped to grow in low-nutrient conditions compared to AD strains. To assess how AD and B2 strains grow in a patient's urine isolated across different time points in a given day, we performed comparative growth curves. We further sought to define the microbiome fluctuations throughout the day, by performing 16S rRNA sequencing on the V3-V4 region of the ribosome.

This work demonstrated that the more pathogenic B2 strains thrive in urine and outcompete other species when nutrients are limited, which could contribute to the effectiveness of B2 strains as pathogens compared to AD strains. Furthermore, we demonstrate that the urinary microbiome remains static throughout most of the day with minor fluctuations in the morning compared to later in the day.

UP16

Characterizing *Coxiella burnetii* transposon mutants during alveolar macrophage infection

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The causative agent of Q fever, *Coxiella burnetii*, is an obligate intracellular pathogen that employs several virulence factors to co-opt host functions during its pathogenesis. The fundamental mechanism supporting its pathogenesis is the Type IVB Dot/Icm secretion system to deliver secreted effector proteins involved in establishing infection and manipulating host immune responses. Previous studies have identified numerous secreted effectors, but the precise role of many effectors is unknown. However, several are hypothesized to modulate innate immune response signaling. Advancements in generating *C. burnetii* transposon mutants for effectors improved the means of characterizing these secreted effectors. This comparative platform of wild-type and mutant *C. burnetii* phenotypes helps predict the function and significance of individual effectors during infection. To approach the question of how *C. burnetii* effector proteins impact innate immunity, particularly the type-II interferon response, this study is focused on characterizing effector mutants predicted to influence this signaling cascade in alveolar macrophages (AMs). Mutants of interest were axenically cultured and quantified, yielding genomic equivalents for comparative challenge. Analyzing AMs infected with *C. burnetii* effector mutants defined effectors/open reading frames that exhibited a growth defect. These results suggest that AMs are capable of attenuating *C. burnetii* when the function of the effector is disrupted and supports the hypothesis that *C. burnetii* effector proteins are involved in the innate immune response. Future experiments characterizing the behaviors of *C. burnetii* effectors in AMs will contribute to defining specific pathways targeted by individual effectors in the innate immune response to *C. burnetii* infection.

UP17

Developing Phage Cocktails for Treatment of AMR-*Staphylococcus aureus* Osteomyelitis

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Osteomyelitis ([OM], bone infections) are a common complication of orthopedic device surgeries, affecting ~200,000 Americans annually. *Staphylococcus aureus* is the most frequent pathogen in these cases, and its high rates of biofilm formation and antimicrobial resistance (AMR) make antibiotic treatment challenging. As resistance to antibiotics spreads across our healthcare systems, bacteriophages, viruses that selectively lyse bacterial cells, are being investigated as an alternative treatment for AMR infections. We are developing anti-*S. aureus* phage therapy for AMR osteomyelitis utilizing poly(lactic-co-glycolic) acid (PLGA) microspheres as a local delivery system. Although we have successfully identified phage species that reduce *S. aureus* biofilm populations *in vitro*, there are several concerns when developing a phage treatment regime. One important consideration is that bacteria can develop phage resistance, rendering therapy useless. This can be avoided using phage cocktails in which multiple phage species with different infection mechanisms are combined so that the bacteria is lysed before resistance develops. We first plan to characterize at least 10 phage species based on their infection mechanisms using two methods: growing bacteria with phage over 24 hours and testing for the occurrence of resistant bacteria in both liquid media and on agar plates. Using this method, we have already identified two phage species that share infection mechanisms. Once the phage have been grouped, we will test the effectiveness of various group combinations against different *S. aureus* OM clinical isolates. We hypothesize that this will increase treatment efficiency as resistance should no longer develop within a relevant time scale.

UP18

Dual Infection of *Campylobacter jejuni* and *Salmonella enterica* in *Galleria mellonella*: A Novel Oral Infection Model

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Campylobacter jejuni and *Salmonella enterica* are two significant pathogens responsible for foodborne illnesses, and understanding their mechanisms of infection individually is essential for improving public health. *Galleria mellonella* (wax moth larvae) has proven to be a valuable model for studying bacterial infections. While typically *Galleria mellonella* is used for injections to model septic infections, we have developed a novel oral gut infection system.

This study aims to investigate the effects of dual infection by *C. jejuni* and *S. enterica* on wax moth larvae. It will focus on immune responses such as melanization (darkening) and behavioral changes like silking or death. The study will also assess bacterial survival within the larvae, monitored over a seven-day period. Signs of infection, including body and midline darkening and abnormal behavior, will be recorded. Additionally, bacterial concentrations will be measured to ensure proper dosing.

We predict that the combination of bacteria will be more lethal than when individually infecting. Investigating the interactions between *C. jejuni* and *S. enterica* in a common host will offer important insights into how these pathogens contribute to disease development and how the host's survival mechanisms are activated. These results could ultimately lead to the development of more effective strategies to prevent foodborne diseases in humans.

UP19

Investigating the Mechanisms by Which Cytomegalovirus Induces Alzheimer's Disease Markers

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In Alzheimer’s disease, there are two major markers: the increased production of amyloid beta peptide and the hyperphosphorylation of tau protein. Our lab has previously demonstrated that infection of cells with mouse cytomegalovirus (MCMV) leads to increased tau phosphorylation, resembling the changes observed in Alzheimer's disease. In this study, we aim to investigate whether MCMV influences amyloid beta levels and to understand what is happening to each of these proteins, as well as the mechanisms behind how the virus causes changes in these markers of Alzheimer’s disease. We hypothesize that kinases play a larger role than phosphatases in the increased phosphorylation of tau and that the levels of amyloid beta peptide will be elevated after MCMV infection of neuronal cells. Methods include mock infection of B35 neuroblastoma cells as a neuronal model, followed by ELISA to test for changes in amyloid beta levels at various times after infection in both cell supernatants and cell lysates. For the phosphorylation of tau, B35 neuroblastoma cell lysates are prepared after either infection with MCMV or mock infection for various time points. Western blot analysis using antibodies specific for kinases which are known to target tau is being done to examine changes in levels. We plan to examine phosphorylation changes to assess activity alterations. As a result we expect to better understand the timing of induction and mechanisms of changes in markers of Alzheimer’s disease following CMV infection to help develop earlier diagnostics or possible treatments for preventions of dementia such as Alzheimer’s disease.

UP20

Influence of Enterococcus on the Environmental Resilience of Campylobacter

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Campylobacter jejuni is the leading cause of diarrheal illness in developed countries, including the United States. It is commonly found in retail chicken products, often coexisting with *Enterococcus* species, which are frequent co-contaminants. *Enterococcus species* are a significant clinical concern due to their higher levels of antimicrobial resistance. Research has shown that *Campylobacter* exhibits enhanced survival in harsh environmental conditions when co-cultured with other common contaminants like *Staphylococcus aureus.* This study aims to investigate how polymicrobial conditions impact *Campylobacter*'s behavior under adverse factors, such as low temperature, aerotolerance, biofilm formation, antibiotic resistance, and low pH, when co-cultured with *Enterococcus faecalis.* We hypothesize that the presence of *E. faecalis* might increase *Campylobacter's* ability to persist in challenging environments. Thus, the study seeks to explore how *Enterococcus* as a co-contaminant might contribute to the resilience of *Campylobacter*, potentially influencing its transmission from farm to fork.

UP21

Characterizing a Putrescine Biosynthetic Mutant in Uropathogenic *Escherichia coli*

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Putrescine is a polyamine shown to play a crucial role in modulating pili synthesis, and consequently, surface motility in *Escherichia coli*. During a urinary tract infection, *E. coli* must attach to the urothelial layer and invade into the host which is facilitated by pili. Furthermore, putrescine increases in abundance in infected urines making the biosynthesis of polyamines a key research topic for these infections. Genes in the two putrescine biosynthetic pathways are increased in the highly pathogenic group B2 clade of *E. coli*. One of these genes, *speB*, encodes the enzyme agmatinase which converts agmatine to putrescine in a single step. We aim to characterize the physiology of a putrescine deficient mutant in two *E. coli* uropathogens by deleting the *speB* gene, hypothesizing the deletion will result in a decrease in pili synthesis and reduced motility. *speB* mutants were constructed in the recently isolated uropathogenic *E. coli* strains KE40 and KE47. Swarming motility assays performed on the two *speB* mutants, revealed a significant decrease in motility diameter compared to their respective wild-types, suggesting decreased pili synthesis. Transmission electron microscopy of these samples confirmed this mechanism, as pili were absent in both mutant strains while their wild-type counterparts displayed abundant pili. Characterizing the physiology of the *speB* mutants revealed that the loss of putrescine biosynthesis disrupts pili synthesis, thus hindering swarming motility. We conclude that putrescine plays an essential role in pili production, and a role in *E. coli* pathogenesis by facilitation of adhesion to the urothelial layer during a UTI.

UP22

Does Exposure to Sub-Lethal Electron Beam Doses Cause Stress-Induced Bacterial Conjugation?

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Bacterial conjugation involves the transfer of plasmid DNA from one donor bacterium to another recipient bacterium through direct contact and is prevalent in environments like soil, water, and biofilms. The mechanism of conjugation mediates the propagation of numerous metabolic features, such as pathogenicity, biofilm formation, and, most critically, resistance to antibiotics. Pathogens such as *Streptococcus pneumoniae, Acinetobacter baumannii,* and *Staphylococcus aureus* found in natural and man-made environments have developed multi-drug resistance. Ionizing radiation technologies such as electron beam (eBeam) and gamma are routinely used in commercial applications, including medical device sterilization, wastewater treatment, and food preservation. There is a high probability that microbial populations will be exposed to sub-lethal doses. We hypothesize that exposure to sub-lethal eBeam doses will influence bacterial conjugation processes. This study investigates how sub-lethal eBeam doses affect bacterial conjugation and transfer of antibiotic resistance-coding plasmids using a model non-pathogenic *Escherichia coli* conjugation system.

Ongoing studies indicate that transconjugants are more resistant to irradiation than separate donor and recipient cells. *E. coli* BB4(F+) is a donor strain carrying a tetracycline resistance gene associated with the fertility (F) plasmid, while *E. coli* SCS1(F-) serves as the recipient with an ampicillin resistance gene. These strains are subjected to sub-lethal eBeam doses ranging from 0.4 kGy to 2 kGy, evaluating the creation of transconjugants exhibiting the tet-amp-resistance phenotype. By examining a range of doses, we can better understand how increasing sub-lethal dose exposure affects bacterial conjugation efficiency.

