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ABSTRACT BOOK

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UNDERGRADUATE ORAL PRESENTATIONS

UO1

From Jenner to Energetic Electrons - We Have Come a Long Way for Vaccines

Petra Clark[1,2], Chandni Praveen[1], Muzaffar Ali[1], Suresh Pillai[1]
[1]National Center for Electron Beam Research Texas A&M University, College Station, Texas.
[2]Biomedical Sciences Undergraduate Program Texas A&M University, College Station, Texas

Abstract

Have we come a long way since Edward Jenner's smallpox vaccine? Since Edward Jenner's heterologous smallpox vaccine, viral inactivation technologies have evolved tremendously. Beginning with heat-killed viruses, which produce inconsistencies in serological assays critical for vaccine development. Chemical inactivation damages the structural proteins associated with antigenicity and requires downstream processes to remove chemicals. To overcome these challenges, gamma inactivation does not require downstreaming processes, but it has nuclear security implications. Alternatively, eBeam technology eliminates the need for radioactive disposal, regulatory constraints, or downstream processes. Electron beam technology involves the use of commercial electricity to generate energetic electrons. This study investigates the effectiveness of eBeam technology as a sustainable alternative to gamma irradiation in viral inactivation.

Human rotavirus and influenza A virus preparations were subjected to high-energy (HEEB), medium-energy eBeam (MEEB), and gamma irradiation under defined target doses (0, 2, 4, 6, 8, and 10kGy).

Both medium and high-energy eBeam technology were effective at viral inactivation. The D10 values for HEEB (1.25 ± 0.25), MEEB (1.29 ± 0.22), and gamma (1.03 ± 0.06) were found to be comparable for the rotavirus inactivation. For influenza A virus, the D10 values were found to be 1.95 ± 0.04 for HEEB, 2.27 ± 0.01 for MEEB, and 1.62 ± 0.003 for gamma irradiation. These findings clearly show that eBeam technology is an effective and sustainable alternative to heat, chemicals, and radioactive cobalt-60-based gamma technology for vaccine development. We have come a long way since Jenner’s vaccine approach!

UO2

Interkingdom Interactions as a Platform for Antifungal Drug Discovery

Vina Manouchehri, Jesús A Romo
University of Texas at San Antonio, San Antonio, Texas

Abstract

Introduction: While we are just beginning to understand how *bacterial* microorganisms communicate with each other, interactions between bacteria and fungi are underexplored. Previous studies, including from our own group, have shown that *C. albicans* interacts directly with bacteria and these interactions can be synergistic (e.g. *Streptococcus mutans*) or antagonistic (e.g. *Pseudomonas aeruginosa*).

Hypotheses: We hypothesize that defining interkingdom interactions, can reveal novel therapeutic targets. The goal of the present study is to co-culture a library of bacterial species with *C. albicans* to identify interkingdom interactions.

Methodology: *C. albicans* SC5314 was co-cultured with 18 distinct bacterial strains using a biofilm formation assay in 1:1 media (TSB:RPMI). *C. albicans* (2×10^6 cells/mL) was cultured with a 1:20 or 1:200 dilution of each bacterium and seeded into 96 well plates. Plates were incubated for 24 hours at 37 °C in 5% CO_2. Biofilm biomass was quantified using the Crystal violet assay.

Results: We have identified interactions that enhance or prevent overall biofilm biomass with some recapitulating previously reported findings (e.g. *Enterococcus faecalis* and *Streptococcus pyogenes*) while others are novel phenotypes (e.g. *Alcaligens faecalis*). All interactions reduced or fully inhibited *C. albicans* filamentation, underscoring their complexity.

Conclusions: Our findings i) underscore the value of our screening platform as several interactions recapitulate previous published findings, ii) have identified multiple interkingdom interactions that inhibit *C. albicans* filamentation, a key virulence factor. In future studies, we will test distinct culturing conditions (e.g. media) and characterize interactions in the context of mature *C. albicans* biofilms.

UO3

Effect of Enterococcus faecalis Coculture on Eschericha coli Growth and Physiology

Akhil M Nelapolu[1], Jacob B Hogins[1], Yihan Liu[1], Shreya Mekala[1], Jose Ramirez[1], Mithun Mekala[1], Satya Vedula[1], Marjan Ganjali[1], Philippe E Zimmern[2], Lydia Freddolino[3], Larry Reitzer[1]

[1]The University of Texas at Dallas, Richardson, Texas. [2]UT Southwestern Medical Center, Dallas, Texas. [3]University of Michigan Medical School, Ann Arbor, Michigan

Abstract

Urinary tract infections (UTIs) are among the most common community-acquired infections, with Escherichia coli causing ~75% of cases, approximately half attributed to phylogenetic group B2 strains. Non-B2 E. coli (Ec) strains are frequently co-isolated with Enterococcus faecalis (Ef), prompting investigation into their interaction. We characterized the interaction between three co-isolated Ec-Ef pairs and other Ec-Ef combinations in a nutrient-rich medium which was intended to emulate the rapid growth condition of the bladder. In this medium, Ef had little effect on Ec growth but resulted in major transcriptome differences. Non-B2 strains upregulated genes involved in ornithine degradation via putrescine to succinate, feeding into the TCA cycle, while B2 strains showed increased transcripts for macromolecular synthesis and similar metabolic pathways, excluding putrescine-to-succinate degradation. Notably, both groups exhibited upregulation of glyoxylate shunt enzymes, suggesting that the disparate and constantly changing environments encountered during UTI lead to the potential for multiple types of Ec-Ef interactions, and these results provide strong evidence for nutrient cross-feeding as one such mechanism.

To determine whether these findings extend beyond the urinary tract, we cocultured three urinary Ef isolates with E. coli derived from IBD patients and assessed competitive outcomes via overnight coculture and selective plating. Contrary to expectations, IBD-derived *E. faecalis* outcompeted *E. coli* across all pairings, suggesting that the rich gut environment may select for a less aggressive *E. coli* phenotype compared to the nutrient poor bladder. Future studies will explore the underlying mechanisms and therapeutic implications for IBD pathogenesis.

UO4

The Role of Genetic Differences and NMD-Dependent Regulation on Cadmium Detoxification in Saccharomyces cerevisiae

Hannah J Ohms, Sunday Olaniyan
Baylor University, Waco, TX

Abstract

Heavy metal detoxification is a crucial mechanism for survival of living organisms. Cadmium (Cd) is a heavy metal and toxic environmental contaminant that disrupts cellular homeostasis in eukaryotic cells. Cd tolerance in yeast *Saccharomyces cerevisiae* is dependent upon PCA1, a P_{1B}-type cadmium-transporting ATPase, which acts as a plasma membrane cadmium efflux pump. However, the extent to which Cd detoxification in yeast is influenced by post-transcriptional regulatory pathways such as nonsense-mediated mRNA decay (NMD) remains unclear. In this study, we examined Cd tolerance across three distinct yeast strains (BY4741, W303a, and RM11-1a) and the role of NMD in regulating these strain-specific phenotypes. Previous growth assays revealed distinct differences in Cd tolerance between these strains, with RM11-1a exhibiting robust tolerance compared to W303a's increased sensitivity when grown across increasing Cd concentrations. Additionally, when NMD factor *UPF1* was deleted in a BY4741pca1Δ background, Cd tolerance was enhanced, suggesting the activation of compensatory detoxification pathways in the absence of PCA1-mediated Cd efflux. To understand the genetic basis for these differences, we will introduce PCA1 alleles from RM11-a and W303a into the NMD mutant BY4741 background and assess the Cd tolerance of each variant under increasing Cd concentrations to see if these allele-specific differences are sufficient to shift the BY4741 phenotype towards the tolerance of RM11-1a or the sensitivity of W303a. The results will illuminate how NMD-dependent regulation and genetic background interact with each other to shape the cadmium tolerance and detoxification capacity of *S. cerevisiae*.

UO5

Root endophytic fungi from marshes: Growth and performance under salinity stres

Linzee D Pacheco, Candice Lumibao
Texas A&M university Corpus Christi, Corpus Christi, Texas

Abstract

Fungal endophytes are types of fungi that colonize and inhabit plant root cells without causing any damage or harm to the plant. Some endophytes provide plants with benefits such as stress tolerance, while the plants provide the endophyte with shelter and nutrients. Despite their importance to host plants, knowledge of fungal endophytes' ecology and biology, for instance, their salinity tolerance, remains limited. In this study, salinity assays were conducted to test growth of different fungal endophyte species/strains previously isolated from the roots of *Batis maritima*, a halophyte plant in Oso Bay salt marshes in South Texas. Five strains were re-cultured onto Malt Extract Agar (MEA) and then grown across a range of salinity concentrations with seven replicates for 84 days. Using ImageJ analyses, weekly hyphal radial and colony growth were measured. Results indicate that all strains showed the fastest and highest growth in 20ppt salinity levels compared to others. For example, by Day 35, most of the strains have mean area growth of ~0.06 cm^2. However, there were intraspecific differences in the patterns of growth: BM12-R4 strain outcompeted others regardless of salinity levels. Taken together, the root endophytes demonstrated high salinity tolerance, likely contributing to the salinity tolerance of the host plant, *B. maritima*. Understanding how fungal endophytes adapt under saline conditions will allow for potential applications for agriculture such as growth promotion, soil health, and the aid of marsh ecosystem management.

UO6

Identification of HLA-I Presented Peptides in *Trypanosoma cruzi*-Infected Human Cardiomyocytes

Jacob J Williams[1,2,3], Leroy Versteeg[2,3], Jong Min Choi[4], Jeroen Pollet[2,3]
[1]Rice University, Houston, Texas. [2]Department of Pediatrics, Baylor College of Medicine, Houston, Texas. [3]Texas Children's Hospital Center for Vaccine Development, Houston, Texas. [4]Lester and Sue Smith Breast Center, Baylor College of Medicine, Houston, Texas

Abstract

Trypanosoma cruzi (*T. cruzi*) is a protozoan parasite that infects an estimated 6-7 million people, predominantly in low-income, rural regions of South America and Mexico. Chronic infection can lead to severe and potentially fatal complications, most notably chronic Chagas cardiomyopathy. Currently, no clinically approved vaccine exists to prevent Chagas disease, and developing an effective vaccine is challenging due to the parasite's complex, multi-stage intracellular life cycle. To identify improved vaccine targets, we applied mass spectrometry-based immunopeptidomics to characterize *T. cruzi* peptides presented by human leukocyte antigen class I (HLA-I) molecules on infected human cardiomyocytes (AC16). Cell lysates from infected cultures were then purified and analyzed by liquid chromatography-tandem mass spectrometry (LC-MS/MS).

By comparing our data with the *T. cruzi* proteome, we identified 105 peptides, many of which originated from *T. cruzi* proteins with unknown functions. Interestingly, in this study using infected human cells, we identified multiple *T. cruzi* peptides derived from the Tcj2 protein, consistent with previously published murine immunopeptidomics data. These results further support Tcj2 as a promising vaccine target, as its peptides are presented by major histocompatibility complex class I (MHC-I) molecules across diverse cell types and species. Furthermore, we identified four *T. cruzi* peptides that are predicted to cross-bind with two or more HLA types using online prediction tools, making them potential broad-spectrum targets for vaccines or diagnostics.

This work highlights the utility of immunopeptidomics and contributes to the broader effort to develop vaccines for complex neglected tropical parasitic diseases that disproportionately affect underserved populations worldwide.

GRADUATE ORAL PRESENTATIONS

GO1

Impaired HIF-1α stabilization underlies syncytiotrophoblast-mediated restriction of *Toxoplasma gondii*

Dawood Ackom Abbas, Lamba Omar Sangare
Texas A&M University, College Station, Texas

Abstract

Toxoplasma gondii (*T. gondii*) is an obligate intracellular parasite that exploits host hypoxia-inducible factor 1α (HIF-1α) to promote intracellular replication. Although fibroblasts readily support parasite growth, human syncytiotrophoblasts (STBs), the multinucleated cells that form the placental barrier, restrict replication through a mechanism that remains unclear. We hypothesize that STB-mediated restriction reflects the parasite's failure to activate the HIF-1α signaling pathway. Using trophoblast stem cell-derived STBs, we demonstrate that *T. gondii* fails to stabilize HIF-1α in STBs, in contrast to human foreskin fibroblasts (HFF). Inability to stabilize HIF correlates with reduced tachyzoite replication and increased formation of the parasite's dormant stage, called cyst. We found that HIF-1α stabilization with $CoCl_2$ significantly reduces cyst formation. To explore potential host determinants, we measured mRNA expression of host factors linked to parasite-mediated HIF-1α regulation. Compared to HFFs, STBs showed reduced transcript levels of prolyl hydroxylase domain protein 2 (PHD2) and activin receptor-like kinases (ALK4/5/7), important host factors *T. gondii* exploits to mediate HIF-1α stabilization during infection. These findings suggest that STBs possess a distinct signaling landscape that limits parasite-driven HIF-1α stabilization. Together, our findings reveal a distinct cell-intrinsic defense mechanism that impairs *T. gondii*'s capacity to replicate and colonize the placenta during pregnancy.

GO2

Role of Beta-2 Adrenergic Receptor Signaling in Streptococcus pneumoniae Infection of the Blood Brain Barrier

Taryn E Keyzer, Brandon J Kim
The University of Texas at Dallas, Richardson, TX

Abstract

The Blood Brain Barrier (BBB) is a highly specialized cellular barrier composed of brain endothelial cells (BECs) that prevent the passage of toxins and pathogens into the Central Nervous System (CNS). *Streptococcus pneumoniae* (pneumococcus) is the leading cause of bacterial meningitis world-wide, however, the mechanisms through which the BBB fails during pneumococcal meningitis remains poorly understood. Our lab models the BBB using induced-pluripotent stem cell-derived brain-like endothelial cells (iBECs), as they recapitulate important barrier properties such as tight junctions and transendothelial electrical resistance (TEER). A human population-based study determined that patients with the single nucleotide polymorphism (SNP) rs1042714 in the beta-2 adrenergic receptor (B2AR) were more susceptible to pneumococcal meningitis than patients with wild type B2AR. We therefore hypothesize that B2ARs play an important role in pneumococcal interaction and disruption of the BBB. To initially test this hypothesis that B2ARs play a critical role, we employed well described FDA approved beta-blockers. When we treated iBECs with the pan beta-blocker propranolol, we observed a significant decrease in pneumococcal invasion. Treatment with the beta-1 adrenergic receptor inhibitor metoprolol did not result in a change in pneumococcal invasion of iBECs. Recently, we have also generated a rs1042714 knock-in induced pluripotent stem cell line which is being characterized and used in pneumococcal adherence and invasion assays after differentiation into iBECs. Here, for the first time, we are leveraging population-based studies, CRISPR technologies, and our stem-cell based models to discover a mechanistic role for host genetic variation and its contribution to bacterial meningitis.

GO3

Urea induces a hormetic response in group B2 uropathogenic *E. coli*

Yihan Liu, Jacob B Hogins, Lawrence Reitzer
University of Texas at Dallas, Richardson, Texas

Abstract

Escherichia coli (*E. coli*) is commonly found in the intestines of humans and animals. *E. coli* strains fall into several phylogroups, including A, B2, and D, each characterized by distinct genotypes. In addition, 50% of the core genes are differentially transcribed between B2 and groups A and D. Phylogenetic group B2 of *E. coli* is associated with several pathologies and is the most common causative agent of urinary tract infections (UTIs). Our long-term goal is to understand the basis for the dominance of B2 strains in UTIs.

During UTIs, uropathogenic *E. coli* moves from the bladder to the intracellular environment of the bladder epithelium -- moving from a urea-containing to a urea-free environment. Almost nothing is known about the effects of urea on gene expression. Our goal is to determine whether urea differentially affected gene expression between group B2 and A strains.

To test this hypothesis, we performed RNAseq on cultures with and without urea in two strains KE21 (B2) and W3110 (A). KE21 responded to the urea by increasing the amount of transcripts of biosynthetic arginine decarboxylase -*speA*, magnesium-transporting ATPase -*mgtA*, and genes related to stress responses (*dnaK*, *rpoE*), genomic stability (*gyrA*, *glnA*) and energy generation (*atp* & *sdh* operons). However, the transcriptome of W3110 remained largely unchanged by the urea addition. These results suggested that urea differentially affected gene transcription between B2 and A strains. Urea induced a hormetic response to stress in B2 strains that primes cells to additional stresses, and potentially contributes to their virulence.