UP23

Analysis of Plant Extracts for Toxicity to Normal and Cancer Cell Lines

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Various plant extracts have been studied for their potential antimicrobial, antiviral and therapeutic properties. Our initial investigation focused on the discrepancies in results across different studies using plant extracts. We found that extraction temperature, storage conditions and seasonality can play a role in their antimicrobial efficacy. To expand further, and allow antiviral testing, we began to test for toxicity effects of *Croton texensis*, *Maclura pomifera*, *Physostegia virginiana*, and *Lantana urticoides* on J774 macrophage cells. Using microscopy and CellTiter-Glo, which measures ATP production, our initial findings indicate that most of the plant extracts exhibit low to moderate toxicity to macrophages, with the exception of the *Physostegia virginiana*, which was highly inhibitory. These results are not encouraging for further analysis of antiviral activity, if the extracts inhibit cellular metabolism. However, preliminary analysis of the B35 neuroblastoma cancer cell line showed evidence of toxicity to these cells at similar concentrations by a wider array of plant extracts. To further confirm toxicity, the CellTiter-Glo results showed highly reduced ATP production for multiple plant extracts. This suggests that some of these plant extracts may differently impact normal cell lines versus cancer cell lines. We are continuing our experiments with additional normal and tumor cells to determine if this is reproducible beyond these two cell lines. Future analysis for doses which inhibit the tumor cells without inhibiting normal cells as well as identification of the active components has potential to ultimately lead to novel anticancer therapies.

UP24

Immune Response of Lung Epithelial Cells upon Exposure to Sub-Lytic Concentrations of Pneumolysin from Streptococcus Pneumoniae

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Streptococcus Pneumoniae, a Gram-positive bacterium, is a leading cause of community acquired pneumonia. While often asymptomatic in healthy adults, those who are children, elderly, and immunocompromised are at higher risk of severe disease. Pneumolysin (Ply), a pore-forming toxin produced by *Streptococcus Pneumoniae*, disrupts epithelial barrier integrity and induces cell death via pyroptosis/apoptosis. In a preliminary study using Calu-3 lung epithelial cells, exposure to 5 μg/mL of Ply for 24 hours caused an immediate drop in transepithelial electrical resistance (TEER), a measure of membrane paracellular barrier function. Partial recovery of the epithelium to ~60% occurred within 5 hours, followed by a sustained decline over 24 hours. We hypothesized that overproduction of proinflammatory cytokines in response to Ply exposure exacerbates cellular damage, impairs recovery, and ultimately drives cells toward Pyroptosis. We utilized enzyme-linked immunosorbent assay (ELISA) for interleukin (IL) -1β and IL-18, two prominent proinflammatory cytokines involved in Pyroptosis. Calu-3 cells grown on Transwell® inserts were treated with 5 μg/mL of Ply for 5, 10 and 24 hours. The apical supernatants were collected for ELISA. We found that IL-1β levels increased substantially, reaching 16.10 μg/mL at 10 hours and 21.13 μg/mL at 24 hours. IL-18 concentrations also increased substantially, averaging 26.96 pg/mL at 5 hours and 31.91 pg/mL at 10 hours, with a significant p-value of 0.04 (n=3) compared to carrier-treated monolayers. Future studies will include pyroptosis reporter assay and profiling the full cytokines response of Calu-3 cells to Ply toxin using multiarray ELISA.

Molecular & Environmental Microbiology

UP25

Integrative Python Workflow for Comparative Genome Analyses of Shiga toxin-producing Escherichia coli

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Background: As one of the "Big Six" serotypes, O45 Shiga toxin-producing *Escherichia coli* (STEC) is an emerging foodborne pathogen associated with severe human disease. Besides Stx production, another key virulence factor is the Locus of Enterocyte Effacement (LEE). This Pathogenicity Island (PI) is responsible for causing and effacing (A/E) lesions on intestinal epithelial cells, facilitating colonization. To gain insights into the genome make up of this emerging STEC lineage, we sequenced the genomes of 16 O45 strains in collaboration with USDA. **Material and Methods:** We developed a Python-based visualization pipeline that integrates Tkinter, Biopython, and BLASTN to visualize and compare genomes in either linear or circular mode. Our workflow applies user-defined color schemes, shapes, and identity thresholds for comparison. Here, we exemplify this tool by comparing and visualizing the composition of the LEE locus. **Results:** The resulting figure revealed substantial conservation in gene content and organization of this 60 kb PI, integrated into either the *pheU* or *pheV* tRNA**,** and associated with the epsilon-intimin subtype. Automated BLAST comparison with functional color-coding identified the island boundaries, operons (LEE1 to 5), and strain-specific IS element insertions. **Conclusions:** This pipeline enables user-friendly multi-strain comparisons of entire genomes or genes of interest depicted. Future development plans are geared towards the implementation of adjustable font sizes, rotation parameters, and additional shapes to further enhance customization and visualization options.

UP26

Comparative analysis of Campylobacter plasmids: Types and core genes

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Campylobacter remains the leading cause of gastrointestinal disease in the United States. *Campylobacter* plasmids are carriers of antimicrobial resistance genes and virulence genes and have been categorized to multiple groups according to shared core genes in previous study. With the number of available plasmid sequences now more than tripled, the objective of this study is to revisit these previous categories by expanding the analysis and identifying any unknown core genes. From the most updated version of the PSLDB database (v.2024_05_31_v2), 468 circular and complete *Campylobacter* plasmids were annotated via Bakta (v5.1_2024), and pangenome analysis was carried out using Roary (v3.13.0). The plasmids were then categorized according to their genomic content and core genes were identified. The core genes constitute only a small portion of the *Campylobacter* plasmid's genetic content, highlighting the genome's flexibility.

UP27

Influence of Microgravity on Growth Characteristics of *Rhodobacter sphaeroides* and *Campylobacter jejuni*

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The effect of microgravity on several organisms, including bacteria has become a topic of interest due to the progressive intrigue in space exploration. Previous studies on bacterial species, such as *Salmonella* and *Escherichia coli* resulted in a significant difference in cellular growth and development between microgravity and normal growth conditions. *Rhodobacter spaheroides* is a free-living, nonpathogenic bacterium that has a complex genome structure and is capable of extensive metabolic versatility, while *Campylobacter jejuni* is a well-known foodborne pathogen commonly found in poultry and prevalent as a source of widespread infectious diseases. In this study, we investigated the characteristics of growth kinetics and morphology of *R. sphaeroides* (nonpathogenic) and *Campylobacter jejuni* (pathogenic) grown under normal and microgravity growth conditions. The results revealed that *R. sphaeroides* exhibits a significantly increased growth under microgravity conditions, while *C. jejuni* does not show a significant difference in cellular mass between normal and microgravity growth conditions. Ongoing study will examine the effects of microgravity on cellular morphology using scanning electron microscopy (SEM) and analyze differential gene expression under microgravity in these two species.

UP28

Comparative Analysis of Bacterial Contamination in Personal Water Bottles throughout Abilene Christian University: Analysis of Microbial Growth in Water Bottles

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Personal reusable water bottles are seen all around college campuses and come in a variety of models, materials, and types. These bottles allow the users to be environmentally conscious and are convenient due to ease of availability. Water bottles are carried around through many environments and may be placed on a multitude of surfaces. This experiment was performed in order to determine the significance of multiple factors on personal stainless steel water bottles on the campus of Abilene Christian University. Variables tested for significance included type of closure, washing method, time since the last wash, and reported overall health of the bottle owner. Samples were collected by swabbing the inside of 78 bottles from students across campus. After incubation for 48 hours at 37°C, the colony counts were recorded and analyzed based on the respondents' answers to component questions. Once 78 samples were collected and sorted, A/B testing was performed to determine the p-values for variables of straw presence and brand. For the relationship of straw/no straw the p-value of 0.023 indicated there was significance in the increased growth observed with no straw. However for Owala/Stanley brand comparison, the p-value showed no significance. The sample size of 25 and 17 for Owala and Stanley respectively is very small which limits the ability to draw a strong conclusion for these variables.

UP29

Characterization of the Ferric-Cupric Reductase, *FRE5,* as Regulated by the Nonsense-Mediated mRNA Decay Pathway in *Saccharomyces cerevisiae.*

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The nonsense-mediated mRNA decay (NMD) pathway is a conserved pathway in eukaryotes known to degrade mRNAs with premature termination codons, as well as natural mRNAs with unique features such as atypically long 3' untranslated regions (3'UTR). NMD function is also known to be affected by environmental stimuli, such as varying concentrations of extracellular iron. Iron homeostasis is paramount to energy production and oxygen transport in mammals and is relatively underexplored in relation to the NMD pathway. *Saccharomyces cerevisiae* bears extensive gene homology to humans as well as similar NMD mechanisms and iron homeostatic processes. In *S. cerevisiae*, the genes involved in maintaining iron homeostasis are referred to as the Iron Regulon. In iron deficient conditions, transcription factors *AFT1* and *AFT2* activate the Iron Regulon, which includes *FRE5*, a ferric-cupric reductase located in the mitochondrial membrane. Prior research demonstrates NMD-mediated regulation of a related cellular membrane homolog, *FRE2*. Due to the connection between mitochondrial and cytosolic iron homeostasis, it is hypothesized that *FRE5* also experiences NMD-mediated regulation. Preliminary data has since been collected indicating that *FRE5* is sensitive to NMD degradation and is predicted to have an atypically long 3'UTR. Further clarification of the relationship between NMD and iron homeostasis within *S. cerevisiae* will improve our understanding in humans and elucidate potential therapies for iron-related diseases, such as iron-deficiency anemia, Friedrich's ataxia, myopathies, and respiratory complex deficiencies.

UP30

Mercury Reduction Gene merA Detection in Caddo Lake

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Caddo Lake is a cypress-Spanish moss dominated lake in Northeast Texas. Studies have shown that Caddo Lake fish contain a dangerously high level of mercury (Hg), beyond the EPA criterion. Hg pollution in lake sediments is a primary factor contributing to contamination. Lake sediment is contaminated by deposited atmospheric Hg from nearby coal-burned power stations, which undergoes transformations by sediment microbes. The Hg transformation mechanism, particularly the microbial *merA* gene activities, has not been investigated. To address these issues in Caddo Lake, lake sediment samples, collected in 2018 over four seasons in 7 sites, were utilized. Biogeochemical analysis showed an average of 6.47 for pH, 108.51g/kg for total carbon, 33.03 µM for sulfate, and 0.61 mg/g for iron (III). Genomic DNA was extracted from the samples to target the *merA* gene. Seven sets of primers were utilized to detect microbes containing the *merA* gene. Hg analysis was also conducted to detect Hg levels in the samples. It was hypothesized that the lake would contain Hg reducing microbes in at least some of the sites over the four seasons. Initial results showed that multiple sites in Caddo lake contained Hg reducing bacteria with *merA* genes over three of the four seasons. Current Hg analysis studies are underway to understand the total Hg (THg) levels in the sediment itself. In past studies, the average THg level between sites in Caddo Lake was 181.4 µg/kg, with a minimum value of 113 µg/kg and a maximum value of 333.6 µg/kg.

UP31

Escherichia coli populations from the Canada Goose (*Branta canadensis*) microbiome: what do migratory gulls add to the mix?

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Antimicrobial resistance (AMR) is a major global public health threat, complicating infectious disease treatment. Genetic transfer between bacterial cells can spread antimicrobial resistance and other virulence factors, resulting in multidrug-resistant strains. Migratory birds can act as carriers, introducing new bacterial strains into the habitat. We hypothesized that gulls arriving in Wichita Falls each winter play this role and that the virulence factors present in the bacterial population of resident birds would change each time the gulls arrive. For a year, fecal samples were collected monthly from resident Canada geese (*Branta canadensis*), and bacterial isolates, presumably Escherichia coli, were selected. The presence of genes coding for AMR and other virulence factors was screened by multiplex PCR. While initial results suggested that virulence factors were present in some isolates, the absence of an expected amplicon for the *uidA* gene indicated that these bands may have been artifacts, and multiplex PCR required a confirmed identity. Isolates were characterized using a screening protocol based on four phenotypes, which was sufficient to identify an isolate as E. coli. Identity was further confirmed by 16S rDNA sequencing. Of 88 isolates screened, 40 isolates tested positive for all four phenotypes. After identity confirmation using sequencing, the pattern of genes present using multiplex PCR will allow us to group the isolates by pathotype and to determine temporal pattern changes. Understanding how virulence genes are introduced to our local environment will help inform decisions related to public health and antibiotic stewardship

UP32

Genomic Profiling of Enterococcus faecalis from Retail Liver Products Using Whole Genome Sequencing

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Enterococcus faecalis is a facultative Gram-positive bacterium naturally present in the human gut microbiome and poultry. However, it also acts as an opportunistic pathogen, contributing to nosocomial infections and posing a significant clinical challenge due to its high rate of multiple antimicrobial resistance. In our previous study, we detected a high prevalence of *Enterococcus* in retail chicken liver products from Walker County, TX, using standard microbiological methods and PCR confirmation using diagnostic genes. In this study, we sequenced twelve selected strains of *Enterococcus faecalis* using long-read sequencing with Oxford Nanopore Technology to analyze their genomic markers. This research provides valuable insights into the molecular characterization of these pathogens, including their phylogeny, genomic traits, antimicrobial resistance genes, horizontal gene transfer capability, and virulence-associated factors.

UP33

Comparison of Chemically Defined and Complex Media to Cultivate Bacteria from Estuaries and Galveston Bay

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Extreme weather events such as hurricanes, tropical storms, oil spills, and flooding have large impacts on bays and estuaries that are important to their communities. Currently, there is a buildup of pollution within bays and estuaries due to urbanization and human interference with these environments. When extreme weather events occur, pollution overflows these systems and can lead to antibiotic resistant genes, diversity, and bacterial virulence. These alterations in the watersheds surrounding affected areas can impact the health of people in the communities and animals in the environment. In this experiment, water samples will be analyzed and cultured for bacteria in areas that have been affected by extreme weather events using chemically defined medias MR2A, JW1, and JW4. These samples are collected from a less affected area and are compared against samples from a more polluted area. Due to increased diversity and virulence factors, the effects could lead to diseases such as “superbugs” that are harder to treat with common prescribed antibiotics. This relates to the issue in estuaries where prescribed medications are often being thrown away or released in fecal matter which leads to them being introduced into the environment and increases resistance in the microbiome. There will be 16s metagenomic analysis, nutrient and MALDI-TOF analysis for the samples that were collected in the Galveston area. Testing for these factors and antibiotic resistance will be a key indicator to see how the environmental microbiome is impacted by these disturbances.

UP34

Comparing UV-Mutagenized and Evolved Strains of Saccharomyces cerevisiae UCDFSD 09-448 in Ethanol Stress for Enhanced Biofuel Production

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Dependence on petroleum for gasoline is a major contributor to global warming, and alternatives are being sought in order to reduce the impact of fossil fuels. Bioethanol is produced as a byproduct of fermentation by microbes such as *Saccharomyces cerevisiae.* A limitation of S. cerevisiae is its viability in industrial fermentation conditions like high ethanol concentrations, osmotic stress and temperatures, and acidic pH. The Phaff Yeast Culture Strain *S. cerevisiae* UCDFST 09-448 (09-448) possesses a pectinase which can break down pectin, a polysaccharide present in some plant cell walls, into polygalacturonic acid, which makes the cell membrane more permeable to sugars that can be utilized for fermentation.

Mutant 09-448 strains were created via UV mutagenesis, mutants M6 and M12, directed evolution either in ethanol (E1, E2, and E3 EtOH strains), or in sodium chloride (E1, E2, and E3 NaCl strains). The mutant and evolved strains were exposed to varying concentrations of ethanol to determine if they outperformed the wild-type strain using 96 well-plates, and examining the growth of each strain over the course of 48 hours in the Biotek Synergy H1 plate reader. The minimum generation time was calculated to determine if the mutant and evolved strains outperformed the wild-type. However, the minimum generation time for the genetically modified strains was longer than that of 09-448, which is why future studies will be looking into utilizing CRISPR to target specific genes for mutation.

UP35

Shell fungal community of Texas tortoises apparently free of fungal disease

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Necrotizing scute disease (NSD) is a fungal infection in Texas tortoises, which degrades the keratin on their shells. The keratin degrades over time displaying white blemishes on the shells, causing bone to occasionally be seen. Currently our knowledge of NSD is limited to the known causal agent *Fusarium emitectum*. NSD is observed on Texas tortoises along the Texas coast. Chaparral Wildlife Management Area (CWMA) is in Cotulla, TX, and NSD has not been observed on this local tortoise population. To understand the fungal community in tortoises without NSD, we collected samples from wild Texas tortoises at CWMA for the study.

UP36

Quantifying the Class 1 Integron-Integrase Gene to Understand Anthropogenic Environmental Pollution in Baffin Bay

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Baffin Bay provides habitat for wildlife and recreational spaces for Texans. This rural, mixed-use watershed supports extensive cattle ranching and agriculture. However, three major freshwater inflows have been classified as impaired due to high fecal indicator bacteria (FIB) concentrations. An ongoing microbial source tracking project within the watershed examines the contribution of nonpoint fecal pollution sources (human, cow, pig, and gull). This study builds on this work by using the Class 1 Integron-Integrase gene, *intI1*, a mobile genetic element associated with antimicrobial resistance, as an additional marker for anthropogenic pollution. *intI1* was quantified in water samples (N = 142) from Baffin Bay and Petronila, San Fernando and Los Olmos creek using a droplet digital PCR assay. The gene was detected at all sites (mean = 269,079.3 gene copies/100mL, median = 2,800 gene copies/100mL, min = 0 gene copies/100mL, max = 4,749,620 gene copies/100mL) and showed significant spatial variation in abundance. San Fernando creek had the highest quantities of *intI1* followed by Los Olmos, Petronila, and bay sites respectively. The *intI1* gene concentration was also positively correlated with human ($p = 0.0013$), cow ($p = 1.56E-15$), and pig ($p = 9.36E-8$) fecal markers, as well as FIB enterococci ($p = 0.0088$), indicating that *intI1* has broad utility for estimating anthropogenic pollution. Adding the *intI1* component to microbial source tracking studies bridges a knowledge gap between fecal pollution monitoring and antimicrobial resistance genes, and it provides evidence for including *intI1* as an indicator for anthropogenic pollution.

Bacteriophage Microbiology

UP37 - Withdrawn

UP38

Isolation and Characterization of *Mycobacterium* phage Gavriela

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Bacteriophages (phages) are viruses that strictly use bacterial hosts in their replication cycles. These viruses have practical uses vital to human and animal health, including phage therapy, genetic engineering, food safety, and vaccine development. Gavriela was isolated from a soil sample collected at LeTourneau University in Longview, Texas (32.468056° N, 94.725417° W), on August 27, 2024. The enrichment method was used with incubation at 37°C using Middlebrook 7H9 as the medium and *Mycobacterium smegmatis* mc^2 155 as the bacterial host. A spot test was performed to confirm phage presence. 10-fold serial dilutions and three rounds of plating were used to purify the phage. Gavriela's plaques looked cloudy and had an average diameter of 1.12 mm (range 0.6-1.8 mm; n =15). Phage Gavriela yielded a high titer lysate of 1.10 $x10^{11}$ PFU/mL which was used for DNA extraction, TEM imaging preparation, and archiving. Gavriela displayed a siphovirus morphotype. Genome sequencing was done using Illumina MiSeq sequencing with ~1312 shotgun coverage. Annotation was done using various software and databases, including DNA Master, PhagesDB, NCBI, HHPred, Phamerator, Starterator, GeneMark, Glimmer, DeepTMHMM, ARAGORN, and tRNAscan-SE. Sequence data showed Gavriela to belong to subcluster P1 with a 13 bp 3' sticky overhang (CCTGCCGCCCCGA), genome length 47,916 bp, and 67.3% GC content. 77 putative protein-coding genes (size range, 90 to 3651 bp) were predicted. Functions were assignable to only 52% (40/77) of the genes. Gabriela had the three key genes associated with a lysogenic life cycle, namely integrase, immunity repressor, and excise.

UP39

Isolation and Characterization of Mycobacterium phage Izel

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Bacteriophages are viruses that infect bacteria and are important in developing treatments for antibiotic-resistant bacterial infections. Phage Izel was isolated from a soil sample collected at LeTourneau University in East Texas on August 28, 2024. The bacterial host used in isolating Izel was *Mycobacterium smegmatis* Mc^2 155, and the medium was Middlebrook 7H9. After confirming phage presence using a spot test, Izel was purified via 10-fold serial dilutions and 2 rounds of plating with a 48-hour incubation at 37 °C. Izel produced small, round, turbid plaques with an average diameter of 1.02 mm (range 0.7-1.4 mm; n =15). Amplification yielded a high titer lysate of 6.0 $x10^{10}$ PFU/mL. The lysate was used for gDNA extraction, TEM imaging preparation, and archiving. Izel displayed a Siphoviridae morphotype with an isometric capsid (average diameter ~55.5 nm) and a flexible tail (average length ~338.8 nm). The gDNA was sequenced using the Illumina method, with a shotgun coverage of ~2340. Izel's genome was annotated using a range of software and databases, including DNA Master, Genemark, Phamerator, Starterator, HHPred, NCBI, PhagesDB, DeepTMHMM, ARAGORN, and tRNAscan-SE. Using PhagesDB's gene content similarity tool, Izel was assigned to subcluster M1. Preliminary analysis shows Izel to contain 135 putative protein-coding genes and 19 tRNAs. Izel was confirmed to be a temperate phage, evidenced by having a serine integrase gene, but notably lacking other key lysogenic genes; namely the immunity repressor and excise. Izel adds to the pool of phages with potential for future use in therapy and research.