GO4

DEVELOPING AN IMMUNOGENIC AND CROSS-PROTECTIVE FLAVIVIRUS MRNA VACCINE

Maxwell A Ross[1,2], Megan N Carey[2,3], Allyson N Hildago[4,5], Daniel P Rehm[4,2], Jeroen Pollet[6,7], Athos S.D. Oliveira[2,6], Jennifer L.S. Clinton[8,2]

[1]Graduate School of Biomedical Sciences, Baylor College of Medicine, Houston, TX. [2]Department of Pediatrics, Division of Tropical Medicine, Baylor College of Medicine and Texas Children's Hospital, Houston, TX. [3]National School of Tropical Medicine, Baylor College of Medicine, Houston, TX. [4]William T. Shearer Center for Human Immunobiology, Texas Children's Hospital, Houston, TX. [5]Department of Pediatrics, Division of Neurology and Developmental Neuroscience, Baylor College of Medicine and Texas Children's Hospital, Houston, TX. [6]Center for Vaccine Development, Texas Children's Hospital, Houston, TX. [7]Department of Medicine, Section of Immunology, Allergy, and Rheumatology, Baylor College of Medicine, Houston, TX. [8]Department of Molecular Virology and Microbiology, Baylor College of Medicine, Houston, TX

Abstract

Zika (ZIKV), West Nile (WNV), and dengue are positive-stranded, RNA flaviviruses transmitted by arthropod vectors, including *Aedes* and *Culex* mosquitoes. Collectively, flaviviruses cause >400 million acute infections and >25,000 deaths annually. Clinical disease ranges from flu-like symptoms to severe neuroinvasive (encephalitis, meningitis), hemorrhagic, or congenital (microcephaly, miscarriage, stillbirths) outcomes. Licensed vaccines remain unavailable for most flaviviruses, including ZIKV and WNV, leaving supportive care as the only option. This project seeks to develop an mRNA vaccine that elicits both humoral and cellular immunity and confers cross-protection against multiple flaviviruses. Our vaccine candidates target the ZIKV envelope (Env) protein, a highly conserved antigen, to enhance immune cross-protection. Five ZIKV Env mRNA constructs containing different signal sequences were generated and formulated in lipid nanoparticles. Protein expression was confirmed *in vitro* by Western blots and immunofluorescence. Immunogenicity was evaluated in C57BL/6 mice (n=30) using a prime-boost regimen, with sera and spleens collected three weeks post-boost. Flow cytometry of splenocytes revealed that Env-vaccinated mice mounted significantly higher antigen-specific CD4+, CD8+, and IFN-γ producing CD8+ responses compared to controls. ELISA quantification demonstrated Env-specific antibody responses, with 2 of 5 vaccine groups showing approximately threefold higher titers compared to controls. To assess neutralization, we plan to conduct plaque-reduction neutralization tests using pooled serum from these groups. Future studies will evaluate efficacy in a ZIKV challenge in immunocompromised mice. As climate change drives mosquito expansion into new regions, a cross-protective vaccine would help mitigate emerging outbreaks, protect vulnerable populations, and reduce the global burden of multiple flavivirus infections.

EDUCATION ORAL PRESENTATIONS

EO1

Socio-scientific Topics in the Microbiology Classroom

Stacy Vasquez
Dallas College, Dallas, TX

Abstract

The use of culturally responsive teaching practices in STEM has been shown to increase student engagement, performance, and retention in the classroom and lab. This oral presentation will cover various socio-scientific topics that have been integrated into a microbiology course at a two-year college. In addition to focusing on the objective, face-based content, socio-scientific topics were integrated to make the course more relevant to the students' lived experiences. The presentation will also address the following: 1) hesitancies to integrating such topics into the microbiology classroom, and 2) rebuttals to criticisms related to culturally responsive teaching. As educators aim to increase interest in STEM and encourage traditionally underserved students toward careers in STEM, it is vital they utilize culturally relevant instructional practices to assist them in achieving their goals.

EO2

Toward a Common Vision: Faculty Consensus Building for Introductory Biology Learning Objectives

Alex R Paine[1], Jonathan Lawson[2], Christopher Lopez[2]
[1]Baylor Univeristy, Waco, Texas. [2]Baylor University, Waco, Texas

Abstract

Across higher education, introductory biology courses are frequently taught by multiple instructors, yet many departments lack a coordinated, department-endorsed set of learning objectives (LOs). While national frameworks articulate what introductory biology should include, fewer studies document how departments move from variation to shared LOs.

The Department of Biology at Baylor University, a large R1 research university serving approximately 1,200 biology majors, offers introductory biology courses taught by as many as 13 faculty members per semester, creating substantial potential for variation across sections. To initiate a structured consensus-building process, we developed and distributed a survey based on nationally endorsed lesson-level LOs. Faculty indicated whether they currently teach each objective and rated its importance for inclusion in the introductory sequence.

Approximately 64% of department faculty participated, providing a snapshot of shared and divergent priorities that served as the foundation for department-wide dialogue. Over multiple faculty meetings, instructors collectively reviewed survey findings and engaged in structured discussion to determine which objectives should be retained, revised, removed, or added. These conversations clarified shared expectations for the introductory sequence and prompted increased cross-course communication, including discussions extending into upper-division courses. Through iterative refinement, faculty are collaboratively working toward selecting a unified set of LOs to be implemented within the introductory biology series.

This presentation will outline the steps of our consensus-building process and present a practical model for fostering shared vision and curricular coherence in multi-instructor gateway STEM courses. The approach may be especially valuable for departments seeking structured, data-informed pathways to curricular alignment.

EO3

The Power of Community in Biology Education

Lee Hughes
University of North Texas

Abstract

Academic careers are often thought of as having a traditional path that most faculty will follow, yet in reality there are many unique ways that individuals enter and navigate through their academic careers. Along the path, I benefited from several supporting and collaborative communities. There is power in finding your community and my successes in microbiology education and research are directly related to the communities to which I belong. I will share how communities have supported me in my biology education career.

HIGH SCHOOL POSTER PRESENTATION

HP1

Longitudinal surveillance of acetic acid bacteria (AAB) and lactic acid bacteria (LAB) in a Californian winery during the 2025 harvest season

Julian Gill[1], Ethan Wang[1], Justin Boeckman[2,3], Mei Liu[2,4]

[1]A&M Consolidated High School, College Station, TX. [2]Center for Phage Technology, Texas A&M University, College Station, TX. [3]Inphasol Inc., College Station, TX. [4]Department of Biochemistry and Biophysics, Texas A&M University, College Station, TX

Abstract

Spoilage of wine from volatile acidity or off flavors caused by uncontrolled growth of acetic acid bacteria (AAB) and lactic acid bacteria (LAB) results in significant economic losses. We aim to survey the longitudinal diversity of AAB and LAB throughout the wine processing stages, to evaluate the potential of phages as targeted microbial control. A total of 23 Cabernet Sauvignon samples were collected from a winery in Livermore, California during 2025 harvest season, covering grape rinse (4 samples), juice/must (7 samples), the end of primary fermentation (6 samples) and malolactic fermentation (6 samples). The samples were enriched and analyzed for bacterial microbiome composition via 16S amplicon sequencing, and individual AAB and LAB were isolated by plating the samples on selective media followed by 16S sequencing. *Acetobacter oryzoeni* was the dominant AAB throughout grape rinse, juice/must, and primary fermentation comprising over 50% of the population in each sample, followed by *Acetobacter pasteurianus* at 13-23% of the population. *Lactococcus lactis* dominated the LAB in the grape rinse, juice/mast samples and *Lactiplantibacillus plantarum* at the end of primary fermentation (both > 34% in each sample). *Oenococcus oeni* took over at the end of the malolactic fermentation (97% of the total population) due to its inoculation by winemakers as a desired strain for this process. A collection of 100 AAB and LAB isolates were established, covering the dominant species reported by microbiome results. In conclusion, our results identified the key AAB and LAB players as potential targets for phage as precision control.

UNDERGRADUATE POSTER PRESENTATIONS

Antimicrobial Microbiology

UP1

Exploring Ficus carica and Psidium guajava as antimicrobial agents to combat Antimicrobial Resistance

Mary Palasota, Adysen Norris, Trish Baynham
St. Edwards University, Austin, Texas

Abstract

Over 2.8 million antimicrobial resistant (AMR) infections occur annually in the U.S., indicating an urgent need for new treatments. This study examined the antibacterial activity and modes of action (MOA) of plant extracts from *Psidium guajava* (guava) and Ficus carica (fig), both of which are used in traditional medicine but lack well defined cellular targets. Dried guava leaves were extracted in methanol and ethanol and, and reconstituted to 100 mg/mL in water, while dried fig leaves were extracted in methanol and reconstituted to 50 mg/mL in 50% DMSO. Antimicrobial activity was assessed using a Kirby-Bauer disk diffusion assay against E. coli lptd4213, which has a porous lipopolysaccharide layer. The average zones of inhibition were 8.8 mm (fig) and 12 mm (guava). Minimum Inhibitory Concentration (MIC) assays revealed MIC's of 5000 ug/mL (fig) and 312.5 ug/mL (guava). To investigate the MOA, bacterial cytological profiling (BCP) was performed using confocal microscopy. Cells were treated with FM4 -64 to stain the cell membranes red and DAPI to dye the DNA blue. Known antibiotics, including tetracycline and ampicillin, produced condensed DNA and elongated cell morphologies, respectively. Cells treated with either extract exhibited cellular elongation as well as DNA that appeared to be condensed and circular. Additionally, KBDD was used to address the effectiveness of these extracts against AMR strains of *Pseudomonas aeruginosa* and Vancomycin Intermediate Staphylococcus aureus. In the future these plant extracts may be used to develop new therapeutics on their own or combined with current antibiotics.

UP2

Antibacterial effect of American beautyberry and chamomile extracts on Vancomycin-intermediate Staphylococcus aureus

Mariana Momenso Candido da Silva, Mary Palasota, Adysen Norris, Patricia J Baynham
St. Edward's University, Austin, Texas

Abstract

Antimicrobial resistance (AMR) has become an increasing issue since the onset of antibiotic use in the 1940s. As part of this phenomenon, methicillin-resistant *S. aureus* (MRSA) infections have increased since the 1960s, and S. aureus infections caused over 1.27 million deaths globally in 2019. The use of vancomycin as a last resort therapy against MRSA has been crucial, so this study aimed to assess the antimicrobial activity of American beautyberry (*Callicarpa americana*) and chamomile (Chamomilla matricaria) extracts against Vancomycin-Intermediate *S. aureus* (VISA) strains. Methanolic American beautyberry and ethanolic chamomile extracts were prepared and evaporated using a rotary evaporator before being resuspended in water. Kirby-Bauer disk diffusion assays were performed to assess antimicrobial activity. Both plant extracts demonstrated inhibitory activity against the VISA strains studied: American beautyberry yielded 16 to 19 mm zones of inhibition, while the zones of inhibition for chamomile ranged from 13 to 18 mm. To quantify the antimicrobial effects of the extracts, minimum inhibitory concentration (MIC) assays were used and MICs were identified as 1.25-2.50 mg/mL for American beautyberry and 1.56-6.25 mg/mL for chamomile. Given these results, future plans include expanding the scope of the research to other bacterial strains of pathological interest and investigating the mode of action of the extracts through bacterial cytological profiling (BCP). This work highlights the potential of these plants as sources of antimicrobials or as complements to other antibiotics in the treatment of AMR S. aureus infections.

UP3

Exploring Sorcerer's Cave: A Possible New Source of Antibiotics from Pseudomonas species SC1154

Amy Osborn, Faith Jackson, Kathleen Lee, Jennifer Huddleston
Abilene Christian University, Abilene, TX

Abstract

Antimicrobial resistance (AMR) occurs when a bacterium overcomes or withstands antibiotics or drugs intended to stop its proliferation and growth. New sources of antibacterial treatment are critical in combating the rising epidemic of AMR infections. In their quest for microbial diversity, microbiologists consider the cave microbiome of particular interest. Untouched and scarcely explored, caves contain pristine ecosystems, which are thought to contain novel microbes that produce unique and potentially beneficial metabolites. Previously, a *Pseudomonas* strain, SC1154, was isolated from Sorcerer's Cave. This strain excretes natural products capable of killing or inhibiting eight clinically important bacterial strains. This study seeks to identify the growth profile of SC1154 as well as isolate, characterize, and optimize the production of the antimicrobial secondary metabolites it excretes. A 24-hour growth curve of SC1154 indicated that the lag phase was hours 0-4, the exponential phase was hours 4-9, and the stationary phase was hours 9-24. Antibiotic production occurred from hour five and reached its maximum at nine hours. A comparison of extraction methods revealed that solid-phase extraction (SPE) was more effective than liquid-phase extraction in isolating bioactive secondary metabolites. Of the SPE resins tested, XAD-4 was most effective as demonstrated by disc diffusion assays. Crude XAD-4 extracts were fractionated by silica gel column chromatography. Further purification will be performed using HPLC, and mass data will be acquired using LC-MS.

UP4

The Battle Against Superbugs: Fought by Locally Discovered Antibiotics

Isabella B. Howard, Adam J Bruton, Aaron M Lynne
Sam Houston State University, Huntsville, TX

Abstract

Antimicrobial resistance is becoming increasingly prevalent globally due to strengthened microorganisms rendering antibiotics unproductive. Fortunately, there is still an opportunity to counteract this; Through the collection of soil, the main goal was to isolate a compound locally, that exhibits antibiotic properties and could be a potential solution to this microbial uprise. To investigate this, collected soil was diluted and isolated to a pure form to study the bacteria against safe relatives of ESKAPE pathogens. Once antibiotic properties were present in a species of bacteria, that species was further tested with multiple bioassays, selective and differential media, and sequencing identification to classify and explore the bacteria. The tested bacteria was classified as a gram-positive streptobacillus, a mesophile, and grows facultatively. Sequencing of the 16S rRNA gene indicated that this bacterium is a member of the *Bacillus* genus. To conclude, the tested bacteria can be considered a potential compound for the development of a new antibiotic with further testing recommended to determine concentrations, actions, and range in which the potential antibiotic should be used.

UP5

Evaluating the Antifungal Effects of Avocado Fatty Alcohols on Postharvest Spoilage Fungi

Carlos Castro[1], Shirley Mora[2], Alejandro Castillo[3]

[1]St. Edward's University, Austin, Texas. [2]Tecnológico de Monterrey, Monterrey, Nuevo Leon. [3]Texas A&M University, College Station, Texas

Abstract

Avocado is a highly consumed fruit with 2.68 billion pounds imported from Mexico annually. During importation, avocados are vulnerable to fungal contamination, which can spoil them and shorten shelf life. Globally, 10-20% of postharvest crops are lost to fungal contamination and it costs the US more than $19 billion each year. Overuse of fungicides can select for resistance to these treatments making them ineffective. This project analyzed substances to determine if they could decrease fungal growth. Fungi included in this project (*C. gloeosporioides, C. siamense,* Aspergillus) are associated with postharvest spoilage and were used for testing these substances. Potential antifungal agents tested include: avocado fatty alcohols (AFA), propylene glycol (PG, a possible solvent of AFAs) and sodium metabisulfite (SM, a current antimicrobial food preservative). These compounds were evaluated using a mycelial growth inhibition assay in which treatments were mixed with agar, fungi were inoculated, and the colony diameter was measured to calculate percent inhibition relative to controls. The assays showed a concentration dependent pattern. AFA + PG treatments reached up to 84% inhibition by day 6, while 1000 ppm SM reached 89%. Overall, inhibition strengthened as AFA and PG concentrations increased, and higher AFA treatments were similar in inhibition to 1000 ppm SM. These findings suggest that AFAs formulated in PG can approach the effectiveness of SM at higher concentrations. Further research could lead to the development of AFAs as a postharvest treatment that extends avocado shelf life and reduces dependence on traditional chemical preservatives that have become less effective.