UP40

Isolation and Characteristics of *Mycobacterium* phage Raddgar

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Bacteriophages are important in research, sanitation, and medical practice worldwide. *Mycobacterium* phage Raddgar was isolated from a soil sample collected at the bank of a pond in Longview, Texas (32.4635 N, 94.72586 W) on August 27, 2024, using the enriched method. The soil sample was mixed with *Middlebrook* 7H9 and *Mycobacterium smegmatis* m^2 155 and incubated at 37°C with shaking at 210 rpm for 4 days, then filtered (0.22 µm). The filtrate was used on a spot test to confirm phage presence, evidenced by the appearance of turbid plaques. Plaques picked during purification were ≥ 5 cm from neighboring plaques. Two rounds of 10-fold serial dilution and plating with incubation at 37°C for 48 hours were done to ensure phage purity. The plaque picked from the final round of purification plating was mixed with 100 µL of phage buffer and used to make 8 webbed plates. Each webbed plate was flooded with 5 mL of phage buffer to prepare a high titer lysate. The lysate titer was 2.1 x 10^{10} PFU/ml. The lysate was used for gDNA extraction, TEM imaging, and sample archiving. Raddgar's gDNA awaits sequencing. Based on plaque morphology and TEM image analysis of 6 virus particles, we can conclude that Raddgar is a temperate phage having a siphovirus morphotype with an isometric capsid (average diameter 47.5 nm, range ~48.2 to 55.6 nm) and a flexible tail (average length 136. 3 nm, range ~108.0 to 152.4 nm). Raddgar's lysates were archived at the University of Pittsburg.

UP41

Isolation and Characteristics of *Mycobacterium* phage Athanasius

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Mycobacterium phage Athanasius was isolated from a soil sample collected in Longview, Texas (32.465077 N, 94.727987 W) on August 27, 2024. Following the SEA-PHAGES Phage Discovery Guide, we utilized the enriched method with Middlebrook 7H9 medium and *Mycobacterium smegmatis* mc^2 155 as the bacterial host. Phage presence was confirmed using a spot test, where turbid plaques appeared on the top agar in the areas where 5 µL, 10 µL, and 100 µL of 0.22 µm-filtered samples were applied. We selected a plaque from the 5 µL area and performed 4 rounds of ten-fold serial dilutions with plating on Middlebrook 7H9 top agar and incubating at 37°C for 48 hours. Individual plaques were chosen during purification to ensure a minimum distance of 2 cm from neighboring plaques. The plaques were relatively large, averaging 4.7 mm (range, 3.0 - 6.0 mm, n = 15). The final selected plaque was mixed with 100 µL of phage buffer and used to create 8 webbed plates. Each plate was subsequently flooded with 5 mL of phage buffer to achieve a high titer lysate. The resultant titer was 4.0×10^{10}PFU/mL, from which gDNA was extracted and yielded a total of 6.84 µg with a 57 µg/ml titer. Athanasius's gDNA awaits sequencing. TEM image analysis of 4 virus particles showed Athanasius to have a siphovirus morphotype with an isometric capsid (diameter, ~48.2 to 55.6 nm) and a flexible tail (length, ~108.0 to 152.4 nm). Athanasius's lysates were archived at the University of Pittsburg.

UP42

Isolation and Characterization of *Mycobacterium* phage GoldenChild

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Bacteriophages are viruses that infect and replicate in bacteria. Bacteriophage isolation is important for treating antibiotic-resistant bacterial infections, and for genomics and phylogenetics research. Phage *GoldenChild* was discovered in August 2024, in Longview, Texas on the LeTourneau University campus (32.46165° N, 94.72697° W). *GoldenChild* was isolated using the enriched method, with *Mycobacterium smegmatis* mc^2 155 as the host, following the SEA-PHAGES Phage Discovery Guide protocols. GoldenChild's plaques had a bullseye-like appearance, with an average diameter of 1.7 mm (range 1mm-2mm; n = 15). GoldenChild's lysate had a titer of 1.3×10^{11} PFU/mL, and it was used for gDNA extraction, negative-stain TEM preparation, and archiving. DNA sequencing is still pending, but TEM image analysis of four virus particles revealed this phage as having an isometric capsid (average diameter 51.1 nm; range ~51.1 to 55.6 nm) and a long flexible tail (average length 201.1, ~182.4 to 214.0 nm).

UP43

Isolation and Characterization of a Novel, Lytic Myovirus, *Mycobacterium* phage Jezreel

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Isolation of naturally occurring bacteria-infecting viruses (bacteriophages) is important to treat infections that won't respond to antibiotics or in cases where patients are allergic to antibiotics. *Mycobacterium* phage Jezreel was isolated from a soil sample collected on August 26, 2024, from a turmeric garden near a pond in Longview, Texas (32.52199° N, 94.6983° W). Using the enriched method, the soil sample was mixed with Middlebrook 7H9 broth and *Mycobacterium smegmatis* mc^2 155 and incubated at 37°C for 4 days with shaking at 210 rpm, then centrifuged and 0.22 µm-filtered. Phage presence was confirmed using a spot test. Phage purification was done through 3 rounds of 10-fold serial dilutions and plating. Phage Jezreel's plaques were clear and tiny (average diameter 0.89 mm; range 0.5-1.1 mm; n=15). The lysate titer was 1.10 $x10^{11}$ PFU/mL. The lysate was used for DNA extraction, TEM imaging preparation, and archiving. Jezreel displayed a Myoviridae morphotype. Genome sequencing was done using Illumina MiSeq sequencing with ~3804 shotgun coverage. Annotation was done using various software and databases, including DNA Master, PhagesDB, HHPred, Phamerator, Starterator, GeneMark, DeepTMHMM, and ARAGORN. Sequence data showed Jezreel subcluster C1. Its genome size was 155,660 bp with a circularly permuted end and 64.7% G+C content. Preliminary data predicts 229 putative protein-coding genes and 33 tRNAs and no evidence of the integrase, immunity repressor, and excise genes that are associated with the lysogenic life cycle. Given that it is a lytic phage, Jezreel has great potential for being used in phage therapy.

UP44 - Withdrawn

UP45

A Tale of Two Phages: Isolation and Characterization of Two Novel Caulobacter Phages

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Bacteriophages (phages) are the most abundant organisms on the planet. There are more phages present on Earth than atoms in a single human body. These phages represent a vast amount of genetic diversity and have played a prominent role in uncovering the fundamental mechanisms of their host cell behaviors. Known phages of Caulobacter crescentus provided the tools to study cell division and cell cycle in this model organism. *C. crescentus* relatives with different characteristics in their cell cycle patterns remain relatively unstudied due to a lack of available tools, including phage. One such relative is Caulobacter henricii, a stalked environmental bacterium. Here, we isolated two new bacteriophages from local water samples that infect both *C. henricii* and C. crescentus, suggesting a shared receptor on both hosts. Using plaque propagation, we observed one phage had turbid plaques and the other had clear plaques, indicating they use distinct replication cycles. We used short-read Illumina sequencing results to assemble their genomes and ongoing genome annotation will identify and characterize genes of both known and unknown function. Comparative analysis with previously reported *Caulobacter* phages suggests similarity to other phages in public databases. Further experimental characterization will establish these two phages as new tools for host and viral studies.

UP46

Discovery and Characterization of Maruru, a Novel Arthrobacter Phage

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The discovery of novel bacteriophages provides information that can prove helpful in medicine and agriculture. This study aimed to isolate, purify, amplify, characterize, and annotate a novel bacteriophage that infects the soil bacteria *Arthrobacter globiformis* B-2979. A bacteriophage was isolated from soil samples collected in Waco, Texas. This was done by enriching soil filtrates with the host bacteria in PYD broth before filtering and performing plaque assays. An identified plaque underwent two rounds of purification to ensure only one phage was being studied. This resulted in consistent lytic plaques measuring 1 mm in diameter and the phage was named Maruru. High titer ($5x10^9$ pfu/ml) lysates were created to support additional analysis. Electron microscopy revealed Maruru is the morphotype Siphoviridae, with a capsid size of 61.9 nm and a tail length of 242 nm. DNA was extracted and preliminary cluster determination was made using PCR before being sent to the University of Pittsburgh to be sequenced using the Illumina method. The bacteriophage was determined to be circularly permuted with a genome length of 60191 bp with a GC content of 67.80%. BLAST analysis was used to assign Maruru to the FG cluster. Preliminary gene annotations done by GeneMark and Glimmer within DNAMaster suggest that Maruru has 99 genes and one tRNA. Additionally, Maruru shows high synteny with phages such as Sonali and Mufasa8. Current efforts to manually annotate Maruru are expected to be completed and later uploaded to GenBank, where it can be used to assist scientific explorations.

UP47

Discovery and Characterization of the Novel Arthrobacter phage, Altostratus

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Bacteriophage infect and kill bacteria and exist abundantly throughout the Earth. However, bacteriophage research and their potential real-world application remain largely unstudied. This research was conducted to increase our knowledge of bacteriophage by isolating, purifying, amplifying, characterizing, and sequencing a new bacteriophage. The bacteriophage Altostratus was isolated from loamy topsoil sourced in Celina, TX, using the host bacteria *Arthobacter globiformis* B-2979. Altostratus was then characterized through plaque morphology, electron microscopy imaging, and cluster-typing PCR. DNA was extracted and sequenced, revealing that it belongs to the FS cluster of Actinobacteriophage. The phage produces lytic plaques approximately 1.5mm in diameter. The bacteriophage has a siphoviridae morphology with a capsid diameter of 48.78 nm and a tail length of 150.3 nm. The genome is 39715 base pairs in length with a GC content of 63.70%. Following an initial auto-annotation, 70 genes are predicted. The initial annotation is currently being refined and predicted gene functions are determined using software such as DNA Master, Genemark, BLAST, and HHPred. Data related to Altostratus have been logged into the PhagesDB database. Further analysis of the genetic sequence will continue to provide information on the nature and behavior of the phage. Altostratus's addition to the database will aid our overall understanding of phage genomics and the annotation of other phages yet to be discovered.

UP48

Isolation and Annotation of Cluster EB Bacteriophage Softsoap

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Members of the genus Microbacterium infect fish, humans, and plants and are a potential target for phage therapy. *Microbacterium paraoxydans* outbreaks in farmed Nile tilapia (*Oreochromis niloticus*) contribute to a 40-100% mortality rate in isolated cases. Bacteriophage Softsoap was directly isolated from a soil sample under a Live oak tree (*Quercus virginiana*) in Salado, Texas, and incubated in a related host, *Microbacterium foliorum* NRRL-24224 SEA. Following two rounds of serial dilutions and plaque assays with a soft agar overlay, Softsoap formed small, defined lytic plaques. Negative-staining transmission electron microscopy revealed *Siphoviridae* morphology with an approximate tail length of 155 nm and capsid diameter of 60 nm. Phage DNA was extracted with a modified zinc chloride precipitation method and sequenced to 906-fold genome coverage by the Pittsburgh Bacteriophage Institute using Illumina Next Generation Sequencing. A double-stranded DNA genome of 41,652 base-pairs with a 10 base 3' sticky overhang (TCTCCCGGCA) was determined, making Softsoap the 42nd largest member of cluster EB, with an average G+C content of 66.5% for the cluster, and most closely related to Microbacterium phages BubbaBear (96.06% coverage) and Albedo (93.98%). Whole-genome sequence analysis using PECAAN, PhagesDB, NCBI BLASTn and BLASTp, HHPRED, and TmHmm revealed 71 protein-coding genes transcribed rightwards (94.4%) and leftwards (5.6%). Putative genes include structural proteins, an HNH endonuclease, Holliday junction resolvase, and Cas4 family exonuclease have been identified.

UP49

The Annotation of the BD3 *Streptomyces bicolor* Actinobacteriophage, Pavo

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Pavo, a BD3 sub-cluster *Streptomyces bicolor* actinobacteriophage, was annotated using bioinformatic tools to analyze its genetic composition and functional elements. Its 48,065 bp genome contains 77 genes, with 38 identified functions, a GC content of 65.9%, and no orphams or tRNAs. Comparative analysis identified Conan as its closest relative. Further research revealed 20 conserved motifs, five Rho-independent terminators, and bidirectional promoters. Pavo exhibits a GTG start codon preference (65.4% of genes), and DeepTMHMM identified one potential membrane-associated protein. These findings contribute to understanding actinobacteriophage diversity and phage-host interaction mechanisms.

UP50

Isolation and Annotation of *Streptomyces antibioticus* Bacteriophage Greenbelt

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Bacteriophages, or phages, are viruses unable to reproduce independently; therefore, they must infect a bacterium to replicate via a lytic or lysogenic cycle. Greenbelt, a phage isolated on *Streptomyces antibioticus* UNT16F3, was analyzed for the University of North Texas Science Education Alliance-Phage Hunters Advancing Genomics and Evolutionary Science (SEA-PHAGES) program. Greenbelt was enriched from a soil sample, purified, and amplified to achieve a high-titer lysate of 6 x 10^9 pfu/mL. After DNA extraction, this phage yielded a DNA concentration of 779.4 ng/µl, with a 260/280 ratio of 1.89. The total DNA yield for this phage was 72.8 µg. The purified DNA underwent restriction analysis and gel electrophoresis, producing a fragment pattern that was unique relative to *Streptomyces* bacteriophages previously discovered through the UNT branch of the SEA-PHAGES program. This sample was sent to Pittsburgh Bacteriophage Institute for genome sequencing. Following sequencing, Greenbelt was identified to be within cluster BN with a genome length of 66149 base pairs, and a total GC content of 64.8%. Initially, the autoannotation of the genome identified 168 genes, but this quantity is subject to change as the genome is currently being manually curated.

UP51

Isolation and Annotation of a Novel Subcluster BE1 Phage, Marsus

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Bacteriophages, or phages for short, are viruses that infect bacterial hosts. The phage Marsus was discovered in Denton, Texas in 2019 as part of the SEA-PHAGES program. *Streptomyces sangleri* UNT16F27A was the enrichment host used in this lab. Marsus was enriched, purified, and then amplified to yield a titer of $1.4x10^8$ pfu/mL. Following DNA extraction and sequencing, Marsus was shown to have 134686 total base pairs and a 10982 bp terminal repeat, starting with 249 autoannotated protein-coding genes, 41 tRNAs, and 1 tmRNA. Marsus is from the cluster BE and subcluster BE1. The GC content is 49.5%. This phage is currently under manual annotation review.

UP52

Analyzing the Minor Tail Proteins of A4 Bacteriophages to Determine Product Differences

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The minor tail proteins (MTP) in bacteriophages allows recognition of host and play a role in moving the phage genome into the bacterial host; this associates the minor tail proteins with their primary function of host infection. We observed differences between the products of SenorClean MTP and other A4 members with MTP during annotation. Our experimental design included collecting 3 MTP, using *Phamerator*, from all A4 bacteriophages at the 18-25 Kbp range, which had a product >150 amino acids (AA). The 'draft' A4s were removed leaving 137 phages for analysis. The 3 products were concatenated, meaning products were combined from upstream to downstream, to determine the AA sequence variation in a single product averaged 1,678 residues in length. *Muscle*, the multiple sequence alignment, from EMBL-EBI was used for analysis. A phylogenetic Tree was constructed to determine phage representatives for separated nodes. The shorten list of representative phages numbered 18 and was used to detect amino acid residue differences through an alignment with *Muscle*. There was a range of variability: (1) single AA changes at residues 178 (2) triple replacements at residues 248/250 in phage Stink (3) deletions of residues 449/450 in phages Noelle and Cindaradix (4) insertions of residues 858/863 for multiple phages. The product variation we observed should be followed with alignments of individual gene products to determine which MTP has the most variation and if the variation translates to different protein structures; and thereafter, wet-lab work could determine if there is a difference in host range.

UP53

Isolation, Purification, and Characterization of Phage SenorClean and the use of Bioinformatics in Genomic Analysis in Order to Annotate it's Genome

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Phages offer more than just therapy for antibiotic-resistant bacterial strains; they also are under investigation for being used as transformative agents to circumvent climate change. Our team isolated SenorClean, an A4 Mycobacteriophage with a siphovirus morphotype, at LeTourneau University (32.568397 N, 94.725734 W) from a soil sample using the enriched method with Middlebrook 7H9 complete medium, host Mycobacterium smegmatis Mc2155, and incubation at 37°C. A spot plate was created to test if our sample contained a phage. After positive confirmation, a 10-fold serial dilution was performed through 2 rounds of purification. Phage SenorClean produced small, cloudy plaques (average diameter 3.40 mm, range 3.00 – 4.00 mm; n = 15). Our phage titer was increased by creating webbed plates that ultimately yielded a high-titer phage lysate of 1.1 X 1011 pfu/mL. We extracted DNA from the high-titer lysate which resulted in a DNA extract with a concentration of 171 µg /ml that we calculated based on our gel electrophoresis results. SenorClean's gDNA was sequenced using Illumina Platform sequencing, revealing a GC content (61.5%), genome length (48724 bp), overhang length (10 bp), and the overhang sequence (CGGTCGGTAA). The genome of SenorClean was annotated using various software, including DNA Master and Phamerator that compare gene products with other A4 phages, Lunsford and YoSam321. Notably, the longer minor tail proteins showed differences in homology, which sparked another investigation. The discovery and annotation of SenorClean provided many learning opportunities along with the possibilities of becoming a valuable phage for future research.

UP54

Assessing Cytotoxicity of Bacteriophage Peaches Genes in Mycobacterium smegmatis

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Bacteriophages are viruses that infect and replicate within specific bacterial hosts. Peaches, a bacteriophage isolated and sequenced through the SEA-PHAGES program in 2008 by the University of Louisiana, belongs to the A4 cluster and infects Mycobacterium smegmatis. This study aims to clone and express individual genes from Peaches to assess their cytotoxicity to the host, contributing to understanding phage-host interactions and discovering strategies for controlling pathogenic bacteria. Each gene is cloned using Polymerase Chain Reaction (PCR) amplification and isothermal assembly in the pExtra plasmid. The plasmids are initially transformed into E. coli. Once a sufficiently concentrated plasmid preparation has been verified through gel electrophoresis, Mycobacterium smegmatis is transformed by electroporation, and cytotoxicity assays are conducted using an inducible expression protocol. Cytotoxic protein expression is recognized by the lack of growth of the host when grown in the presence of the induced gene. Each potential protein is also modeled using AlphaFold to add to the possible functions of the bioinformatically predicted genes. Preliminary results indicate that cytotoxicity assays conducted on the first eight genes showed no significant cytotoxic effects. However, by continuing to apply cytotoxicity assays to genes of both known and unknown function, we aim to identify potential cytotoxic genes within Peaches. This research contributes to a deeper understanding of bacteriophage gene function and their possible applications in biotechnology and medicine.

UP55

The Isolation and Characterization of the Bacteriophage 'DuneStem'

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Bacteriophages, a known virus, attack and kill their host bacteria. It has been estimated that there are over 1031 bacteriophages present on our planet; that have been used as vectors for gene therapy, and treatments for antibiotics resistant bacterial infections.

In this study, the isolation of a novel bacteriophage 'DuneStem', initiated with a soil enrichment procedure followed by several experiments to characterize the isolated phage using its bacteria host Mycobacterium smegmatis. A high titer lysate was harvested for isolation of the genomic DNA. This allowed for the restriction digest analysis and genomic sequencing. The phage morphology of 'DuneStem' was studied by transmission electron microscope imaging; utilizing uranyl acetate negative staining. A spot test was performed to isolate the lysogen from 'DuneStem', which was used in a Lysogen Efficiency test.

The plaques of 'DuneStem' indicated a lytic life cycle at the time of isolation. The TEM images show 'DuneStem' has a capsid with a diameter of 81nm, and a tail 217 nm in length. The restriction digest patterns suggested the 'DuneStem' genome contains multiple recognition sites for BamHI, ClaI, HaeIII and few sites for HindIII; while EcoR1 has none. Both the TEM image and restriction pattern imply that 'DuneStem' belongs to the cluster A. The lysogen efficiency test of 'DuneStem' indicated that 73.9% of the host *Mycobacterium smegmatis* could be destroyed by this bacteriophage, which makes 'DuneStem' a potential candidate to develop a phage treatment for pathogenic *Mycobacterium tuberculosis*, a close species to *M. smegmatis*.

UP56

The Isolation and Characterization of the Bacteriophage 'Ditch'

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Bacteriophages are viruses that specifically target and destroy bacteria. It is estimated that more than 10^{31} bacteriophages exist on Earth, and an increasing number of them are being utilized as vectors for gene therapy and as treatments for antibiotic-resistant bacterial infections, in clinical trials.

In this study, the bacteriophage named 'Ditch' was first isolated using host *Mycobacterium smegmatis.* Several experiments were then conducted to study and characterize this phage, including *M. smegmatis* enrichment, spot/streak test, HTL harvest, DNA isolation, Restriction Enzyme Digest (GEL), TEM Imaging, Lysogen Isolation test, Lysogen Efficiency test, Lysogen Antibiotic Resistance Disc test, and Lysogen Immunity test.

The plaque morphology for "Ditch" displays a more lytic life cycle. The restriction digest patterns suggested the "Ditch" has multiple cutting sites for *Hind*III, *Hae*III, and none for *EcoR*I in its genome. The TEM images show the tail of 'Ditch' is estimated to be ~260nm in length, and capsid is ~70nm in diameter. The lysogen of M. smeg post virus infection was isolated and confirmed. The antibiotic resistance test on the lysogen display the decrease on Kanamycin, Novbicine, and Tetracycline compared to wildtype. The lysogen efficiency is 51.4%, indicating half of the host *M. smegmatis* are destroyed by this bacteriophage. This work is published to PhagesDB and "Ditch" can be viewed publicly.