UP6

Synthesis and Assessment of Prodrug Activity Against Gram-Positive Bacteria

Katherine Richey, Aidan Duffield, Braden Chadwick, Emma Kulla, Emily Rathke, Jean-Luc Montchamp, Shauna M McGillivray
Texas Christian University, Fort Worth, Texas

Abstract

Conversion of an active antibiotic compound into a clinically viable pharmaceutical includes optimizing the drug's pharmacokinetic profile, such as its stability, bioavailability, or permeability. If a drug is too hydrophilic, it cannot effectively penetrate the hydrophobic bacterial cell membrane and reach its target. One strategy to overcome this delivery challenge is the use of prodrugs, which are inactive or chemically modified derivatives of parent compounds that are converted to their active form *in vivo*. The addition of different prodrug moieties to an active compound can improve permeability without altering its mechanism of action. Our goal is to assess the efficacy of novel prodrugs and determine which prodrug structures can be attached to antibiotics while maintaining activity. Because some prodrugs can be difficult to synthesize, Penicillin G, containing a single acidic site, was selected as a suitable parent compound with well-documented antibiotic activity. We first examined how different prodrug structures altered the minimum inhibitory concentration (MIC) in *Bacillus anthracis*, which has lower susceptibility to the non-derivatized penicillin G (240 μM) in comparison to other gram-positive bacteria. We identified several prodrug moieties, some previously categorized and some novel, that increased activity of penicillin against *B. anthracis*. These compounds were then tested against additional gram-positive bacteria, *Staphylococcus aureus* and Vancomycin-resistant *Enterococcus faecalis,* which are more susceptible to penicillin, to determine whether the produgs influenced penicillin activity in these species. Promising prodrugs will be used in future studies with antibiotics possessing challenging pharmacokinetic properties to determine if antibiotic efficacy can be improved.

UP7

Identifying Antibiotic Properties of Compost Microbes

Tu-Anh Le
St. Edward's University, Pflugerville, Texas

Abstract

Composting increases soil health leading to better agriculture production, but may also play a role in spreading antimicrobial resistance. This poses a danger to public health as people may be exposed to these antibiotic resistant bacteria (ARB) through contact with compost-amended soil, water, and crops.

In this study, the prevalence of ARB in compost was analyzed using composite samples of compost from three compost stages at Becker Elementary. Samples were serially diluted and spread plated on Luria-Bertani (LB), MacConkey (MAC) and LB+antibiotic (tetracycline (40µg/mL), ampicillin (200µg/mL), trimethoprim (200µg/mL), erythromycin (40µg/mL)) agar plates, then cell density was calculated. Minimum Inhibitory Concentration (MIC) tests were conducted on isolates Am1-1 and Er3-1. More bacteria were resistant to ampicillin (6.3E+05 CFU/g), trimethoprim (1.2E+06 to 2.0E+06 CFU/g), and erythromycin (9.7E+05 to 4.9E+06 CFU/g) than tetracycline (2.3E+03 to 5.3E+03 CFU/g) across all compost stages. From patch plating 300 isolates from all three bins, over 200 isolates were multidrug resistant to three or more tested antibiotics. From the MIC test, the MIC of Am1-1 on tetracycline was 32µg/mL and Er3-1 was 64µg/mL. Both isolates had a MIC on ampicillin, trimethoprim, and erythromycin of 2000µg/mL, 128µg/mL, and 256µg/mL, respectively. These findings call attention to the environmental and public health risks associated with the prevalence of ARB in compost.

UP8

Spontaneous Mutations for Antibiotic Resistance in *Escherichia coli*

Julian F ramirez, Cristian Buford, Brent Orji, Natalie Zarate, Wei-Chin Ho
University of Texas at Tyler, Tyler, Texas

Abstract

Understanding how microbes evolve is crucial, particularly regarding antibiotic resistance. Evolution is largely driven by random genetic changes known as spontaneous mutations, but we have limited information about them. For example, environmental conditions might influence which types of mutations occur. Gaining insight into this process is key to predicting how microbes adapt to new environments. Usually, the evolution of traits like antibiotic resistance results from a combination of mutation, natural selection, and genetic drift. However, the relative contribution of each factor is not always clear. This uncertainty makes it difficult to predict or control how quickly and in what way resistance develops in bacterial populations. In this research, we aimed to isolate the impact of mutations alone. We did this by repeatedly streaking single bacterial colonies. Doing this helps maintain random mutations, increases genetic drift, and reduces selective pressure. Using this approach, we were able to focus on how mutations alone affect the development of antibiotic resistance. We cultured E. coli on nutrient-rich media, both with and without the antibiotics ampicillin and streptomycin. Then we measured how resistant the bacteria had become to these antibiotics using MIC tests. Our results showed that the bacteria developed higher resistance to streptomycin, but not to ampicillin. This indicates that spontaneous mutations tend to generate more resistance-related changes for streptomycin. These findings improve our understanding of how different antibiotics may influence the supply of resistance mutations. They also explain how mutations and environmental factors interact to shape microbial evolution and antibiotic resistance over time.

UP9

Targeting Carbapenem Resistance: Dynamic Mechanisms of KPC-2 Inhibition by DBO and β-Lactam Sulfone Compounds

True Russell
Stephen F. Austin State University, Nacogdoches, TX

Abstract

Carbapenem-resistant Enterobacteriaceae pose a critical global health threat, largely due to the production of KPC-2, a class A serine β-lactamase that hydrolyzes the beta-lactam ring in last resort carbapenems. KPC-2-producing organisms are classified as urgent threats by the CDC, with mortality rates exceeding 40% in bloodstream infections when effective therapy is delayed. Clinically deployed β-lactamase inhibitors span two mechanistic classes. Diazabicyclooctane (DBO) compounds form reversible covalent bonds with the catalytic serine (Ser70) and are not hydrolyzed by the enzyme, whereas beta-lactam sulfones act as suicide substrates, irreversibly acylating Ser70 but undergoing slow hydrolytic turnover. Understanding how these mechanistic differences translate into active-site stability is essential for rational inhibitor optimization. This project screens six β-lactamase inhibitors (BLIs) against KPC-2 using molecular docking and molecular dynamics (MD) simulations. The high-resolution crystal structure of KPC-2 (PDB: 6TD0) served as the template for docking with AutoDock Vina, followed by MD simulations in NAMD to assess binding stability, active-site interactions, and conformational dynamics over time. Four DBO compounds, avibactam, relebactam, zidebactam, and durlobactam are compared, with the β-lactam sulfones sulbactam and tazobactam (older inhibitors with weaker KPC-2 activity) used as negative controls. Docking simulations identified favorable active-site positioning for all inhibitors, with distinct interaction patterns observed between DBO compounds and β-lactam sulfones. By directly comparing the two classes, this study seeks to illustrate the binding dynamics differences that explain the clinical superiority of the DBO class. Such computational approaches can complement microbiological and clinical data and may guide next-generation inhibitor design targeting carbapenem-resistant pathogens.

UP10

Isolating Bacteria with Antibiotic Resistance Phenotype in East and North Texas

Christian Buford, Wei-Chin Ho
University of Texas at Tyler, Tyler, Tx

Abstract

Some bacteria, such as Escherichia coli, can be found in the environment and animal hosts. For example, while some bacteria strains are symbionts that support gut health or commensals, some strains can be harmful, damaging livestock, wildlife, and humans. Antibiotic resistance of bacteria makes the situation more difficult. Understanding how the pathogenesis of bacteria strains varies across seasons and spread through water bodies is important for public health, agriculture, and wildlife management. This research aims to study the pathogenesis of bacteria in North and East Texas, including sample sites in Collin, Hunt, Kauffman, Madison, Navarro, and Smith Counties. We attempted to focus on Gram negative bacteria such as *E. coli* or other fecal coliform microorganisms, by isolating green/purple colonies on eosin-methylene blue agar. Antibiotic resistance phenotyping was then performed on these isolates by disc diffusion assay with Mueller-Hinton agar. In winter, we found no strains resistant to antibiotics Tetracycline, Sulfamethoxazole-Trimethoprim, Streptomycin, and Nalidixic Acid. For spring, we found one strain resistant to Nalidixic Acid. During the summer, we found two strains resistant to Streptomycin and Nalidixic Acid. Finally, in the fall, we found one strain assistant to Nalidixic Acid. The finding helps inform wildlife management strategies by identifying patterns of antibiotic resistance in the wild. Additionally, it provides insight into environmental antimicrobial resistance for clinical applications. Our future plan includes sequencing the isolates and identifying their genetic diversity.

UP11

Microbial March Madness: Assessing Ginkgo biloba's Effects on Alzheimer's Disease-Associated Gut Microbiomes

Kaeden T James, Vamessa Ruiz-Ceballos, Jacqueline Miralles-Salazar, Catherine Wakeman
Texas Tech University, Lubbock, Texas

Abstract

Alzheimer's disease (AD) is a neurodegerative disorder associated with chronic gut inflammation and dysbiosis; emerging evidence highlights the significance of the gut-brain axis in AD pathology, where microbial imbalance may contribute to neuroinflammation and cognitive decline. Ginkgo biloba (GB), a medicinal plant recognized for neuroprotective, antioxidant, and anti-inflammatory properties, may influence gut microbial composition and activity due to antimicrobial properties. This study evaluates GBs affects on gut microbiota both in monoculture and coculture. Using a "March Madness" bracket-style elimination design, bacterial mono and cocultures were grown in the presence and absence of GB to determine relative growth advantage and interactions between gram-positive and gram-negative gut microbes. After incubation with GB, cultures were diluted and plated on selective media to assess its effects on proinflammatory microbes (*E. coli*, *E. cloacae*, *S. enterica*, *S. marcescens*, and *C. freundii*) and anti-inflammatory microbes (*B. subtilis*, *S. epidermidis*, and *E. faecalis*). An active ingredient of GB is specific terpenes that express antimicrobial properties that differentially suppress the anti-inflammatory microbes in this study while allowing proinflammatory microbes to “win” the games. In the bracket studied gram-negative bacteria *E. coli*, *E. cloacae*, *S. enterica*, and *S. marcescens* won the first 4 games over the gram-positive bacteria *B. subtilis*, *S. epidermidis*, *E. faecalis* and gram-negative *C. freundii* lost these games; with the final winner of the bracket being *S. marcescens.* These interactions can provide an insight into the potential of GB as a modulator of gut microbiota and its relevance in altering gut dysbiosis associated with Alzheimer's disease.

UP12

Probiotics Beverages: Using Yakult and Kombucha to determine their Impact on the Oral Microbiome

Alba Ramirez, Anthony Castillo, Sin Man (Sabrina) Mak, Megan Romeo
Dallas College, Dallas, Texas

Abstract

While probiotics are widely recognized for supporting gut health, emerging research suggests they may also help promote microbial balance in the mouth. This study set out to explore how two popular probiotic beverages, Yakult and Kombucha, might influence the oral microbiome over time. Across four weeks, we monitored four participants, assessing their oral bacteria using two different media. Though small in scale, our findings suggest that probiotic drinks could be a promising supplement for enhancing oral microbial health.

UP13

Assessment of Anti-Biofilm Antimicrobial Activity of Wound Care–Associated Compounds

Adil Miah, Allie C Smith
Texas Tech University, Lubbock, TX

Abstract

Chronic wound infections are a significant cause of morbidity and mortality in a growing patient population and are a major clinical concern. Chronic wounds are wounds that fail to progress through the normal stages of wound healing and are often characterized as polymicrobial and biofilm-associated. The biofilm environment is unique and requires treatment considerations distinct from acute infections, for which traditional antimicrobials are designed to treat. Many topical agents are marketed for use in wound care and boast-antibiofilm efficacy, however for some compounds, there is no evidence in the literature discussing testing of these compounds in biofilm specific assays. The goal of this project is to test compounds for anti-biofilm efficacy by comparing susceptibility profiles mimicking acute versus biofilm environments.

UP14

Evaluating the Antibacterial Effects of Selected Plant Extracts Against Staphylococcus aureus

Ashley Y Trejo[1], Mary Palasota[2], Patricia J Baynham[1]
[1]St.Edwards, Austin, TX. [2]St.Edwards, Austin, Tx

Abstract

Antimicrobial resistance (AMR) is an escalating global health crisis that compromises the effectiveness of antibiotics used to treat infections. In 2019, AMR infections were directly responsible for an estimated 1.27 million deaths worldwide and contributed to nearly 4.95 million deaths globally. In the United States, AMR infections affect over 2.8 million individuals annually. Among these pathogens. *Staphylococcus aureus* and methicillin-resistant S. aureus (MRSA), remain significant contributors to hospital and community acquired infections. Additional therapeutics will be valuable in treating these AMR infections.

Plant-derived compounds contain diverse bioactive phytochemicals with documented antimicrobial properties however, systematic evaluation of their activity against clinically relevant bacterial pathogens remains limited. My study evaluates the antibacterial activity of 60 South American plant extracts against *S. aureus* using Kirby-Bauer disk diffusion assays. Sterile paper disks impregnated with plant extracts were applied to agar plates inoculated with *S. aureus* and incubated for 16-24 hours. Sixteen of the 60 extracts tested produced zones of inhibition ranging from 7-15mm, while the remaining extracts showed no observable antibacterial activity.

These findings suggest that a subset of plant derived compounds exhibits inhibitory effects against S. aureus. Although activity varied among samples, these findings highlight the potential of specific phytochemicals as complementary antimicrobial agents. Additional future testing, including minimum inhibitory concentration (MIC) analysis and compound isolation, are necessary to evaluate their potential development as a therapeutic treatment.

UP15

Electron Beam Processing of Chewing Tobacco for Reducing Denitrifying Microbial Load

Alyssa N Kubesch
Texas A&M University, College Station, Texas

Abstract

Microbial populations present in smokeless tobacco products contribute to the formation of tobacco-specific nitrosamines (TSNAs), carcinogenic compounds associated with increased oral cancer risk. Certain bacteria involved in nitrate metabolism convert naturally occurring plant nitrates into nitrites, which can subsequently react with tobacco alkaloids to form nitrosamines. Reducing or eliminating these microbial populations may therefore decrease the potential for nitrosamine formation during product storage and use.

The objective of this study was to evaluate the effectiveness of electron beam (eBeam) processing as a microbial reduction intervention for commercially available chewing tobacco products. It was hypothesized that energetic electrons generated during eBeam treatment would significantly reduce or inactivate denitrifying microorganisms present in these products.

Multiple commercial chewing tobacco brands representing different product styles were analyzed to determine baseline microbial loads and community composition. Microbial populations were quantified using standard aerobic and anaerobic culture techniques. Samples were then subjected to increasing doses of electron beam processing, and surviving microbial populations were enumerated to assess dose-response effects.

Commercial products exhibited substantial and variable microbial populations, including facultative anaerobic *Bacillus spp.* known to participate in nitrate reduction pathways. Electron beam processing produced a clear dose-dependent reduction in viable microorganisms. Progressive increases in treatment dose resulted in significant microbial inactivation, and at the highest dose evaluated, no viable anaerobic microorganisms were detected using standard culture methods.

These findings demonstrate that electron beam processing is an effective intervention for substantially reducing denitrifying microbial populations in chewing tobacco, potentially lowering the risk of TSNA formation.

UP16

The Hospital-Soil Interface: Investigating Antibiotic Resistance Near Clinical Environments

Nina K. Parvari
University of Texas at Arlington, Arlington, Texas

Abstract

The dangers of antimicrobial resistance continue to escalate global health crises. Consequences of antimicrobial resistance include detrimental outcomes for human health and may also effectuate the proliferation of new antimicrobial resistant strains. While studies have correlated antimicrobial resistance with anthropogenic effects such as antibiotic misuse in healthcare, the extent of clinically relevant soil bacteria in proximity to healthcare settings is under-researched. Here, tetracycline-resistant soil species are investigated to determine relevant frequencies and extent of resistance in proximity to hospitals as well as metabolic or enzymatic trends of clinically relevant resistant species. Culture-based methods using soil samples were utilized on MacConkey agar to isolate gram-negative bacteria and test for lactose fermentation. Colony-forming units were analyzed to determine relative frequencies of resistant species per gram of soil. The extent of tetracycline resistance was examined via minimum inhibitory concentration testing. Analytical Profile Index tests were performed to evaluate metabolic and enzymatic trends of clinically relevant resistant species. An inverse correlation was observed with resistant species becoming less frequent with increased hospital proximity. Most species showed moderate to high tetracycline-resistance thresholds of 10-15 ug/ml. Metabolic and enzymatic analysis of resistant species showed that the majority produced urease, gelatinase, and lysine decarboxylase enzyme, utilized citrate, and fermented lactose. This profile suggests biofilm-mediated protection, pH-dependent modulation, possible presence of broad-spectrum efflux pumps, and potential porin modification. These findings indicate that resistant species possess a vigorous combination of intrinsic, phenotypic, and possibly acquired resistance mechanisms.

UP17

Growth Responses of Three Sulfate Reducing Bacteria to Fluoroquinolone Exposure Under Anaerobic Conditions

Perla Guzman, Neida Perez, Riqing Yu
University of Texas at Tyler, Tyler, Texas

Abstract

Mercury (Hg) methylation is a critical microbial process because it generates methylmercury (MeHg), a neurotoxic compound that threatens human and wildlife health through transfer along the food web. This transformation is mediated by anaerobic microorganisms, particularly sulfate-reducing bacteria (SRB) as key Hg methylating microbes. Antibiotic fluoroquinolones (FQs) are used in human infection treatments, in veterinary medicine, and in aquaculture, and have become emerging contaminants. Our recent field investigations in paddy soils revealed that FQs can significantly increase microbial MeHg production in rice fields. This enhancement appears to be driven by stimulated MeHg synthesis by SRB. However, the mechanisms underlying the interactions between FQs and SRB-mediated MeHg production remain poorly understood. In this study, three SRB species as the known Hg methylators including *Desulfotomaculum nigrificans*, *Desulfobacterium autotrophicum*, and *Desulfovibrio desulfuricans* ND132 were selected to explore how fluoroquinolone antibiotics affect their growth. Each SRB strain was grown anaerobically and exposed to different concentrations of ofloxacin, norfloxacin, ciprofloxacin, and enrofloxacin. Bacterial growth was measured using optical density to determine how antibiotics impacted cell growth. Our preliminary results show that growth decreased as antibiotic concentration increased, but the level of inhibition varied between species and between antibiotics. ND132 showed a significant growth inhibition compared to *D. nigrificans* and *D. autotrophicum*. These results suggest that environmental antibiotic FQs may influence the growth of bacteria that are involved in mercury cycling. Understanding how these stressors affect bacterial growth is an important step toward evaluating their potential impact on Hg methylation and MeHg contamination in natural environments.

Pathogenic Microbiology

UP18

Rapid Detection of Echinocandin Resistance Markers in *Candidozyma auris* using Nanopore sequencing of *FKS1* hotspot 1.

Guillermo Nunez, Alexis Vargas, Asael Garcia, Abisai Garcia, Weijing He
UT Health San Antonio, San Antonio, Texas

Abstract

Candidozyma auris is an emerging multidrug-resistant fungal pathogen for which rapid detection of echinocandin resistance is critical for patient management and infection control. Echinocandin resistance is primarily mediated by mutations in hotspot regions of the *FKS1* gene, which encodes the catalytic subunit of 1,3-β-D-glucan synthase. While these mutations are well described, routine detection still relies largely on phenotypic susceptibility testing, which may delay identification of resistant isolates. In this study, we evaluated the feasibility and performance of targeted Nanopore sequencing of the entire *FKS1* gene for rapid genotypic prediction of echinocandin resistance in *C. auris*.

A total of 64 clinical *C. auris* isolates (9 echinocandin-resistant and 55 susceptible by broth microdilution MIC testing) were subjected to amplicon sequencing of *FKS1* using the Rapid Barcoding kit and MinION sequencer from Oxford Nanopore Technologies. All resistant isolates harbored nonsynonymous mutations within hotspot 1 of *FKS1*, whereas all susceptible isolates exhibited wild-type sequences and synonymous variants across the *FKS1* gene. Genotypic results demonstrated complete concordance with phenotypic susceptibility profiles. Full-gene coverage further enabled comprehensive assessment of sequence variation outside canonical hotspots and confirmed the absence of resistance-associated substitutions in susceptible isolates.

These findings demonstrate that targeted Nanopore sequencing of *FKS1* provides an accurate and rapid method for detecting echinocandin resistance markers in *C. auris*. This approach offers a practical framework for fast and accurate molecular resistance screening. Additionally, this supports the integration of real-time sequencing into clinical and public health laboratories for early detection and surveillance of antifungal resistance in this important pathogen.

UP19

Robust detection of infectious rotavirus by integrating sample pooling and ICC-qPCR

Caroline L Readinger[1,2], Muzaffar Ali[1,2], Chandni Praveen[1,2], Krishna Narayanan[3], Suresh D Pillai[1,2]

[1]National Center for Electron Beam Research, College Station, TX. [2]Department of Food Science and Technology, Texas A&M University, College Station, TX. [3]Department of Electrical & Computer Engineering, Texas A&M University, College Station, TX

Abstract

Rotaviral gastroenteritis is a major public health concern globally, especially in children under five. Rotavirus is contagious with 10-100 particles necessary to infect susceptible individuals, and outbreaks begin simply from contaminated vegetable consumption. Therefore, food surveillance is critical for outbreak preparedness. Conventional cell culture requires long incubation time (3-5 days) and qPCR cannot distinguish live from dead viruses. In this study, integration of sample pooling with ICC-qPCR addresses these challenges, producing a robust, sensitive, and cost-efficient assay.

During the experiment, 100 samples mimic a large case volume with 4-5 randomly spiked with log-2 titer/sample. The pooling strategy which included row-wise, column-wise, and diagonal-wise resulted in 30 pooled samples (10mL each) that were concentrated to 200uL. The virus was allowed to propagate in MA104 monolayer cells for 24 hours. Following propagation, the cell monolayer was scraped, harvested and subjected to a freeze-thaw cycle to release virus particles. The samples were heat-treated at 99°C for 5 minutes and tested by qPCR without RNA extraction. Positive pools were algorithmically processed to determine their corresponding positive samples.

The results demonstrated that ICC-qPCR is highly efficient, detecting 100% (11/11) of positive samples with higher confidence (evidenced by lower Ct values) while propagation confirmed the rotavirus as infectious and viable. Further, sample pooling alongside ICC-qPCR reduces costs by 70%. Together, these findings show that ICC-qPCR with sample pooling made the assay a robust, sensitive, and high-throughput platform that could be used globally to address the challenge of large-scale surveillance during outbreaks.

UP20

Comparison of the Conventional Methods and ICC-qPCR for Detection of Rotavirus

Kailee R Meyer[1,2], Muzaffar Ali[1,2], Chandni Praveen[1,2], Suresh D Pillai[1,2]

[1]National Center for Electron Beam Research, Texas A&M University, College Station, Texas. [2]Department of Food Science and Technology, Texas A&M University, College Station, Texas

Abstract

Food surveillance allows for swift and vital interventions to prevent foodborne infections like rotavirus, which causes 600M illness with 420K deaths annually. However the conventional rotavirus detection methods such as cell culture and qPCR are slow (5-7 days) and can not distinguish between infectious and non-infectious rotavirus. Therefore, it is of dire need to develop an assay with greater sensitivity and robustness to combat food contamination challenges. In the current study, cell culture was integrated with qPCR to eliminate the need for long incubation and detect the infectious rotavirus.

A total of 30 distilled water samples (10mL each) were spiked with 1 TCID50 rotavirus titer and tested with cell culture, qPCR and ICC-qPCR methods. The pooled samples (n-10) were concentrated to 10uL to directly test with qPCR. The remaining 20 samples were concentrated to 200 uL and used to infect monolayers. For the ICC-qPCR assay, 10 cell infected samples were harvested 24 hrs post-infection to test with qPCR. For the cell culture assay, the remaining 10 samples were incubated to observe the cytopathic effects for seven days.

The results showed that the ICC-qPCR, where the virus was cultured in the cell monolayer before qPCR was able to detect 9/10 (90%) rotavirus. In contrast, the direct qPCR was only able to detect 2/10 (20%) and the cell culture assay was able to detect 4/10 (40%). These findings demonstrate that ICC-qPCR is more sensitive and robust than the conventional cell culture and qPCR assays.

UP21

Alterations in gut biota associated with stress-sensitivity in the Chick Social Separation Stress test

Layla R Behrens, Madison M Schultz, Danielle G Garbarino, Todd P Primm, Anand B Karki, Stephen W White
Sam Houston State University, Huntsville, Texas

Abstract

The microbiota-gut-brain axis is responsible for programming stress hormone production early in life and may influence stress-vulnerability. The chick social-separation stress test is a well validated preclinical simulation of anxiety and depression utilizing neonatal chicks. The model has identified two unique genetic strains that show differences in latency to enter behavioral despair. Production Reds (PR) display longer latency to behavioral despair whereas Black Australorps (BA) display shorter latency to behavioral despair, representing stress-resiliency and -vulnerability, respectively. Additionally, the BA genetic line presents homologies with treatment-resistant depression. Differences in gut microbiota communities between the two strains were determined. Fecal samples from both strains at two time points, day 3 and day 8 post-hatch, were collected and bacterial DNA was extracted and sequenced, and community diversity and evenness determined. Overall, more community stability was displayed in PR with less stability and more variability over time in BA. Multiple significant differences in species relative abundance between chicken strains were observed. More work may lead to better understanding of the gut-brain axis role in depression.

UP22

The Characterization of Broad Host Range Antimicrobial Agent BceT44681

Allyson Sokolowski, Andrej Momiroski, Carlos Gonzalez, Jason Gill
Texas A&M University, College Station, Texas

Abstract

The *Burkholderia cepacia* complex (Bcc) consists of over twenty species of bacteria that can cause cepacia syndrome in patients with cystic fibrosis (CF) and chronic granulomatous disease (CGD). Cepacia syndrome is a life-threatening condition leading to lung decline, sepsis, and death. Prevalence of intrinsic drug resistance mechanisms in Bcc species poses challenges against treating cepacia syndrome. Phage therapy is currently being studied as a potential treatment option alongside antibiotics to treat severe infections. Antimicrobial agents, such as tailocins, or phage tail-like bacteriocins, have been harnessed to develop therapeutic phages for cepacia syndrome patients. *Burkholderia cenocepacia* strain AU44681 constitutively releases BceT44681, a putative tailocin. The distinct zoning and behavior are consistent with high molecular weight activity seen with other Bcc tailocins. BceT44681 can be produced at high concentrations without UV induction and cause inhibition activity against other B. cenocepacia isolates. Of the 19 strains tested, 13 were targeted by the putative tailocin. The genome of strain AU44681 contains a locus with high similarity to tailocin BceTMilo indicating that AU44681 contains a Milo-like defective prophage. This locus could be responsible for the production of BceT44681. The relatively broad host range of BceT44681 suggests that the genetic determinants of BceT44681's broad inhibitory spectrum could prove useful for developing therapeutic phages for cepacia syndrome. Genetic efforts to confirm the locus responsible for the production of BceT44681 are ongoing.

UP23

The Role of Clinical Environmental Factors and Polymicrobial Interactions in Modulating Antimicrobial Susceptibility Testing Results

Maya K Panday[1], Nina Walker[2], Taylor Barr[2], Corey Swackhammer[2], Allie C Smith[1]
[1]Texas Tech University, Lubbock, Texas. [2]Texas Tech University Health Sciences Center, Lubbock, Texas

Abstract

Chronic polymicrobial infections represent a significant clinical challenge due to their persistence, variability, and recalcitrance to standard antimicrobial therapies. These infections frequently fail to resolve within expected timeframes, contributing to significant morbidity and mortality. Current antimicrobial susceptibility testing is conducted under monomicrobial conditions in nutrient-rich laboratory media that do not replicate the polymicrobial and physiologically complex environment of chronic wounds. While certain host-pathogen interactions are conserved across infection sites, local microenvironmental factors uniquely shape microbial behavior and antimicrobial susceptibility. In addition, bacterial communities may exhibit synergistic interactions that alter susceptibility compared to individual species. Previously, we have evaluated the chronic wound pathogens *Enterococcus faecalis, Staphylococcus aureus,* and *Pseudomonas aeruginosa* in monomicrobial suspensions. Building on this foundation, we investigated how polymicrobial interactions influence antimicrobial susceptibility results generated from Gram-positive and Gram-negative Minimum Inhibitory Concentration (MIC) panels within an automated diagnostic system. We aim to further this line of research by examining the impact of bacterial grouping and physiologically relevant media compositions. Incorporating clinically representative conditions into susceptibility testing may enable more targeted antimicrobial selection and ultimately improve patient outcomes.

UP24

Polymicrobial Interactions Among Foodborne Pathogens: Coexistence or Competition

Esmeralda G Mendoza Lara, Marbella Canaca, Todd P Primm, Anand B Karki
Sam Houston State Univeristy, Huntsville, Texas

Abstract

Salmonella enterica and *Campylobacter jejuni* are major foodborne pathogens often associated with raw or undercooked retail poultry. In these environments, they coexist with *Enterococcus* species, which are commonly found as gut microbiota and are known for antimicrobial resistance, the production of enterocins, and other inhibitory metabolites. Depending on environmental conditions, these interactions may promote coexistence or result in competitive exclusion. Polymicrobial interactions can influence pathogen survival; however, the role of *Enterococcus* in these relationships remains unclear. This study evaluates the antimicrobial properties of *Enterococcus faecalis* and examines its interactions with *S. enterica* and *C. jejuni*, including its relationship with *S. enterica* under different pH conditions. We hypothesize that *E. faecalis* may enhance the survival of *S. enterica* under stress in polymicrobial environments. The findings of this study enhance our understanding of the antagonistic or beneficial effects of *Enterococcus* species on co-contaminant foodborne pathogens relevant to food safety.

UP25

Can the Oral Microbiome Impact Cardiovascular Health?

Umang Mehta, Sin Man (Sabrina) Mak, Megan Romeo
Dallas College, Dallas, Texas

Abstract

Streptococci bacteria are a group of Gram-positive bacteria commonly found as a major component of the human oral microbiome. Previous studies have shown a correlation between streptococci that enter the bloodstream and an inflammatory reaction that can lead to heart damage. In this study, we will investigate whether it is possible to increase or decrease the relative concentration of oral streptococci within the oral cavity, and if there is any measurable impact on the cardiovascular system by assessing heart rate recovery, blood pressure changes, and EKG.

UP26

APEX as a proximity labeling technique for G3BP1 during during viral infection

Micah D Day, Maxine Cañelas, Jared Bard
Texas A&M University, College Station, Texas

Abstract

G3BP1 is a eukaryotic protein that nucleates cytoplasmic assemblies of RNA and protein termed stress granules, which are thought to be antiviral. G3BP1 regulates RNA synthesis during cellular stress and viral infection and features both proviral and antiviral characteristics, as some viruses, such as single-stranded RNA alphaviruses, are dependent on G3BP1 for replication. Alphavirus non-structural protein 3 (nsP3) disrupts stress granule formation through direct interaction with G3BP1 by modifying its post-translational modifications and binding properties, as well as competitively displacing cellular partners, ultimately causing alterations in the G3BP1 interactome. From this study, we propose that APEX can be used as a proximity labeling technique to better define interactions between G3BP1 and RNA during baseline and stressed cellular conditions. By comparing these two variables, it will be possible to determine how viral strain reshapes the interactions between G3BP1 and cellular RNA. Overall, this work will provide a basis for understanding how viral modification of G3BP1 alters RNA localization and stress granule composition, clarifying the proviral and antiviral characteristics of G3BP1 during viral infection,

UP27

Modulating the Ability to Form Stress Granules Causes Translational Changes in Mammalian Cells

Zane A Richter, Thu TU Nguyen, Jared AM Bard
Texas A&M University, College Station, Texas

Abstract

Eukaryotic cells employ a variety of mechanisms to overcome the stressors they encounter such as toxins, heat shock, and viral infection. A cornerstone of this stress response is the formation of stress granules (SGs)-dynamic, membraneless organelles engineered to promote cell survival and adaptation. These granules are known to be both consequences and causes of translational shifts in cells. SGs have been shown to have effects on translation initiation components such as eIF2 as well as some downstream effectors of the mTOR pathway like S6-Kinase and 4E-BP1. By modulating cells' abilities to form stress granules, we explore how global translation changes when stress granule formation is inhibited. Additionally, we investigate some of the large effectors of translation and how those controllers are modified in response to controlling SG forming capabilities. We found that the loss of ability to form SGs had a negative effect on global translation rates in cells. This proves to be contrary to some schools of thought, as SGs are often depicted as sites of translational depression or having a minimal effect on translation. Our findings suggest there may be more to translational modifications by SGs than previously believed and that SGs may have an effect on major translational regulators. Still, the role of SGs in controlling translation state remains unclear. These findings could lead to more effective treatments for conditions such as cancer and viral infection.