UP57

The Isolation and Characterization of the Bacteriophage 'DuckPond'

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Bacteriophage virus are viruses that infect and replicate only in bacterial cells. In recent years, studies have shown that Phages can be a strong solution to treating antibiotic resistant bacterial infections.

In this study, the isolation of bacteriophage 'DuckPond' was accomplished by bacteria *Mycobacterium smegmatis and a* soil sample from the Corpus Christi Duckpond. Followed by a spot test and streak test to confirm the presence of one single virus. A high titer lysate was harvested for phage genomic DNA isolation, followed by a restriction digest analysis. The phage morphology of 'Duckpond' was then studied by uranyl acetate negative staining and transmission electron microscope imaging. The lysogen of 'Duckpond' was isolated from a spot/patch test with extra incubation time and was used for a phage efficiency and lysogen Antibiotic Resistance study.

The plaques of 'Duckpond' indicated a lytic life cycle. The TEM images show that 'Duckpond' contains a capsid of `70nm in diameter and a tail of `100nm in length. The restriction digest patterns suggested the 'Duckpond' genome contains multiple recognition sites for *Bam*HI, *Cla*I, and few for *Hae*III. The lysogen efficiency test of 'Duckpond' indicated that 91% of the host *Mycobacterium smegmatis* could be destroyed by this bacteriophage, which makes 'Duckpond' a good model to study phage therapy for pathogenic *Mycobacterium tuberculosis infection.* As for the antibiotic disc test, Kanamycin, Tetracycline, and Novobiocin showed a decrease in resistance and Nalidixic Acid and Chloramphenicol showed a increase in resistance. This information was published on PhageDB.org.

UP58

Genetic Diversity of Cluster P Bacteriophage Integrases and Possible Implications on Host Range, Infection Dynamics, Identification, Phylogenetic Analyses, and Gene Therapy

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The bacteria-infecting viruses, known as bacteriophages, are extremely host genus or strain specific. When a temperate phage infects a bacterium, its DNA is integrated into the bacterial genome. This process is facilitated by a phage integrase enzyme, which enables the site-specific recombination of the attachment (*attP*) site on the phage with the target site (*attB*) on the bacterium. Phage integrases therefore play key roles in determining the range of bacterial hosts a phage can infect. Integrases could thus serve as tools for identifying bacterial pathogens, determining host range, and bacteriophage phylogenetic studies. Moreover, since phage integrases facilitate site-specific recombination between different DNA sequences, they have the potential to be utilized in gene therapy and cell line manipulation. To enhance our knowledge of phage host range and infection dynamics, it is necessary to investigate the sequence and structural diversity of phage integrases. Phage integrases from all non-draft cluster P members (P1, P2, P3, P4, P5, P6), including phage Gavriela which we isolated and recently annotated were analyzed. A total of 46 integrases were analyzed. All P phages had tyrosine integrases. Maximum likelihood phylograms were constructed using NGPhylogeny.fr and MEGA12. Using Alphafold 2.0, three-dimensional protein folding models were constructed. Inter and intra-subcluster nucleotide and structural differences were observed among the integrases. Further studies are needed across a wider range of temperate phages to determine the impact of integrase gene diversity and structural conservation on bacterial host range, identification, and phage phylogenetic analysis

GRADUATE POSTER PRESENTATIONS
Pathogenic Microbiology

GP1

Deciphering HCMV US27: Unveiling Control Mechanisms in Endocytosis

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Human Cytomegalovirus (HCMV) is a common pathogen, with nearly 100% prevalence in Africa and Asia and about 80% in Europe and North America. It's often asymptomatic in healthy individuals but can pose serious risks to those who are immunocompromised. The virus's capability to establish lifelong latency and its strategies for manipulating host immune responses underscore its clinical importance. This study focuses on two viral proteins encoded by HCMV, US27 and US28, which play a role in immune evasion and are homologous to host G-protein coupled receptors (GPCRs). GPCRs are the largest family of 7-transmembrane receptors and are critical for various cellular processes. They are also the target of ~30% of FDA-approved drugs. US28, currently being considered as an antiviral target, regulates host signaling and cellular trafficking. It has been studied more than US27, which activates antioxidant genes and enters the endocytic pathway. The regulation of US27 signaling is crucial for HCMV fitness, and it is hypothesized that this signaling is regulated by its endocytosis. We aimed to determine whether US27 interacts with proteins involved in endocytosis and recycling, including β-arrestin, AP-2, NSF, and GABARAP, which is the first step in understanding how US27 enters the endocytic pathway. Using a glutathione S-transferase pull-down assay, we observed that all four proteins bind to the C-terminal tail of US27. Co-immunoprecipitation in mammalian cells confirmed these findings. Understanding these interactions is essential for clarifying how US27 regulates endocytosis and signaling, which is significant for understanding its role in HCMV pathogenesis.

GP2

A Dual-Disease Model Framework: Analyzing Influenza and COVID-19 Co-Infection Dynamics

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Infectious diseases pose a significant challenge to global health. The co-occurrence of various infectious diseases enhances load on the healthcare systems further, adding a level of complexity in their control. The simultaneous occurrence of two infectious diseases Influenza and COVID-19 is well documented in many clinical and epidemiological studies. Though mathematical models have been applied extensively to study single disease transmission, co-infection dynamics are somewhat less studied. To bridge this gap, we developed a new model that integrates two well-established compartmental models: SVIR (Susceptible-Vaccinated-Infected-Recovered) for influenza and SVEA/IHR (Susceptible-Vaccinated-Exposed-(Asymptomatic/Symptomatic)-Infected-Hospitalized-Recovered) for COVID-19. Our SVIR-SVEA/IHR model simulates the concurrent transmission of influenza and COVID-19 in a common population. Unlike previous studies that primarily addressed secondary infections, our model accounts secondary infections and co-infections simultaneously. Using epidemiological determinants from U.S. population, we made an estimate of the effective reproduction number when vaccination is implemented as a preventive tool. Our findings indicate that for containment of co-infection with influenza and COVID-19, at least 54% needs to be vaccinated against influenza (assuming 64% vaccine effectiveness) and at least 74% needs to be vaccinated against COVID-19 (assuming 95% vaccine effectiveness). These vaccination threshold levels could be reduced when additional interventions are implemented along with the vaccination. Although our model is influenza- and COVID-19-specific, it offers a generalizable framework to model other co-circulating infectious diseases, including different strains of the same pathogens. Our model thus facilitates further understanding of co-infections and can be leveraged to address co-circulating infectious diseases in a population.

GP3

Investigating the Growth Kinetics and Plaque Morphology of Diverse Dengue Virus Strains

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Dengue virus (DENV) (Flaviviridae, *Orthoflavivirus*) is the causative agent of dengue fever. Globally, dengue fever affects 400 million people, with 50% of the world population at risk of infection. There has been a dramatic global increase of dengue outbreaks in recent decades, and currently, it is the fastest spreading mosquito-borne viral disease in the world. As of June 24, 2024, more than 9.7 million dengue cases have been reported in the Americas, twice as many as in all of 2023 (4.6 million cases).

DENV is transmitted through the bite of infected mosquitoes, with initial replication occurring in keratinocytes. The virus exists in four distinct serotypes (DENV-1 to DENV-4), with DENV-2 being the most frequently associated with severe disease. Severe dengue, or dengue hemorrhagic fever, is characterized by vascular leakage and primarily affects tropical and subtropical regions, including Southeast Asia, Latin America, and the Western Pacific. Although dengue is endemic in Africa, severe cases are rare.

To elucidate the viral factors contributing to regional disparities in severe dengue, we are studying the differences in the biology of regionally distinct DENV. Our preliminary findings demonstrate a significantly higher viral titer and cytopathic effects on cell monolayer associated with the Southeast Asian compared to the West African DENV strain. Our study's findings will contribute to improving our understanding of the mechanisms by which DENV interacts with the host cells and tissues to lead to disease pathology.

GP4

Harnessing the Power of 10 MeV Electron Beam Technology to Reduce Allergen Levels in Peanuts

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Peanut (*Arachis hypogaea*) allergy is one of the most prevalent and serious food allergies due to the widespread consumption of peanuts. Despite this, no established processing method effectively reduces peanut protein sensitization.

This study aimed to evaluate the effects of 10 MeV Electron Beam treatment at various doses and physical states on reducing or eliminating peanut allergens.
Two grams of each peanut sample were prepared under six different irradiation conditions: dry with shell, dry without shell, immersed in 10 mL of water, and immersed in 10 mL of 20 mM buffer solutions at pH 4.5, 6.5, and 8.5. Each condition was applied to three peanut forms: powder, crushed, and whole. Samples were irradiated at five doses, including a control (0, 5, 10, 15, 20 kGy), with three replicates per combination. The Gold Standard Diagnostics SENSIStrip Peanut Lateral-flow Device was used to detect peanut proteins via immunoassay.

Among all tests, dry samples: whole, crushed, and powdered, treated with doses above 10 kGy consistently fell below the test's detection limit (LOD: 0.17 mg/L), yielding negative results. This suggests a significant reduction in major peanut allergens, making these peanuts potentially safer for allergic individuals. Samples in 20 mM buffer at pH 4.5 also showed promising results at 5 kGy but emitted a rancid odor.

As peanut consumption can trigger severe allergic reactions, including anaphylaxis, decreasing peanut allergenicity is a critical food safety priority amid growing global concerns.

GP5

Characterization of *Staphylococcus aureus* Clinical Isolates from Osteomyelitis Infections

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The rise of antimicrobial resistant (AMR) infections is a growing concern in the treatment of osteomyelitis (OM) and orthopaedic device-related infections (ODRIs). *Staphylococcus aureus* is responsible for 33-43% of these infections due to its prevalence in hospital settings, association with mammalian skin, numerous antibiotic-resistant variants, and ability to form biofilms. Bacteriophage (phage) therapy is now being studied as a treatment for AMR OM/ODRIs. We are investigating the use of phage encapsulated into poly(lactic-co-glycolic) acid microspheres for local delivery to the site of AMR OM/ODRIs infections. We determined that the anti-staphylococcal phage K effectively lyses the *S. aureus* osteomyelitis clinical isolate UAMS-1 growing in liquid culture, on agar plates, and as an *in vitro* biofilm. Our UAMS-1 *in vitro* biofilm model closely mimics the bone infection environment.To validate UAMS-1 as a representative strain, we are characterizing 28 *S. aureus* OM clinical isolates, which includes10 methicillin-resistant strains (MRSA) by assessing biofilm formation, metabolic activity, and antibiotic and phage susceptibility in nutrient broth and artificial synovial fluid. Whole genome sequencing will identify genetic differences related to resistance, virulence, and persistence, and clinical data will help correlate phenotypic traits with infection outcomes. The UAMS-1 strain is resistant to two antibiotics, whereas the recently isolated 10 MRSA strains exhibit resistance against one to ten different antibiotics.This study will determine whether UAMS-1 adequately models clinical MRSA infections and provide insights into biofilm disruption strategies using phages. These findings will inform the development of phage-based therapeutics for AMR OM/ODRIs.