UP28

How Herpes Virus Might Help Us Understand Alzheimer's.

Domenica P Gaibor Verdezoto, Laura K Hanson
Texas Woman's University, Denton, Texas

Abstract

Alzheimer's disease (AD) is the most common form of dementia and is linked to the buildup of abnormally modified tau protein. Recent research suggests that infection with herpes virus might contribute to Alzheimer's disease. One such virus is cytomegalovirus (CMV), which infects a large portion of the population. This project explores how CMV infection may cause changes to tau protein and possible outcomes in neuronal and immune cells. Using biochemical tools, we are comparing different cell types respond to CMV over time, and how localization may play a role in tau's functions. We are also investigating how two cellular enzymes, Protein Kinase A (PKA) and Protein Phosphatase 2A (PP2A), may influence these changes. Understanding how a common virus like CMV alters tau phosphorylation and its link to the cytoskeleton could reveal new insights into the early steps in development of Alzheimer's pathology, leading to improved diagnosis and targets for prevention.

UP29

Formulation And Characterization of Synthetic Lipid Droplets Loaded with the Novel Antimicrobial Silver Ibuprofen.

Ashlyn G. Wolf, Ricardo Muñoz-Medina, Carolyn L. Cannon
Department of Microbial Pathogenesis and Immunology, Texas A&M University, Bryan, TX

Abstract

Pseudomonas aeruginosa (Pa) is a Gram-negative opportunistic pathogen with growing resistance to standard of care antimicrobials, and consequently, has been designated as a serious threat by the World Health Organization. Thus, there is a need for alternative therapeutic formulations to combat these resistant strains. Biocompatible lipid droplets (LDs) are promising drug delivery devices for water insoluble drugs. These hydrophobic drugs can be loaded in the LD core that provides protection from enzymatic degradation or other harsh conditions. Additionally, LDs can be coated with ligands that target them to tissues or cells including bacteria. Targeting to bacteria results in accumulation at the site of infection, which may overcome antimicrobial resistance. We aimed to develop a LD formulation containing the novel antimicrobial, silver ibuprofen (AgIBU), for the treatment of antimicrobial resistant Pa infections. LDs are composed of a neutral lipid core surrounded by a phospholipid monolayer. Our LDs were characterized by dynamic and electrophoretic light scattering, confocal microscopy, and thin layer chromatography. They are spherical with a diameter of ~180 nm and a zeta potential of -8.17 mV. The phospholipids to neutral lipid ratio was 9% confirming a single phospholipid layer. Confocal microscopy of LDs stained with Nile-Red confirmed the neutral lipid core. Preliminary loading for our LDs resulted in a concentrated solution of 6 μg/mL. The minimum inhibitory concentration against clinical Pa isolates was ~4 μg/mL. In conclusion, we have synthesized lipid droplets loaded with silver ibuprofen that may effectively treat infections caused by resistant *Pseudomonas aeruginosa*.

UP30

Streptococcus agalactiae inhibits Breast Cancer Resistant Protein function in brain endothelial cells

Benjamin Klemp[1], Natalie Alexander[1], Alexandra Meyer[2], Elisa Tucker[2], Brandon J Kim[1]
[1]University of Texas at Dallas, Richardson, TX. [2]University of Alabama, Tuscaloosa, AL

Abstract

Infection of the central nervous system (CNS) in the form of bacterial meningitis poses a serious health threat. Bacterial meningitis arises from bacterial interaction and disruption of the blood-brain barrier (BBB), formed by brain endothelial cells (BECS). As a means to maintain CNS homeostasis, BECs possess efflux transporters to return exogenous materials back into circulation. This defense mechanism presents a substantial obstacle to drug delivery and treatment of CNS infections. One such efflux transporter is Breast Cancer Resistance Protein (BCRP), whose activity is known to contribute to multidrug resistance in cancer cells. However, BCRP function is not well characterized during bacterial infection of the BBB. A causative agent of meningitis is the bacterium *Streptococcus agalactiae*, or Group B *Streptococcus* (GBS), an opportunistic pathogen that typically colonizes the genital and gastrointestinal tracts. Reduction of BCRP function and abundance was observed in induced pluripotent stem cell-derived BECs following GBS infection and live bacteria were necessary to observe this phenomenon. Therefore, this improved understanding of how BCRP function is affected by bacterial infection offers avenues toward improved therapeutics for CNS infection.

UP31

Streptococcus pneumoniae-induced Proinflammatory Cytokines Profile in 3D cultures of the Human Lungs Epithelial cells

Joshua Tadegegn, Ali Azghani
The University of Texas at Tyler, Tyler, Texas

Abstract

Pneumolysin (Ply) is a pore-forming toxin produced by *S. pneumoniae.* Ply causes epithelial barrier disruption and influences the expression of inflammatory cytokines. Paracellular permeability of Calu-3 monolayers seeded on culture inserts was measured using the Millipore trans-epithelial electrical resistance (TEER) system. Low concentration of Ply (5 μg/mL) decreases TEER with a transient recovery at 5 hours. Exposure to the same concentration of Ply for 24 hours also increased proinflammatory cytokines production, with notable increases in interleukin-6 (IL-6) ($n = 4$, $p < 0.05$) and IL-18 ($n = 3$, $p < 0.05$). In an effort to understand the nature of PLY -induced cellular injury, we set forth the hypothesis that the early therapeutic intervention to initiate a differential change in cytokine expression and the onset of IL-18 induced pyroptosis. To address this hypothesis, the cells on Transwell inserts were treated with Ply for 5 hours, followed by replacement with serum-free medium for 24 hours. Supernatants collected at 5, 10, and 24 hours post-toxin removal for IL-6 and IL-18 expression using enzyme-linked immunosorbent assay (ELISA). IL-6 shows reduced production through 24 hours post-removal compared to continuous Ply exposure. Conversely, IL-18 showed continuous cell stress responses in the absence of Ply. We are in the process of evaluating the cells' apoptotic and pyroptotic state using FLICA Caspases imaging assays.

UP32

DEHP Exacerbates CVB3 Infection by Promoting the Release of Infectious Extracellular Vesicles

Sanjana Pandian, Ramina Kordbacheh, Jayden Aleman, Jon Joseph Sin
The University of Texas at Dallas, Richardson, Texas

Abstract

Coxsackievirus B3 (CVB) is a positive-sense RNA virus and a member of the *Picornaviridae* family. CVB typically causes mild or asymptomatic infections, but in young children can lead to severe inflammatory diseases such as pancreatitis, myocarditis, and meningoencephalitis. The factors driving this variability remain poorly understood. Di(2-ethylhexyl) phthalate (DEHP), a widely used environmental plasticizer, has recently emerged as a potential modifier of viral susceptibility. We have previously shown that DEHP enhances CVB infection and spread. This study defines how DEHP amplifies viral release and disease severity. DEHP and CVB both contribute to mitochondrial dysfunction, promotes mitophagy, and elevates the production of CVB capsid protein VP1-positive extracellular vesicles (EVs). HeLa cervical cancer cells, Caco-2 colon carcinoma cells, and induced pluripotent stem-cell derived brain endothelial cells were exposed to DEHP for 24 hours prior to infecting with enhanced green fluorescent protein-expressing CVB3 to assess viral replication and EV release. EVs were also isolated from cell culture supernatants of infected HeLa cells and western blot analysis revealed increased levels of VP1 and LC3 in EVs released from DEHP-treated infected cells compared to vehicle-treated infected cells. In vivo DEHP exposure markedly worsens CVB-induced pancreatitis in mouse models, resulting in higher pancreatic viral titers, extensive acinar destruction, and increased immune cell infiltration. Ongoing work investigates how DEHP targets cellular stress pathways, viral egress mechanisms, and the resolution of pancreatic inflammation. Together, these studies reveal how a common environmental pollutant amplifies CVB infection and identify mechanistic targets to mitigate pollutant-exacerbated viral disease.

UP33

A Novel Chronic Infection Model For Investigating Red Queen Hypothesis Dynamics In Host-Pathogen Coevolution

Guy LaMorgese, Noemi Del Campo, William Cody
University of Dallas, Irving, Texas

Abstract

Host-pathogen interaction is an antagonistic dynamic in which each organism must adapt to overcome the other in order to survive. Changes that increase an organism's fitness in the presence of its antagonist are selected and are passed on to offspring. This continuous cycle of reciprocal adaptation, which is necessary for organisms to maintain a relative level of fitness, is known as the Red Queen Hypothesis. To investigate this concept, we developed a chronic infection model of *Pseudomonas aeruginosa* infection, with Drosophila melanogaster serving as the host. Chronic *P. aeruginosa* infection in the airway is the most common cause of morbidity and mortality among patients with Cystic Fibrosis (CF), a genetic disorder characterized by thickened mucus in the lungs that impairs mucociliary clearance and creates a favorable environment for the persistence and adaptation of bacteria, exerting strong selective pressure on pathogens. We utilized three different treatment groups for infection: host evolved, pathogen evolved, and coevolved. These groups measure the adaptive changes of the host, the pathogen, or both together over multiple generations. This infection model allows for the continued propagation of both host and pathogen lineages while monitoring evolutionary changes across generations. Host survival was measured for a nine-day period after oral infection. Within twelve generations of infections, higher survival percentages have been observed among the host evolved group as compared to the pathogen evolved group. Better understanding host-pathogen interactions will provide much needed insight for the development of novel drug treatments and therapeutics.

Molecular & Environmental Microbiology

UP34

Uncovering the Biosynthesis of Phosphatidylglycerol-linked ECA in E. coli K-12: a Genome-screening and Systematic Characterization Approach to Discovering Target Genes

Hannah E Fuhrman, Joseph F Carr, Angela M Mitchell
Texas A&M University, College Station, Texas

Abstract

Antibiotic resistance is an increasing global health threat. Several members of the *Enterobacterales* order including Escherichia coli, a CDC-designated urgent threat, are the focus of research into new small-molecule therapeutics. Enterobacterial common antigen (ECA), is a conserved carbohydrate moiety, specific to *Enterobacterales*, that has a role in maintaining outer membrane permeability. ECA exists in linear outer membrane diacylglycerol-phosphate- (ECA_{PG}) and lipopolysaccharide-linked forms, and a cyclic periplasmic form. This study utilized transposon-directed insertion sequencing (TraDIS) and phenotypic characterization to uncover the biosynthetic pathway of ECA_{PG}. Transposon libraries were constructed in a ECA negative background (*ΔwecAΔwzzEΔwaaL)* and ECA_{PG}-only background (*ΔwzzEΔwaaL)* with the transposome EZ-Tn5<KAN-2>Tnp inserted via electroporation. Fragmented libraries were generated from pooled DNA extracted at t = 0h and t = 5h for Illumina HiSeq 2500 sequencing. We defined conditional essentiality based on the elyC deletion phenotype. ElyC is essential in ECA_{PG}-only strains, but non-essential in wildtype. Utilizing our criteria for conditional essentiality, we identified 53 candidate genes. To study the deletion effects, knockout strains were built using the KEIO collection in a MG1655 and *ΔwzzEΔwaaL* (ECA_{PG}-only) strain. Further phenotypic characterization was performed using EOPs (Vancomycin, SDS-EDTA), which allowed us to determine membrane integrity through antibiotic sensitivity. ECA immunoblots evaluated linear ECA levels, and were used to determine whether the gene is involved in ECA regulation. Using our systematic approach, we noted several promising candidate genes. Elucidation of the specific cellular roles and contributions of these 53 genes will allow for the eventual construction of a complete biosynthetic pathway of ECAPG.

UP35

Surveying Drain Samples From Meat Processing Plants For MDR andBiofilm Formation

Gisela Aviles[1], Sapna Dass[2], Kabilan Mani[2], Kirsten Zwally[2], Vignesh Palanisamy[2], Emma Pousson[1]

[1]St. Edward's University, Austin, Texas. [2]Texas A&M University, College Station, Texas

Abstract

The emergence of multidrug-resistant (MDR) bacteria in meat processing environments is a pressing public health concern, particularly when these organisms also demonstrate the ability to form biofilms. Biofilms enhance bacterial survival by offering protection from environmental stressors, concentrating nutrients, and enabling the horizontal transfer of genetic material, including antibiotic resistance genes. These issues become relevant in settings such as meat processing plants because bacterial pathogens exhibiting biofilm formation and MDR could possibly come into contact with "ready to eat" products. These products are advertised as not needing further processing, so MDR biofilm forming bacterial pathogens would not be destroyed if present. This study investigated the presence of MDR and biofilm-forming bacteria in 164 drain samples collected from meat processing plants across Texas. Completion of several Kirby Bauer disk diffusion assays, susceptibility tests that determine if bacterial specimens are resistant, intermediately affected, or sensitive to certain drugs (specifically eight antibiotics in this study: streptomycin, kanamycin, novobiocin, tetracycline, penicillin, erythromycin, neomycin, and chloramphenicol) revealed a total of 163 bacterial isolates that exhibited resistance to multiple antibiotics. Several Crystal Violet assays, a method of biofilm formation quantification using Crystal Violet dye to highlight polysaccharide bonds created by bacterial organisms to maintain the structure of the biofilm, revealed that 10 isolates formed weak biofilms, 49 moderate, 49 strong, and 16 extreme biofilms. These findings underscore the potential risk posed by MDR biofilm-forming bacteria in meat processing facilities, and highlight the importance of continued monitoring and mitigation strategies to protect consumer health.

UP36

Exploring the Phenotypic Diversity of Environmental *Nakaseomyces glabratus*

Oscar E Reyes Parker, Taylor Hearne, Kendall T Esparrago, Paola Zucchi, Jesús A Romo
University of Texas at San Antonio, San Antonio, TX

Abstract

Nakaseomyces glabratus is an opportunistic pathogenic fungus and colonizer of the human gastrointestinal tract. It is also found in the environment, where it is exposed to diverse stress conditions (high temperatures, desiccation, predators, fungicides, and others). Despite its clinical importance, a significant knowledge gap exists about how adaptations to distinct environmental conditions influence its virulence. We hypothesize that adaptation to non-mammalian environments influences clinically relevant traits in *N. glabratus*. Four environmental isolates of *N. glabratus* were compared to two lab strains in growth phenotype, biofilm formation, antifungal susceptibility, and stress response. To determine growth phenotype, isolates were cultured at 30 °C and 37 °C in YPD or RPMI media. Biofilm assays were performed in RPMI at 37 °C and quantified using crystal violet. Antifungal susceptibility was determined via minimum inhibitory concentration assays. Response to stressors was determined by growing strains in caffeine, Calcofluor white, Congo red, and SDS at three distinct temperatures. We found that two environmental isolates displayed growth defects at 30 °C in YPD, while all isolates grew comparably at 37 °C. No significant differences in fluconazole, amphotericin, or caspofungin susceptibility were observed. Biofilm formation varied among environmental isolates but showed no significant difference from controls. Stress response varied across isolates. Future studies will characterize *in vivo* virulence using a *Galleria mellonella* infection model, as well as determine response to osmotic stress. Overall, our findings suggest that clinically relevant traits of *N. glabratus* are present before encountering a mammal. However, characterization of a larger isolate collection is required.

UP37

Campylobacter in Microgravity: Growth, Physiology, and Genomic Expression

Roberta S Garces, Alexis Ho, Madhusudhan Choudhary, Todd P. Primm, Anand B. Karki
Sam Houston State University, Huntsville, TX

Abstract

Campylobacter jejuni remains a major cause of gastrointestinal infections worldwide and is typically transmitted through the ingestion of undercooked, contaminated meat. However, the behavior of this pathogenic bacterium has not been well characterized under spaceflight conditions, where microgravity is present. This study examined the influence of simulated microgravity on the growth, cell length, and virulence gene expression of *Campylobacter jejuni*. Microbial cultures were maintained under simulated microgravity using a Rotary Cell Culture System (RCCS) and compared with cultures grown under normal gravity conditions. No significant difference in bacterial growth was observed under simulated microgravity compared to normal gravity. However, a significant difference in cell length was detected between gravity conditions, with cells being shorter under microgravity. Gene expression analysis using quantitative PCR (qPCR) was performed on selected virulence genes (*cadF, ciaB, cdtA, luxS*, and *flaA*) associated with adhesion, invasion, toxin production, motility, and chemotaxis. The study found no significant differences in gene expression under simulated microgravity compared to normal gravity for the selected genes. Further investigation using whole-genome transcriptomic and proteomic analyses is warranted to gain a deeper understanding of the effects of microgravity on *Campylobacter* metabolism and physiology.

UP38

The Effect of Media Type on ZnO Cytotoxicity

Marion L Hutchison, Shauna McGillivray, Yuri Strzhemechny
Texas Christian University, Fort Worth, TX

Abstract

The increasing prevalence of antibiotic-resistant bacteria, including *Staphylococcus aureus*, has intensified the search for alternative antimicrobial strategies. Metal oxides have emerged as promising candidates, with zinc oxide (ZnO) attracting particular interest due to its low cost, thermal and mechanical stability, and minimal generation of harmful by-products. ZnO has potential applications in medical device coatings, food preservation, and topical therapeutics. Previous work in our laboratory demonstrated that growth inhibition of *S. aureus* correlates with the release of Zn^{2+} ions from ZnO Sigma particles in Mueller–Hinton broth (MHB) (Caron et al., 2024). However, it has been reported that the media can influence Zn^{2+} dissolution and ZnO toxicity. In support of this, we find that ZnO particles exhibit increased dissolution in saline compared to MHB, resulting in enhanced cytotoxicity toward *S. aureus*. To further investigate the influence of different media types on ZnO dissolution and bacterial survival, we will investigate HEPES and MOPS buffers as media alternatives to assess ZnO toxicity. By evaluating how different chemical environments affect Zn^{2+} release and antimicrobial activity, this work aims to maximize the potential of ZnO-mediated cytotoxicity.

UP39

Isolation and Characterization of Endophytes in Modern and Ancestral Seeds of the Marsh Plant, Schoenoplectus americanus

Trinity A Storr, Candice Lumibao
Texas A&M University - Corpus Christi, Corpus Christi, TX

Abstract

Coastal marshes are highly productive ecosystems that support carbon storage, shoreline stabilization, and diverse biota. Along the Gulf and Atlantic coasts of the United States, coastal marshes are dominated by a few foundational species, such as *Schoenoplectus americanus*, a sedge plant. Coastal marsh sediments can preserve *S. americanus* seeds for over a century, creating a natural seed bank that enables comparisons between modern and ancestral plant populations. While previous studies show that plant traits and genetics can change across time or sediment depth, not much is known about whether seed endophytes (microbes that live inside seeds) also change over time. Here, we isolated bacterial and fungal seed endophytes from *S. americanus* seeds retrieved from soil cores at two Chesapeake Bay marsh sites, Sellman and Corn Island. Seed stratigraphy was previously reconstructed from ^{210}Pb and ^{137}Cs dated soil cores. Representative seeds were sampled from 0-5 cm (modern) and ~16 – 27 cm depth (~100-year-old), surface-sterilized, cut into four 1mm pieces, and plated on nutrient agar or malt extract agar to isolate bacterial and fungal endophytes, respectively. Preliminary results show nine bacterial and five fungal morphospecies isolated from modern seeds. Growth from ancestral seeds is slower, with early cultures having produced one bacterial morphotype so far, with no fungal isolates. Isolated cultures will be genotyped via DNA sequencing to ascertain genetic identity. Understanding how seed endophytes vary across modern and ancestral seed banks may reveal microbial factors that influence plant establishment, stress tolerance, and marsh resilience under changing environmental conditions.

UP40

Generation of a Stable Knock-out of Streptococcus agalactiae Restriction Enzymes Using Cas12a Mutagenesis

Austin D Sandobal, William D Cutts, Sneha Chandrashekar, Krishi Chevendra, Brandon J Kim
University of Texas at Dallas, Richardson, TX

Abstract

Streptococcus agalactiae (Group B Streptococcus, GBS) is a gram-positive, opportunistic pathogen that asymptomatically colonizes the vaginal tracts of women and is the leading cause of neonatal meningitis globally, uniformly resulting in death without treatment. The hypervirulent COH1 strain of GBS is a multi-locus type 17 strain, which is a genetic background that is highly associated with neonatal meningitis. Along with other clinically relevant GBS strains, COH1 has anecdotally proven difficult to transform. Inactivation of restriction systems has improved transformation efficiency in other clinically relevant strains such as *S. pyogenes*. COH1 possesses an endogenous restriction system, with endonucleases annotated as *eco47* and *eco57*, which we hypothesize plays a role in hindering successful transformations. By knocking out the *eco47* and *eco57* restriction endonucleases via CRISPR-Cas gene editing, we hypothesize transformation efficiencies will be improved. To accomplish this, we have designed a repair cassette to produce a clean deletion of each gene and cloned it into plasmid pGBSedit. The resulting transformants were confirmed via plasmidsaurus sequencing. Once a knock-out is confirmed, transformation efficiency will be measured and compared to a wild-type COH1 strain. In the future, this mutant will be used to speed up the mutagenesis process and more quickly facilitate the study of various virulence factors.

UP41

Polymicrobial Interactions Between *Campylobacter jejuni* and *Enterococcus faecalis* Under Simulated Microgravity

Marwa Hemri, Jocelyn Vazquez-Sanchez, Madhusudan Choudhary, Anand B. Karki
Sam Houston State University, Huntsville, Texas

Abstract

Campylobacter jejuni is a leading cause of foodborne illness and is often found alongside *Enterococcus faecalis* in poultry associated microbial communities. In environments such as microgravity, bacterial growth and interactions can shift in ways that differ from those seen on Earth. Although close bacterial interactions are known to influence how *Campylobacter* responds to stress, it is not clear how these relationships change under microgravity conditions. In this study, we examined whether growing *C. jejuni* with *E. faecalis* affects its survival and stress-related behavior during simulated microgravity exposure. We expected that microgravity conditions would alter how the two species interact, leading to differences compared with single-species growth. Observations from simulated microgravity experiments point to possible differences in *Campylobacter* behavior when *Enterococcus* is present compared with monoculture conditions.

UP42

The Effects of Historical Adaptation of Microbes in New Stressful Environments

Ivan Mendez, Kingsley Amoateng, Sora Kim, Julian Ramirez, Natalie Zarate, Wei-Chin Ho
The University of Texas at Tyler, Tyler, Texas

Abstract

Adaptive mutations often improve an organism's fitness in one environment, but the effects of the mutations may change during environment shifts. The historical effects of adaptive mutations are largely unknown, but they substantially determine the evolutionary dynamics in the early adaptation to complex environments. If previous mutations retain their advantages, organisms may transition more easily into new environments. To explore this question, we examined three distinct bacterial populations that had evolved in Luria-Bertani (LB) medium for 900 days. The populations accumulated mutations that improved their fitness within this specific environment. To determine whether these derived mutations were beneficial, neutral, or harmful in new environments, we transferred the adapted microbes into four new stressful environments: LB broth with alcohol, LB broth with MgSO4, LB broth with HCl, and Glucose minimal medium. The results showed an idiosyncratic nature of historical adaptation. In four of the 12 cases studied, historical mutations provided significant fitness advantages in new environments, suggesting some mutations are useful. However, in four cases, beneficial mutations became disadvantageous, indicating that microbes thriving in LB broth struggled in new environments. Interestingly, one population shows advantages in more than one new environment. To further understand the underlying molecular basis, we examined the molecular profile of derived mutations. One candidate mutation for benefits in glucose minimal medium is a nonsense mutation in cytR, which likely removes repression of CRP-mediated carbon utilization pathways. This change may promote improved fitness under carbon limitation by facilitating rapid activation of scavenging metabolism in glucose minimal media.

UP43

Defining Alanine Transaminase Regulation in E. coli

Mithun S Mekala, Jacob B Hoggins, Lawrence Reitzer
University of Texas at Dallas, Richardson, TX

Abstract

Alanine synthesis via the AlaA and AlaC transaminases in *Escherichia coli* coordinates metabolism with cell wall biosynthesis and stress responses. Previous results suggested that alaA provides alanine for cell wall synthesis, while *alaC* provides alanine for protein synthesis. We investigated the regulation of the transaminases by binding of known pyruvate- and alanine-binding regulators, PyrR, PdhR, and MraZ, to promoter regions of alaA and *alaC*. MraZ is associated with cell wall synthesis, while PyrR and PdhR are associated with pyruvate utilization. We successfully isolated PyrR, PdhR, and MraZ: a single band on gel electrophoresis and western blotting the his-tag extension.

For promoter analysis, we used PCR amplified DNA of the promoter region upstream of the *alaC* and *alaA* structural genes. Mobility shift assays to detect protein-DNA interactions were performed with and without pyruvate and alanine which are the precursor and product of the alanine transaminases. MraZ bound to *alaA* promoter DNA with and without alanine and pyruvate. MraZ did not bind to the *alaC* promoter. PyrR and PdhR bound to both *alaA* and *alaC*: pyruvate inhibited and alanine stimulated binding.

We propose that alanine binding to PyrR and PdhR represses transaminase gene transcription, and pyruvate binding prevents this repression. However, MraZ binding to the alaA promoter confirms the role of AlaA in providing alanine for cell wall synthesis. The effector(s) that control MraZ activity are not known. These results show the elaborate mechanisms that control synthesis of alanine which is required for both protein and cell wall synthesis.

UP44

Competition between root fungal endophyte and *Fusarium spp*. under salinity stress

Logan Walker, Candice Lumibao
Texas A&M - Corpus Christi, Corpus Christi, Texas

Abstract

Fungal endophytes are a diverse group of plant symbionts that colonize the cortical cells of plant tissues asymptomatically. They can play an important role in host plants' fitness and stress tolerance, such as aiding the host plant in combating pathogen invasion. Fungal endophyte ecology is generally well-studied but basic understanding on how endophytes interact with fast-growing fungal pathogens and how it shifts abiotic stress remains limited. The fungal pathogen *Fusarium spp*, which typically infects crops, is a fast-growing fungus that grows at an exceptional rate under its typical growth conditions. However, under the presence of other fungi or abiotic stress, growth might be inhibited. In this study, competitive interactions between a fungal root endophyte strain and a Fusarium spp. under two environmental conditions were investigated. Endophytes and *Fusarium* were inoculated into 10 replicates each of competition as monoculture as well as co-inoculated into another 10 plates at 0ppt and 15ppt salinity levels. Observation took place over 15 days. Growth was monitored daily and pictures were taken every 3 days. *ImageJ* was used for analysis, where radial expansion and area were measured as proxy for growth. Results indicate that growth rates for the endophytes were increased under the high salinity and inversely for the pathogen. However, endophytes were outpaced by *Fusarium spp*. in growth. There were also noticeable morphological differences between the salinity treatments for the fungal pathogen, indicating plasticity. Information gained from this research will provide information on the application of endophytes as a potential antifungal treatment in agriculture.

UP45

The Effect of E. faecalis Secreted Metabolites: Lactate and Ornithine, on E.coli's Metabolic Transcriptome

Satya Vedula[1], Jacob Hogins[1], Yihan Liu[1], Akhil Nelapolu[1], Shreya Mekala[1], Jose Resendiz[1], Marjan Ganjali Dashti[1], Philippe E. Zimmern[2], Lydia Freddolino[3], Larry Reitzer[1]
[1]The University of Texas at Dallas, Dallas, Texas. [2]UT Southwestern, Dallas, Texas.
[3]University of Michigan, Ann Arbor, Michigan

Abstract

Urinary tract infections are the most common community-acquired bacterial infection, and Escherichia coli is the primary cause of 50-75% of these infections. 25% of *E. coli*-associated infections include *Enterococcus faecalis* as a co-pathogen. We previously determined that E. faecalis has increased genes for ornithine and lactate generation in the presence of *E. coli* and that E. coli alters its core metabolic transcription patterns in response to *E. faecalis* co-culture. We advance that here by exploring the transcriptional effects Enterococcus faecalis metabolites (lactate and ornithine) have on *Escherichia coli* to see if these chemicals signal the changes seen in coli-faecalis co-cultures.

Three strains of E. *coli* which had been co-isolated with E. faecalis were grown on MacConkey Agar to ensure mono-cultures were used. Multiple colonies were inoculated into 1mL of glucose tryptone media, incubated for two hours, diluted to OD 0.02 in 10mL of fresh medium with and without lactate, ornithine or the two in combination, and grown for 2.5 hours. After the incubation, bacteria were harvested by centrifugation and the pellets froze at –80. The following day, RNA was extracted and sequenced.

Our results suggest that E. coli utilizes lactate as a carbon source, while ornithine is converted to putrescine which has two regulatory functions 1) genes of energy generation and 2) genes of the TCA cycle. Although there could be other factors contributing to the changes seen in *E.coli*'s transcriptome, this discovery shows that some E. coli infections may be multi-species infections, emphasizing the need for novel therapeutic treatments.

UP46

Programmed Frameshifting Drives Ribosome Collisions and SmrB Recruitment on MS2 Viral mRNA

Khalid Abdelhamid, Zachary Hoover, Junjie Zhang
Texas A&M University, College Station, Texas

Abstract

Bacteriophage MS2 uses programmed ribosomal frameshifting (PRF) at the coat–lysis overlap to control production of the lysis protein from the viral mRNA. This recoding signal (slippery site plus downstream RNA structure) can stall elongating ribosomes on the viral transcript, potentially causing trailing ribosomes to collide and form collided disomes. In bacteria, ribosome collisions recruit the endonuclease SmrB, but whether PRF on viral mRNAs is a direct, quantifiable driver of collision formation and SmrB recruitment remains unresolved.

We will quantify collided disome formation on MS2-derived mRNAs by comparing constructs with an intact PRF cassette to matched controls lacking PRF (or carrying a weakened downstream structure). Ribosome assemblies will be resolved by sucrose-gradient fractionation to estimate disome abundance. To independently validate and quantify collision-linked factor recruitment, we will repeat the translation reactions and assay FLAG-tagged SmrB by protein purification followed by anti-FLAG Western blotting, testing whether PRF-positive viral mRNAs yield higher SmrB enrichment than controls.

MS2 exhibits an approximate 25:1 coat-to-lysis output, implying a PRF frequency of about 1 in 26 translation events (~3.85%). Using this known recoding rate as an internal benchmark, we will estimate what fraction of PRF events lead to measurable collisions and whether collision frequency is disproportionately high relative to frameshifting frequency. Establishing PRF as a collision-inducing viral motif would highlight a conserved vulnerability across many phages and viruses that rely on PRF, motivating future strategies to disrupt PRF-dependent translation and limit infection.

UP47

Assessing the Oligomeric Mechanism of Bacteriophage Mu's Lysis Regulatory Protein gp25

Mia Pham[1], Guadalupe Valencia-Toxqui[2], Jolene Ramsey[2]

[1]TAMU Department of Veterinary and Biomedical Sciences, College Station, Texas. [2]TAMU Department of Biology, College Station, Texas

Abstract

In a typical bacteriophage, the main strategy to lyse a host cell relies on a holin-endolysin-spanin system to breach the cell envelope of Gram-negative bacteria. The holin forms pores in the inner membrane to time the release of the endolysin, which ultimately results in lysis. However, bacteriophage Mu lacks a functional holin entirely. Instead, Mu encodes a novel regulatory protein gp25. This protein works in tandem with endolysin gp22 to drive cell lysis via an unknown mechanism. Here, we show that gp25 functions as an oligomer and that its assembly into multimeric complexes is essential for lysis activity. Unpublished data from our lab collected with purified protein suggests that gp25 adopts a trimeric conformation. To test this *in vivo*, we assessed a panel of nonfunctional and functional gp25 mutant alleles for dominant negative activity. Specifically, E.coli Mu lysogens encoding wild-type gp25 were induced in the presence of plasmids expressing the gp25 mutant alleles. By observing lysis timing, we could infer whether mutant subunits could disrupt the formation of wild-type homotrimers. Our results showed that all 11 nonfunctional mutants significantly delayed the lysis cycle, while two of the seven functional mutants accelerated it. Together, these findings reveal that gp25 possesses an intrinsic tendency to oligomerize. We propose Mu induces host cell lysis via an assembly-driven regulatory mechanism. Structural mapping of dominant mutants will identify the molecular determinants governing complex formation. These insights broaden our understanding of phage diversity and the evolution of novel lysis strategies across bacteriophages.