GP6

Metabolically Active yet Non-Viable State of Listeria monocytogenes after Exposure to Sub-Lethal Electron Beam Doses.

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Listeria monocytogenes is a significant foodborne pathogen known to cause severe health issues, particularly in immunocompromised individuals, pregnant women, newborns, and the elderly, with notable resilience at refrigerated temperatures. However, the metabolic state of this pathogen when exposed to sub-lethal doses is unknown. We hypothesize that surviving cells of *Listeria monocytogenes* exposed to sub-lethal eBeam doses will exhibit increased virulence due to enhanced stress responses.

Among the 13 serotypes of *Listeria* monocytogenes, three- 1/2a, 1/2b, and 4b- account for 90% of human infections. Inactivation studies have been performed to determine the D10 value, which tells us the dose at which a bacterial population is reduced by 90%. The D10 values for strains of *Listeria monocytogenes* are 0.3 kGy ± 0.06 for serotype 1/2b (ATCC BAA-839) and 0.4 kGy ± 0.09 for serotype 4b (ATCC 19115). Metabolic activity, indicative of nutrient consumption and potential virulence, remains positive after irradiation. Previous studies have shown that ionizing radiation affects gene expression, particularly in relation to stress response and virulence. We propose to study the gene expression in this pathogen after exposure to sub-lethal eBeam doses and also study virulence using toxin production assays.

Understanding the effects of sub-lethal irradiation on *Listeria monocytogenes* is crucial for ensuring the safety of eBeam or gamma processed foods. Although ionizing radiation significantly reduces bacterial populations, it is extremely important to calibrate the treatment dose such that there are no surviving pathogens. Ultimately, these insights will help enhance food safety protocols and improve public health outcomes.

GP7

Tight Junction Recovery in Pulmonary Epithelial Cells Challenged with Pneumolysin

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Pneumolysin (PLY), a pore-forming toxin produced by *Streptococcus pneumoniae*, disrupts epithelial tight junctions, contributing to lung barrier dysfunction. Our previous findings indicated that 5 µg/mL of PLY disrupts tight junctions, with partial restoration at 5 hours, as indicated by Transepithelial electrical resistance (TEER), western blot, and immunofluorescence imaging. However, tight junction integrity declined again by 10 hours. Based on these findings, we hypothesized that pulmonary epithelial cells (Calu-3) are capable of positively modulating the regulatory pathways in favor of repair and recovery. To address our goals, we used monolayers cultured on transwell plates and confluent monolayers were treated with 5 µg/mL of PLY. After 5 hours of treatment, the toxin was replaced with a complete medium supplemented with serum and antibiotics. TEER and fluorescence imaging were used to assess tight junction integrity and cytoskeleton protein dynamics. We found that TEER gradually improved over 24 hours, correlating with fluorescence imaging analysis of occludin, ZO-1, and F-actin remodeling. Compared to control, intracellular occludin levels increased significantly at 5 hours (±5.16, $p < 0.001$) and 10 hours (±4.74, $p < 0.001$), while junctional occludin increased at 5 hours (±5.28, $p < 0.001$) and improved until we stopped the assay at 24-hour time point. Intracellular ZO-1 remained unchanged, but junctional ZO-1 decreased significantly at all time points ($p < 0.0001$). Notably, F-actin remodeling was evident at 5 and 10 hours. These findings suggest pulmonary epithelium has an intrinsic repair mechanism influenced by its environment. Future research will investigate signaling pathways regulating this recovery.

GP8

A Compendium of Metagenome-Assembled Genomes Recovered from the Urinary Microbiome of Postmenopausal Women

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Recurrent urinary tract infection (rUTI) is a growing clinical concern in postmenopausal (PM) women. Due to widespread antimicrobial resistance, antibiotic therapies can quickly become ineffective. Estrogen hormone therapy is used to manage rUTI in PM women because it may promote putatively protective lactobacilli in the female urinary microbiome (FUM). However, further research is needed to understand the function of the FUM and how it changes during infection and after interventions. Here we present a catalog of metagenome-assembled genomes (MAGs) representing the urinary microbiome of PM women in health and disease. Shotgun metagenomic sequencing was performed on urine samples from 86 women categorized by UTI history and current infection status. The MetaWRAP v1.3.2 pipeline was implemented resulting in 676 bins or putative MAGs, with Kraken2 resolving 324 bins to the species level. Some bins were known contaminants, leading to the development of a parsing script that removed contaminating sequences. *Finegoldia magna* (n=25 bins), a known urogenital microbiome member, was the most common species level MAG recovered. Kraken2 failed to classify a large proportion of urinary MAG sequences, with 271 bins having over 40% unclassified sequences. An NCBI Blastn query taxonomically resolved the unclassified sequences with taxa unique to the FUM. We therefore conclude that the Kraken2 standard database is limited in its utility for FUM MAG classification, likely due to underrepresentation of FUM species. Future directions include identifying biosynthetic and metabolic pathway enrichments and identifying antibiotic resistance genes and mutations associated with the urinary microbiomes of women with rUTI history.

GP9

Investigating the Role of Rhesus Cytomegalovirus (RhCMV) Proteins in Host Cell Signaling

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Human Cytomegalovirus (HCMV), a member of the Herpesviridae family, is highly prevalent in the general population and establishes lifelong latency. Although typically asymptomatic, HCMV can cause serious disease in immuno-compromised individuals including transplant patients and neonates. Cytomegalovirus is highly species-specific making it a challenge to study and develop vaccines in most animal models. Due to its similarities with HCMV, Rhesus macaque cytomegalovirus (RhCMV) has emerged as an ideal model for vaccine studies. One key protein encoded by both HCMV and RhCMV is US28, a G-protein coupled receptor (GPCR) that helps the virus evade immune responses, thereby promoting viral replication and spread. RhUS28, a homolog of HCMV US28, contains 5 gene repeats. Previous studies found that two of these genes, Rh214 and Rh220, promote protective immune responses in RhCMV-based vaccine studies in monkeys. Here, we aim to identify cellular targets of Rh214 and Rh220 in RhCMV-infected cells. The goal is to understand the role of these viral proteins in host cell signaling and protective immune responses, which will help pave the way for a vaccine against HCMV.

GP10

Challenges in predicting zonal trends using a model parametrized with national-level COVID-19 data

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Infectious disease models are vital for public health decision making, but their effectiveness depends on data quality (and the underlying assumptions). During the Coronavirus Disease 2019 (COVID-19) outbreaks, some affected regions had sparse epidemiological data due to various factors. To address this issue, different approaches have been undertaken, including incorporating underreporting and scaling infected fatality rate within the model framework. Often data from different regions are aggregated and country-wide trends are considered as a proxy for regional trends, facilitating an expedient intervention, particularly for regions that lack data. However, studies assessing these aspects are yet lacking. We, therefore, investigated country-wide and zonal COVID-19 trends in India. We first fitted a Susceptible-Exposed-Infected-Recovered-Deceased (SEIRD) model to the COVID-19 data from India and then assessed the predictability of this model across six zones of India. We then fitted the SEIRD model to the COVID-19 data from each zone. We observed that the country-wide model was not able to predict zonal trends in several instances. Our analysis attributed the national-level model's failure to predict the zonal trends to differences in the model parameter values between the national and zonal models. To further assess these limitations, we compared our SEIRD model with the published INDISCIM model, both parameterized on the country-wide epidemiological data. Both models' predictions of the zonal trends were inconsistent, failing to capture trends in cases and deaths in multiple regions. Our findings underscore the need for region-specific models, where feasible, to improve prediction accuracy and guide effective public health interventions.

Molecular Microbiology

GP11

An Assay to measure Resuscitation in Actinobacteria

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It is estimated that 25% of the world's population is infected with latent (dormant) tuberculosis. We define dormancy as Viable But Not Culturable (VBNC) initiated by stress. Actinobacteria which include *Mycobacterium tuberculosis* (MT*),* and *Micrococcus luteus* (ML), are known to exhibit dormancy. Here we use ML and *T. phoenicis* (TP) as substitutes for MT. TP was isolated from NASA spacecraft assembly facility (SAF) clean rooms and can survive stringent cleaning procedures. Dormancy is well studied in ML in which a universal stress protein and a resuscitation factor (RPF) are known to be associated with dormancy. Genes for these proteins are widely found in actinobacteria including TP. We show here that the SAF isolate TP goes into dormancy under nutrient starvation. detailed study of dormancy and especially resuscitation from dormancy requires a reproducible assay. RPF cloned from ML was used to initiate resuscitation of ML and TP in rich and minimal acetate media. Growth curves generated from cells grown in 24-well plates showed reproducible resuscitation of growth dependent on the concentration of RPF. Thus dormant cells of both TP and ML can be revived by supplementing the media with the RPF. Additionally, we observed that bicyclomycin, an inhibitor of the rho transcription termination factor, also resuscitates ML and at sublethal doses as efficiently as RPF. This observation implies that resuscitation is dependent on the inhibition of transcription termination in ML. Dormancy and revival of such strains using RPF has implications for persistence, identification and recovery of such microbes from cleanroom facilities.

GP12

Multiplexing Space Slime: Quantitative insights into a polymicrobial community from the International Space Station

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The success of prolonged human space travel depends on critical life support systems. Onboard the International Space Station (ISS) the clean water supply is maintained by recycling astronaut urine and humidity condensate through the Water Recovery System (WRS). The WRS provides potable water for both consumption and for use in the Oxygen Generator Assembly. A resident five-member mixed bacterial population has persisted in the WRS despite various microbial control methods. The bacteria identified here grow together in polymicrobial communities and have a propensity to form biofilms. In addition to the potential negative impacts on astronaut health, these biofilms need to be closely monitored for their role in malfunctioning spacecraft equipment such as the clogging of WRS lines and filtration units. The aim of this study is to establish a multiplex quantitative PCR (qPCR) assay to both identify and quantify the individual bacterial species in a polymicrobial community obtained from the ISS, to evaluate their relative susceptibility to biofilm control measures. We have identified and validated species-specific gene targets and are in the process of testing specific TaqMan probes for each of the 5 species within our biofilm test model. This assay will drastically reduce the time required for identification from days to hours and will allow us to test the susceptibility and tolerance of individual species within the biofilm to various antimicrobial agents.