UP48

Genetic Modification of Saccharomyces cerevisiae ATCC 201388 Using CRISPR/Cas9 to Increase Pectinase Expression for Industrial Bioethanol Production

Victoria O Hoyt, Hannah Simpson, Claire Edwards
Saint Edwards University, Austin, Texas

Abstract

There continues to be an ongoing demand for renewable energy sources to mitigate greenhouse gas emissions from hydrocarbons and reduce global warming. However, current bioethanol production methods remain inefficient and costly. In second-generation bioethanol production, one of the inefficiencies is due to the lack of yeast strains capable of surviving the industrial fermentation of pectin-rich biomass and producing sufficient quantities of bioethanol. To address this challenge, this study focused on the genetic modification of the industrially robust *Saccharomyces cerevisiae* strain ATCC 201388 using CRISPR/Cas9 to enhance polygalacturonase expression, thereby improving the efficiency and reducing the cost of bioethanol production. Previous studies have demonstrated that disrupting the DLS1 gene in S. cerevisiae increases PGU1 expression and polygalacturonase activity. In this study, L2-01 plasmids containing Cas9 were engineered to include guide DNA targeting the DLS1 gene in *S. cerevisiae* through a transformation inducing the gene disruption. The resulting double-strand break was repaired using a URA3 repair patch amplified via polymerase chain reaction. Successful transformants were identified by growth on leucine and uracil drop out plates. To determine if the DLS1 knockout was achieved, PCR and phenotype testing will be done on successful transformants. Future research will focus on screening other industrial strains for DLS1, PGU1, and URA3 genes to identify other strains to genetically modify and improve the viability and cost of second-generation bioethanol production.

UP49

Decoding the Unknown Lysis Genes in Bacteriophage DAC15

Vedh K Gontuka[1,2], Gillian Brown[1,2], Michael A Debrah[1,2], Jolene Ramsey[1,2]
[1]Texas A&M University, College Station, Texas. [2]Center for Phage Technology, College Station, Texas

Abstract

The gut microbiome in humans is linked to human health, and imbalances can lead to serious chronic diseases. A major component of the healthy microbiome are the bacteriophages (phages), which infect and kill bacteria. Up to 95% of sequenced viruses in the microbiome are crAssphages. Given their abundance, we expect they play an important role in maintaining gut microbe levels. However, little is known about their basic infection cycles and lysis processes. To address this gap, our study aims to identify the lysis genes of DAC15, a crAssphage that infects the highly abundant complex carbohydrate-digesting bacterium, *Bacteroides thetaiotaomicron*. Using genome annotation software, we annotated the DAC15 genome to generate a list of 13 candidate lysis genes. The candidate genes were cloned into pFW6000, a shuttle vector for inducible expression in B. thetaiotaomicron. We are optimizing the conjugation of these plasmids using the donor *E. coli* strain RHO5. The advantage of this optimization is that RHO5 relies on exogenous DAP, a crucial precursor for the E. coli peptidoglycan layer. By counterselecting against *E. coli* in the absence of DAP, and adding Erythromycin to the plates, we are selecting for B. thetaiotaomicron with the lysis gene candidate inducible vectors. Future experiments will use lysis curves and microscopy to detect cell toxicity or morphological differences caused by expression of the candidate lysis genes. By identifying the lysis genes and their role in DAC15 lysis, we will be one step closer to understanding the crAssphage-bacteria dynamics in the gut microbiome.

UP50

Elucidating the Genes Underlying Hydrocarbon Degradation in Pseudomonas aeruginosa

Theodore Nguyen, Margaret Watson, Elinor True
University of Dallas, Irving, Texas

Abstract

Each year, thousands of gallons of aviation fuel is spilled at airbases, airfields, and airports causing significant harm to the environment. Current methods for spill remediation are inefficient, expensive, and logistically challenging. A potential alternative method, bioremediation, employs microorganisms to remove hazardous pollutants while diminishing these difficulties. *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 generated 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.

UP51

Breaking Through Barriers: How Host Factors Enable A Cationic Antimicrobial Peptide to Induce Lysis

Luis A Carrillo, Michael A Debrah, Guadalupe Valencia-Toxqui, Jolene Ramsey
Texas A&M University, College Station, Texas

Abstract

Once production of virion bacteriophage progeny is completed, it must escape through the host's envelope to spread to other hosts. In most well-characterized phages infecting Gram-negative bacteria, escape from the outer membrane is enabled by periplasmic-spanning fusion proteins, spanins, to induce lysis of the host cell. However, in bacteriophage phiKT, which lacks spanins, outer membrane disruption was fully complemented by a novel cationic antimicrobial peptide, gp28, diverging from this canonical bacteriophage lysis pathway. Our aim is to understand the involvement of host factors in the mechanism of action of gp28. To do this we are selecting for survivors of gp28 cytotoxicity in the presence of host factors encoded in the ASKA collection, a genomic overexpression library of every *E.coli* gene ORF. First, we tested methods to increase the throughput of our library screening process. We demonstrated synergy between low levels of gp28 expressed from a plasmid and multiple antibiotics. We observed that B-lactams showed a greater synergistic effect with gp28 expression, however the synergistic thresholds did not permit high-throughput use. Next, we constructed an overexpression plasmid encoding gp28 that is cytotoxic on its own. Into this strain, we transformed ASKA collection ORF clones, in which we expect to see survival when transforming clones encoding E. coli genes that inhibit gp28 function. Ongoing work will isolate these clones of interest. These results will further our understanding of gp28 outer membrane disruption and expand the possibility of using gp28 broadly to disrupt antibiotic-resistant bacterial membranes.

Bacteriophage Microbiology

UP52

When Viruses Come Home: Understanding Spillback Events

Lynley G Sombito[1,2,3], Akiko Kaitlin Balitactac[1], Austen Rowell[1], Enoch Ghosh[1], Lisa M Bono[1]
[1]Department of Biological Sciences, Texas Tech University, Lubbock, Texas. [2]Honors College, Texas Tech University, Lubbock, Texas. [3]TrUE Scholars Undergraduate Research Program, Texas Tech University, Lubbock, Texas

Abstract

Viruses are exposed to various hosts continuously, giving them opportunities to shift to new hosts and re-encounter their ancestral host. Studies have shown that when a virus expands its host range, mutations occur in the attachment gene, which allows the virus to enter the host cell more efficiently. Many studies focus on spillover, but little is known about how a virus responds to being returned to its original host (spillback). Here, we aim to examine how bacteriophage phi6 responds to spillback.

Previously, the host range of phi6 was expanded across novel hosts: *P*. *syringae* pv. *tomato* and *P*. *pseudoalcaligenes* ERA. The initial mutations that allowed host range expansion were costly (antagonistic pleiotropy). These mutants then evolved on either one novel host or alternating between the two novel hosts for 150 generations. Then, we isolated a single plaque from the endpoint of these evolution experiments and passaged them on their original host *P. syringae* pv. *phaseolicola* (Pp) for 100 generations.

Here, we sequenced the viral populations at the beginning and end of the evolution on Pp. We extracted viral RNA from two samples and purified it using gel extraction. Then, samples were sent to undergo cDNA conversion and Illumina sequencing. We assembled the genomes and identified SNPs that reached at least 1% of the population to locate and identify genetic variations that differed from the ancestor. We found that there were mutations in the membrane protein gene, which is responsible for virus structure, attachment to the host, and membrane fusion.

UP53

Isolation of Microbacteriophage PaleRider

Joshua Otero, Jacob Schilhab, Yasmeen Ramirez, Alexandra Aguilar, Gustave Allen, Mckenna Rice, Dustin Edwards
Tarleton State University, Stephenville, Texas

Abstract

Bacteriophages are viruses that infect and replicate within bacterial cells. The goal of this study was to discover, isolate, and analyze new microbacteriophages to generate new insights into their diversity, evolution, and the genetic structure of viruses that infect the phylum Actinobacteria. Microbacteriophage PaleRider was isolated from a silty soil sample in Rockdale, Texas (GPS Coordinates: 30.665 N, 97.0038 W) at 32 . The soil sample was suspended and incubated at 29 in peptone yeast calcium (PYCa) media while shaken at 225 rpm for 1 hour. The supernatant was filtered through a 0.22 μm filter and inoculated with the host bacterium, *Microbacterium foliorum*, on PYCa agar plates for 24 hours. PaleRider presented with small, clear plaques; one was picked, and two rounds of 6-fold serial dilutions followed to create a lysate. Webbed plates were made with undiluted lysate to form a high-titer lysate at 2.7 10^7 pfu/mL. Transmission electron microscopy (TEM) was performed with copper grids, and PaleRider was stained with 1% uranyl acetate to show siphovirus morphology and an approximate capsid diameter of 60 nm and tail length of 200 nm. DNA extraction was conducted with a modified zinc chloride protocol to determine a total DNA of 1.79 μg and a concentration of 46.7 ng/μL (A260/A280 = 1.86; A260/A230 = 2.24). PaleRider was archived at Tarleton State University and the University of Pittsburgh.

UP54

Isolation and Characterization of Microbacteriophage Triri from Central Texas Soil

Olivia Mccutchan, Shelby Perrenoud, Trinity Cobb, Gustave Allen, Mckenna Rice, Dustin Edwards
Tarleton State University, Stephenville, Tx

Abstract

Bacteriophages, also known as phages, are the most abundant biological entities on Earth and offer promising alternatives to antibiotics via phage therapy against resistant infections. Another notable quality of phages are being able to reduce the presence of harmful bacteria in soil, water and so on. Microbacteriophages infect Microbacterium species and exhibit high genetic diversity, many with uncharacterized genes. To expand knowledge of regional diversity, we isolated and characterized Triri, a novel phage from central Texas. Triri was isolated from soil in Stephenville, TX (GPS: 32.20959°N, 98.21471°W). The sample was enriched in PYCa medium, filtered (0.22 μm), and incubated with Microbacterium foliorum at 29°C for 7 days. Plaques were obtained via serial dilution and soft agar overlays on PYCa plates after 2 days. Triri was purified by picking a single plaque and replating. High-titer lysates (10^9–10^{10} PFU/mL) were prepared by flooding webbed plates with phage buffer overnight at 4°C. TEM showed siphovirus morphology (isometric capsid, long non-contractile tail). Triri belongs to Cluster EA1, however the genome sequencing was fragmented. This isolation contributes to cataloging Texas microbacteriophages, highlighting local diversity and potential applications in biotechnology or therapy against Microbacterium infections. Future efforts include full genome sequencing to reveal novel functions.

UP55

Discovery and Isolation of Bacteriophage Blimey from *Microbacterium foliorum*

Angel G Lozano, Adriana Drescher, Kinley Harris, Mckenna Rice, Dustin Edwards
Tarleton State University, Stephenville, Texas

Abstract

Bacteriophages are an abundant class of viruses which target bacteria as part of their replication cycle. This process consists of the phage injecting its DNA into the bacteria to produce more copies of itself. This property makes cataloguing the diversity of phages an attractive realm of study. In the interest of understanding the biodiversity of bacteriophages, Bacteriophage Blimey was extracted from a soil sample collected from sand-like surface soil in Stephenville, TX (32.223988 N, 98.222796 W,). An enriched isolation was performed on the sample. The resulting plaque assay confirmed that the sample contained evidence of bacteriophages. Two serial dilutions were performed to isolate the bacteriophage, followed by a third serial dilution to obtain a low volume lysate (LVL). The LVL was used to create webbed plates, which were used to yield a high volume lysate (HVL). The HVL was serially diluted and a plaque assay was performed (titer: 2.9×10^6 pfu/mL). DNA was successfully extracted from the HVL over two days, and a .8% agarose gel solution was prepared for electrophoresis. The results from electrophoresis are being used for genomic sequencing, which is currently being conducted at the Pittsburgh Bacteriophage Institute. The HVL was also mounted and stained with 1% uranyl acetate to prepare a Transmission Electron Microscopy (TEM) grid. The grid was sent to an external facility for imaging, which yielded a siphovirus morphology. Research on Blimey is ongoing, including sequencing of the genome, with the goal of expanding knowledge of known bacteriophages.

UP56

Isolation and Characterization of a Lytic Bacteriophage Aleia

Abigail Ortega, Calli A Hoesterey, Celeste Gant, Frederick N Baliraine
Department of Natural Sciences, Letourneau University, Longview, Texas

Abstract

Bacteriophages or phages are recognized as powerful tools in combating antibiotic-resistant bacteria, due to their ability to specifically infect and lyse bacterial pathogens. Phage Aleia was isolated from a soil sample collected on August 26, 2025, by the bank of a pond in Longview, Texas (32.52344° N, 94.77375° W). It was isolated using the enrichment method with Middlebrook 7H9 medium and Mycobacterium smegmatis mc^2155 as the host. A spot test was used to confirm phage presence. Aleia was purified through 3 rounds of 10-fold serial dilutions and plaque picking from plates incubated at 37 °C for 48 hours. The plaques were clear to slightly cloudy, with an average diameter of 2.1 mm (range 2.0 - 3.0 mm; n = 10). Amplification produced a high titer lysate of 3.3×10^{10} pfu/mL which was used for gDNA extraction, transmission electron microscopy preparation, and archiving. Aleia has a genome length of 68,626 bp, a G+C content of 66.4%, and a circularly permuted end. Preliminary data show that Aleia has 101 putative protein-coding genes, no tRNA, and no lysogeny-associated genes, indicating a lytic life cycle. Phage Aleia was assigned to subcluster B1, making it the first B phage isolated in East Texas and notably one of the only three subcluster B1 phages reported in the state of Texas so far. Further genomic investigation of Aleia may enhance knowledge about its potential application in treating antibiotic-resistant bacterial infections.

UP57

A Hole-in-One Discovery: OverPar, The First L2 Bacteriophage Identified in East Texas

Reagan L. Chastain, Clarissa A. Martinez, Camila Andrade, Zoe J. Harbison, Frederick N. Baliraine
Department of Natural Sciences, LeTourneau University, Longview, Texas

Abstract

The first L2 bacteriophage isolated in East Texas, OverPar expands our knowledge of regional phage diversity and supports ongoing phage therapy research. OverPar was isolated from a soil sample collected on August 21, 2025, from LeTourneau University in Longview, Texas. The enrichment method was utilized with *Mycobacterium smegmatis* mc^2 155 as the host and Middlebrook 7H9 as the medium. A spot test was used to confirm phage presence and after OverPar was purified via 10-fold serial dilutions and 3 rounds of plating with a 48-hour incubation at 37 °C. Amplification yielded a high titer lysate of 2.1 x 10^9 PFU/mL which was used for gDNA extraction, transmission electron microscopy (TEM) imaging, and archiving. OverPar produced turbid plaques with an average diameter of 0.168 mm (range 0.10-0.28 mm; n = 10). TEM imaging revealed that OverPar has a siphovirus morphotype. The gDNA was sequenced using the Illumina shotgun sequencing method with a 100 bp read length and ~1055x coverage. Sequencing data showed OverPar belongs to subcluster L2 with a 10 bp 3' sticky overhang (TCGATCAGCC) sequence, 69,993 bp genome length, G+C content 58.9%, 126 putative genes, and 8 tRNA. Annotation is ongoing, but so far, phage OverPar has been confirmed to be a temperate phage, evidenced by the presence of a tyrosine integrase, immunity repressor, cro protein, and excise in its genome. OverPar adds to the growing inventory of bacteriophages with therapeutic potential and highlights East Texas as a valuable source of novel phage diversity.