GP13

The role of Wag31 biomolecular condensates in mycobacterial polar growth

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DivIVA, also known as Wag31, is an essential pole-localized cytoplasmic protein with roles in polar growth, morphological maintenance, and the recruitment of other cell wall synthesizing proteins in mycobacteria. However, Wag31's molecular mechanisms in polar growth have not been well described. Predicted structure software models show an over 100 amino acid long intrinsically disorder region (IDR) in Wag31 which is favorable for the formation of biomolecular condensates. Our data show that Wag31 from *Mycobacterium tuberculosis* forms condensates *in vitro* in physiological conditions. Biomolecular condensates are membrane-less organelles that help regulate metabolic events and can dynamically alter their properties in different environmental conditions. *In vitro* Wag31 condensates are reversible and reform when favorable biochemical conditions are met. Our preliminary work suggests that changes in the condensate properties of Wag31 could help regulate polar growth in mycobacteria through the delocalization of essential polar cell wall proteins in response to extracellular signals of starvation.

GP14

LPFA1 and LPFA2 Are Essential for enterohemorrhagic Escherichia coli Adhesion in the Zebrafish Intestinal Tract

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Enterohemorrhagic *Escherichia coli* (EHEC) is a foodborne pathogen that causes severe gastrointestinal disease and hemolytic uremic syndrome. EHEC employs multiple virulence factors to colonize the host intestine, including the locus of enterocyte effacement (LEE)-encoded type 3 secretion system, intimin, and flagella, yet the role of long polar fimbriae (LPF) in adhesion and infection remains less well understood. Long polar fimbriae (LPF) can mediate bacterial attachment to host cells in vitro, yet the regulation and function of its two fimbrial operons, *lpf1* and *lpf2* in vivo remain poorly understood. Here, we utilize a larval zebrafish (*Danio rerio*) model of foodborne EHEC infection to investigate their contribution to gut colonization. As a vehicle for foodborne infection, we use Paramecia, a unicellular protozoan and a natural prey of larval zebrafish. We demonstrate that LPFA1 and LPFA2, the major structural subunits of LPF1 and LPF2, are required for efficient bacterial adherence and persistence within the zebrafish midgut. Deletion of *lpfA1*, *lpfA2*, or both significantly reduced bacterial attachment, leading to lower EHEC recovery from zebrafish intestines. Fluorescence imaging confirmed reduced gut colonization by LPF-deficient strains. Ongoing experiments with LPF transcriptional reporters aim to define the spatiotemporal regulation of *lpf* expression in vivo. These findings provide insight into EHEC pathogenesis and may inform future strategies to mitigate bacterial adhesion and infection.

GP15

***Mycobacterium abscessus* resists lunar, Martian, and terrestrial dust exposures in vitro**

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Nontuberculous mycobacteria (NTM) are opportunistic pathogens found in human built and natural environments globally that cause a recalcitrant lung disease in susceptible individuals through inhalation of NTM-laden dust particles. Extraterrestrial dust exposure will be a significant concern for the NASA Artemis Program, a new space mission to establish a sustained presence on the Moon and Mars. We previously isolated viable *Mycobacterium abscessus* attached to ash recovered from the lava fields of the Kīlauea volcano, Hawai'i, an environment like Mars. We hypothesize *M. abscessus* growth is modulated by exposure to volcanic, moon, and Mars dusts.

Four *M. abscessus* isolates derived from volcanic ash, soil, water biofilm, and respiratory tract were tested. $1x10^5$ *M. abscessus* CFU were inoculated into Middlebrook 7H9 culture media -/+ 0.5mg/ml of volcanic ash, lunar, or Martian dust simulants and changes in CFU were tabulated 1, 24, 48, and 96 hours after inoculation.

No significant differences in growth were observed between any of the environmental *M. abscessus* isolates alone -/+ dust at any of the timepoints tested. However, the respiratory-derived isolate exposed to simulated space dusts showed significantly lower CFU at the 24-hour timepoint ($p<0.05$).

In toto, environmental-derived *M. abscessus* isolates were unaffected by dust exposures. In ongoing work, exposure of alveolar macrophages and airway epithelial cells to *M. abscessus* and lunar and Mars dust will help to understand how lung cells respond to these dual exposures, addressing key gaps in our understanding of pulmonary risks for astronauts, including those participating in upcoming missions like the Artemis program.

GP16

DISTANCE BASED ALIGNMENT FREE APPROCH FOR PRION DISCOVERY

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The level of shared sequence similarity among prion proteins is very low and hydrophobicity is the prevalent deciding factor for a protein to be considered as a prion. The primary sequence level contexts of prions, such as Q/N richness, compositional bias, and physicochemical characteristics, are highly variable. Current computational methods for prion identification are limited in their ability to account for such variability and detect prions with high accuracy. We propose a distance-based alignment-free approach utilizing the physicochemical characteristics of amino acids to address the issue. This method entails transforming a protein sequence to a numeric sequence by assigning each amino acid a combined numeric value accounting for its hydrophobicity and molecular weight. A window-based approach allows comparing query sequence within a moving window against prionic template sequences and the similarity or dissimilarity between sequences is quantified based on distance metrics. Our proposed method demonstrated effectiveness in localizing protein fragments imparting prion characteristics and hence can there be used in identifying prions. The applicability of the proposed method is not limited to discovery of prions in yeast only, which was used initially for validation of the method, but can be used to probe the proteomes of other species as well for the presence of prion or prion-like proteins.

GP17

Understanding mycobacterial cell division assembly and regulation.

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Regulation of cell division is important for bacteria to survive the stressful environment they experience inside the host cells. This regulation is carried out by a complex of proteins called the divisome. Bacteria inhibit their division during DNA damage by blocking their divisome assembly. Neither the divisome assembly process nor the regulation of the divisome in DNA damage is fully known in mycobacteria. We have found that the divisome assembly in mycobacteria involves the Actinobacterial-specific cell division protein SepIVA, which is recruited by FtsQ and we have also shown that SepIVA is delocalized in DNA damage, when cell division is inhibited. We performed a screen to identify genes critical for bacterial fitness under DNA damage. Our analysis revealed several distinct factors essential for bacterial survival during DNA damage along with key DNA repair genes.

GP18

The Impact of Rio Grande Irrigation Waters on Antimicrobial Resistance of Cilantro-Associated Bacteria

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Triclosan (TCS), an antimicrobial, has been introduced by crop irrigation from Rio Grande waters. As a major crop production region, the Rio Grande Valley depends on these surface waters. This project aims to assess the impact of irrigation waters on microbial communities of organic cilantro farm samples and to determine if antimicrobial resistance patterns vary with distance from irrigation pipes. Organic cilantro crops were collected mid- and late-season to enumerate heterotrophic plate counts (HPCs), total coliforms, and *E. coli* from water, soil, phyllosphere, and rhizosphere samples. Heterotrophic bacteria were serially diluted onto a low nutrient agar (R2A) to enumerate total HPCs Total coliform and E. coli counts were quantified with the Coililert QuaniTray2000 for the most probable number (MPNs). To determine antimicrobial resistance (AMR) populations, both media were amended with triclosan (6,000 µg L^{-1} TCS) ampicillin (1,560 µg L^{-1} AMP), and tetracycline (3,130 µg L^{-1} TET). Mean HPCs in irrigation water was 6.06 x 10^2 CFU ml-1, while total coliform and *E. coli* were found at a mean of 16 and 11 MPN 100 ml^{-1} irrigation water, respectively. Water samples contained high TCS-resistant HPCs, AMP-resistant total coliforms, and TET-resistant *E. coli*. Phyllosphere and rhizosphere AMR populations primarily consisted of AMP-resistant bacteria. Preliminary results indicate that distance from the irrigation source affected phyllosphere AMR numbers. Our results indicate that antimicrobial exposure in crop systems poses a food safety issue, regarding both AMR development and exposure of AMR bacteria to the consumer.

Post-doctoral Poster Presentations

PP1

Developing a Cross-protective Flavivirus mRNA Vaccine

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Zika (ZIKV), dengue, and West Nile are arthropod-borne flaviviruses that cause >400 million infections annually, leading to significant morbidities. Climate change is increasing vector distribution and outbreak potential. While individual flavivirus vaccines are in development, none offer cross-protection against viruses with overlapping endemicity. Prior studies indicate that ZIKV envelope € protein may protect against other related flaviviruses. This study aims to develop a pan-flavivirus mRNA vaccine targeting the highly conserved flavivirus E protein and assess immunogenicity in mice. Three mRNA candidate vaccines expressing ZIKV E protein and different signal sequences were generated and confirmed by in vitro expression. Constructs were formulated in lipid nanoparticles as singlevalent E or a multivalent vaccine constructs. C57BL/6 mice (n=6) received prime and boost immunizations of singlevalent, multivalent, or eGFP control vaccines 21 days apart. Sera were collected prior to each immunization. Sera and spleens were harvested 21 days after boosting and are currently being tested for neutralizing antibodies by plaque reduction neutralization tests (PRNTs) and T-cell responses by flow cytometry. Neutralizing antibodies against multiple flaviviruses will be evaluated by PRNTs. We anticipate that the multivalent ZIKV E mRNA vaccine will be the most immunogenic by inducing high levels of CD8 T-cells and effective neutralizing antibodies against multiple flaviviruses. If successful, this study highlights the potential for a broadly protective flavivirus mRNA vaccine. Future studies will assess protection against flavivirus infection and identify correlates of protection. The long-term goal is to generate an effective, safe, and cross-protective flavivirus vaccine readily adaptable for outbreak mitigation.

PP2

Harnessing Electron Beam Technology: A High-Impact Solution for Big Six *E.coli* Decontamination in frozen meat products

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Shiga toxin-producing *Escherichia coli* (STEC), particularly the "Big Six" non-O157 strains (*E. coli* O26, O45, O103, O111, O121, and O145), pose significant public health risks through contaminated food, especially meat. Contamination primarily occurs during slaughtering and grinding, leading to Big six *E.coli* outbreaks that can result in severe complications such as Hemolytic Uremic Syndrome (HUS), which may cause kidney failure, particularly in children and the elderly.

In the study conducted in our laboratory, we evaluated the efficacy of electron beam (eBeam) treatment on big six *E.coli* inoculated frozen beef samples. Bacterial survivors from treated samples were enumerated to assess inactivation kinetics. Results demonstrated that even at the lowest dose (3.2 kGy), STEC titers were reduced to below the detection limit (<2.35 Log CFU/25g), achieving a >5.2–6 log reduction at all tested doses (3.2 kGy, 5.2 kGy, 7.3 kGy, and 10.3 kGy). These findings indicate that an eBeam dose of approximately 3 kGy can effectively achieve a ≥5–6 log reduction in Big Six STEC.

These findings highlight eBeam technology as a safe, effective, and scalable intervention for reducing pathogenic contamination in meat products. Its implementation could significantly mitigate foodborne illness risks and enhance food safety, particularly in light of emerging H5N1 in cattle. Integrating eBeam technology into meat processing could be a crucial step toward ensuring public health and food safety.