UP58

Isolation and Characterization of *Mycobacterium* Phage Ruriko

Alba L. Prince, Brooke A. Bubert, Hailee R. Perry, Frederick N. Baliraine
Department of Natural Sciences, LeTourneau University, Longview, Texas

Abstract

Bacteriophages, or phages, are currently used in research, bioremediation, and in medicine such as in the treatment of antibiotic-resistant infections. Phage Ruriko was isolated from a compost bin in Lindale, Texas (32.49 N, 95.42 W) on August 26, 2025, using the enriched isolation method. The soil sample was mixed with Middlebrook 7H9 medium and *Mycobacterium smegmatis* mc^2 155, 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. Ruriko produced small, bullseye plaques, indicating a temperate lifecycle. The plaques picked during phage purification were ≥5 cm apart from neighboring plaques. Three rounds of 10-fold serial dilutions and plating with incubation at 37 °C for 48 hours were done to ensure that a pure phage was isolated. Nine webbed plates at the 10^{-4} dilution were prepared, each was then flooded with 5 ml of phage buffer, producing a high titer lysate which was collected from all plates and filtered (0.22 µm). The lysate (titer 1 x 10^{10} PFU/ml) was used for gDNA extraction, TEM imaging, and sample archiving. Ruriko's sequencing was completed on January 6, 2026, using the Illumina NextSeq 1000 platform at the Pittsburgh Bacteriophage Institution. Ruriko's genome length is 41903 bp, with an 11 base (CCCCATGGCAT) overhang sequence, and 60 putative genes. Ruriko's genome awaits annotation.

UP59

Isolation and Characterization of Bacteriophage SteMason

Cash H. Dunn, Alba L. Prince, Brooke A. Bubert, Hailee R. Perry, Frederick N. Baliraine
Department of Natural Sciences, LeTourneau University, Longview, Texas

Abstract

Bacteriophages are viruses that utilize bacterial hosts in their life cycle, and are important in various applications including phage therapy and genetic engineering. SteMason was isolated from a soil sample collected behind the Mechanical Engineering Lab l building at GPS coordinates 32.46485^{o}N, 94.72667^{o}W on the campus of Letourneau University in Longview, Texas, on September 15, 2025. The bacterial host cells used for isolation were *Mycobacterium smegmatis* mc^{2} 155 and the medium used was Middlebrook 7H9. After confirming phage presence using a spot test, SteMason was purified through a 10-fold serial dilution and one round of plating with an incubation period of 48 hours at 37 ^{o}C. SteMason produced turbid, bullseye-like plaques with an average diameter of 3.56 mm (range 3-4 mm; n=10). Amplification resulted in a high titer lysate of 3.0 x 10^{10} PFU/mL. The lysate was used for gDNA extraction, TEM imaging preparation, and archiving. TEM micrographs showed SteMason to have a siphovirus morphotype. The gDNA was sequenced using the Illumina NextSeq 1000 method, with a shotgun coverage of ~ 906x. Sequence data showed that SteMason belongs to the A4 cluster. It has a genome length of 51,372 bp with a 10 bp (CGGCCGGTAA) 3’ sticky overhang sequence, 63.9% G+C content, 89 putative genes, and no tRNAs.

UP60

Discovery and Genomic Characterization of Cluster EE Bacteriophage Rira

McKenna Rice, Richard Turner, Aliana Ramos, Lisseth Cervantes, Alexandra Aguilar, Dafne Balderas, Toby Barrera, Adriana Drescher, Kinley Harris, Angel Lozano, Olivia McCutchan, Josh Otero, Shelby Perrenoud, Yasmeen Ramirez, Gustave Allen, Dustin Edwards
Tarleton State University, Stephenville, Texas

Abstract

Bacteriophages are the most abundant microbes on Earth, driving nutrient cycling and bacterial population regulation in terrestrial environments. Yet, those infecting the genus *Microbacterium* remain largely unexplored. Phage Rira was discovered via enriched isolation from dense, rocky soil collected from Rockdale, TX (GPS coordinates: 30.66544° N, 97.00423° W). The soil sample was suspended in PYCa medium, inoculated with bacterial host Microbacterium foliorum, and incubated at 29 °C. Following filtration (0.22 µm), screening via soft-agar overlays revealed small, turbid plaques after 48 h at 30 °C. Optimal plaque visibility was observed at the 10^{-4} dilution. Purification was achieved through serial replating from a single plaque, and high-titer lysates (2.5×10^8 PFU/mL) were yielded via webbed-plate flooding and filtration. A Siphoviridae morphotype was identified through transmission electron microscopy, characterized by an isometric capsid and a long, non-contractile tail. A 16,971 bp double-stranded DNA genome was determined through Illumina sequencing, consistent with the notably small genomes of Cluster EE microbacteriophages. A 68.8% GC content and 9 bp 3’ sticky overhangs (CCCGCCCA) were observed within the sequence. Twenty-five predicted protein-coding genes (including 1 tRNA) were identified through preliminary annotation. Rira was found to cluster with existing EE phages based on sequence similarity, expanding the known diversity of this cluster and highlighting the genetic mosaicism inherent in soil-borne actinophages. Ongoing genome annotation is being performed to further define the gene products responsible for the lytic replication cycle and host-phage dynamics of this isolate.

UP61

Comparative Analysis of the Phosphoesterase Gene Across Subcluster A Bacteriophages

Micah R.R. McCloy, Dylan W Yost, Frederick N Baliraine
Department of Natural Sciences, LeTourneau University, Longview, Texas

Abstract

Bacteriophages must specifically target and insert their genome into a bacterial host's genome to successfully propagate. A phosphoesterase, a.k.a. metallophosphoesterase or metallophosphatase (MPE/MPP) if it contains an HEXXH motif carrying two metal ions, is an accessory protein and virulence factor used by bacteriophages to subvert host antiviral systems to facilitate infection and subsequently aid host DNA degradation. To further knowledge of phage infection dynamics, phosphoesterase gene prevalence, synteny, and conserved domain types and prevalence were analyzed across 10 subcluster A bacteriophages. Ten non-draft phage genomes were analyzed per subcluster (A1-A10), except subcluster A7 where all five genomes were analyzed. Phosphoesterase was found in all the 95 analyzed genomes. Synteny was analyzed using Phamerator to determine if there were any differences in the flanking genes within and among the subclusters. All analyzed MPPs were located downstream the DNA binding protein and transcribed in the reverse direction. Gene length of adjacent genes was found to be of a consistent length within each subcluster. Conserved domains (CDs), analyzed using Microsoft Excel, were found in 75.8 % (72/95) of the studied genes. Notably though, CDs were absent from all (100%) subcluster A1 and 70% subcluster A8 MPP genes. The most common CDs were the calcineurin-like phosphoesterase Metallophos (54.7%), the DNA repair exonuclease SbcD (27.4%), the calcineurin-like phosphoesterase superfamily domain Metallophos_2 (22.1%), and the predicted ICC-like phosphoesterases COG1407 (20.0%). Further studies are warranted to investigate phosphoesterase variation across all cluster A bacteriophages in relation to presence, synteny, and phylogenetic relationships.

UP62

The role of Sim proteins of bacteriophage P1 in superinfection exclusion

Isabel Wang[1], Honorio Negrete-Méndez[2], Jason J Gill[2]

[1]Department of Biochemistry and Biophysics, Texas A&M University, College Station, Texas.

[2]Department of Animal Science, Center for Phage Technology, Texas A&M University, College Station, Texas

Abstract

Superinfection exclusion (SIE) is a trait expressed by a primary infecting phage from the prokaryotic host cell and prevents secondary infections by phages of the same or different species. In *Escherichia coli* phage P1, the *sim* locus (*simABC*) has been implicated in the SIE phenotype. At least one Sim protein, SimC, is necessary to confer superinfection exclusion, but the specific mechanism is unknown. The other proteins, SimA and SimB, have unknown functions. Understanding the interactions between P1, its host, and other phages in the context of SIE will build foundational knowledge of phage biology to advance phage therapeutics. The *simABC* cassette and the individual *sim* genes were cloned into pBAD24 expression plasmids and transformed into *E. coli* BW25113. Infection of SimABC-expressing bacteria by a thermoinducible P1 strain (P1tscm) showed decreased plaque formation by a titer difference of 10^{-3} and cytotoxicity. Under the same conditions, expression of SimC alone produced a reduction in P1 plating efficiency similar to that of SimABC while SimB expression led to cytotoxicity. SimA expression alone produced no observable phenotype. The SIE effect of SimABC was determined to be specific to P1 as SimABC-expressing bacteria did not prevent infection of other *E. coli* phages including T4, T7, λ, and Mu. RNA-seq data of P1tscm lysogens showed that *simABC* has low expression in the prophage state, suggesting it is active during the phage lytic cycle. A strategy of selection for P1tscm mutants that can bypass SIE by SimABC is being pursued to identify the structural point of interaction.

UP63

Tool Development for the Study of CrAss-Like Bacteriophage DAC15

Enya M Hoxha, Gillian D Brown, Micheal A Debrah, Jolene Ramsey
Department of Biology, Center for Phage Technology, Texas A&M University, College Station, TX

Abstract

The gut microbiome plays a critical role in human health. Chronic disease states, such as irritable bowel disease, correlate with shifts in gut microbiome community structure called dysbiosis. Critical members known to contribute to a healthy gut include carbohydrate catabolizing bacteria in the *Bacteroidota* group. Interestingly, the Bacteroidota are predated by the highly abundant crAss-like bacteriophages (phages). The crAss-like phages account for up to 95% of all sequence reads in individual human viromes worldwide. To understand how these phages and their hosts are both balanced and abundant in the healthy microbiome we need to study their infection. Using *Bacteroides thetaiotaomicron* and its phage DAC15, we are establishing tools for genetic, functional, and population dynamic studies. First, we began to define DAC15 gene essentiality. An ongoing screen of ethyl methanesulfonate-mutagenized DAC15 for essential phage genes has tested over 900 plaques for temperature-sensitive phenotypes. Second, we probed phage dependence on essential host cell processes. Preliminary results from the use of rifampin to inhibit transcription, erythromycin to inhibit translation, metal ion chelators and chloroform to disrupt membrane integrity, and a protonophore to disrupt the proton motive force will be presented. Finally, traditional fluorescent reporter proteins are oxygen-dependent, making them incompatible with *B. thetaiotaomicron* infections. Therefore, we are testing the anaerobic-compatible FAST protein fluorogen system to track live phage infections. These tools enable us to perform genetic manipulation of DAC15 as a model for the dynamics between crAss-like phages and their hosts in the gut microbiome.

UP64

Investigation of a Phage Lysis Gene by CRISPR-Cas13a Mediated Deletion

Angelina Zhu, Michael Baffour Awuah, Jolene Ramsey
Texas A&M University, College Station, TX

Abstract

As antibiotic resistance is projected to cause 2 million global deaths annually by 2050, finding alternative therapeutic approaches is crucial. Phage therapy is an alternative to treat antibiotic-resistant bacterial infections that relies on the terminal event in the phage infection cycle: lysis. Host cell lysis is initiated by phage holin proteins when they form holes in the cytoplasmic membrane. *E. coli* bacteriophage N4 encodes a holin called gp63 and a second protein called gp62 with uncharacterized holin regulatory activity. Here, we explore the hypothesis that gp62 is a second holin protein aiding in phage N4 lysis. When expressed alone from plasmids, gp62 exhibits lethal activity on cells. When replacing holins in heterologous phages, gp62 exhibits a delayed complementation phenotype. These two pieces of evidence suggest gp62 may be a holin with altered hole-forming kinetics. We tested this in the phage. Homologous recombination and CRISPR-Cas13a counterselection against the wildtype was used to delete gp62 in the N4 genome. The Δgp62 N4 phage exhibits accelerated asynchronous lysis, initiating 100 minutes earlier than wildtype. However, the lysis timing could not be complemented to the wildtype lysis time by heterologous gp62 expression, suggesting polar effects on downstream gene expression. Future tests will use an amber allele in gp62 to minimize polar effects. By understanding molecular mechanisms regulating critical phage lysis proteins, we can advance future clinical applications to target antibiotic-resistant bacterial infections.

UP65

Bioinformatic Analysis and Genome Annotation of Bacteriophage Kinny

Olivia Kelly[1], Melanie Baires[1], Amber Morton[2], Theoren Markusson[1], George Sander[1], Arya Sreenivas[1], Libertyann Ward-Polonski[1], Damian Al Khayatt[1], Abigail Frank[1], Sophia German-Kavle[1], Morgan Reed[1], Shreya Srivastava[1], Katie Dale[2], Elizabeth C. Williams[2], Sabrina Gregoire[2], Khushi Rathod[2], Victoria Gilchrist[2], Carson Bellew[1], Christian DeLuna[1], Francesca Makilan[1], Austen Roswell[1], Fahareen B. Mosharraf[1], Adam D. Rudner[2], Allie C. Smith[1], Lisa M. Bono[1]

[1]Texas Tech University, Lubbock, Texas. [2]University of Ottawa, N/A, N/A

Abstract

Bacteriophages are viruses that specifically infect bacteria and represent the most genetically diverse groups of biological entities on Earth. Through the Science Education Alliance–Phage Hunters Advancing Genomics and Evolutionary Science (SEA-PHAGES) program, undergraduate researchers contribute to expanding scientific knowledge by discovering and analyzing bacteriophages in the environment. In this study, we investigated Kinny, a bacteriophage that infects the soil bacterium Arthrobacter globiformis, an organism known for its ability to thrive in varied environmental conditions. Following genome sequencing, Kinny's DNA was examined using bioinformatic annotation methods to identify genes and predict their biological roles. We used Glimmer and GeneMark to generate initial gene predictions and then reviewed and refined manually using DNA Master. Each predicted gene was evaluated for the most likely start site by examining coding potential, ribosomal binding sites, and the spacing and overlap between neighboring genes. We assigned gene functions by comparing predicted proteins to known sequences and conserved domains using databases such as NCBI BLAST, HHpred, and Phamerator. These tools allowed us to identify genes likely involved in genome replication, structural assembly, and host infection. Comparative genomic analysis placed Kinny in the AU cluster of Arthrobacter phages. Annotating Kinny contributes to the growing database of bacteriophage genomes, thereby strengthening our understanding of viral diversity and evolution. Future work will focus on a deeper comparative analysis to better understand Kinny's relationship to other AU cluster phages and its potential significance in microbial research.

UP66

Isolation and Characterization of the Novel Arthrobacter Phage, Nandito

Kate Cavenaugh, Dacey Dougherty, Arav Tyagi, Tamarah Adair, Jonathan Lawson
Baylor University, Waco, TX

Abstract

Bacteriophages are the most abundant biological entities on earth and play key roles in microbial ecology. Phage discovery broadens the diversity of known phages and improves understanding of phage evolution. This study reports the isolation and characterization of Nandito, a novel phage that infects *Arthrobacter globiformis* B-2979. The soil sample was collected from damp soil near compost in Woodway, Texas. The phage was isolated from an enriched sample, and the subsequent plaque assays displayed clear, lytic plaques with an average diameter of 1.046 mm. Plaque assays were used to purify the sample and a high-titer lysate (greater than 5.0×10^9 pfu/mL) was made for further studies. The phage was observed by transmission electron microscopy and revealed a Myoviridae morphotype, characterized by the contractile tail measuring 115.7 nm and a capsid diameter of 63.4 nm. The genetic information was then analyzed by Illumina single-end read sequencing, which produced approximately 1266× coverage. The genome is circularly permuted, has a length of 50282 bps, and a GC content of 69.6%. Auto-annotations predicted 79 genes, which are currently being revised using evidence from BLAST, GeneMark, and HHPred to verify genes and, if supported by evidence, make functional calls. Comparative genomic analysis placed Nandito within the FH cluster. The study will continue annotation until the Nandito genome has been fully annotated with gene locations and their respective functional calls. The finalized annotated genome will expand the growing phage database and support future applications in comparative genomics, microbial genetics, and phage-based therapeutic research.

UP67

Mardi, a novel Arthrobacter phage

Chandini Elango, Nancy Li, Tessa Nguyen, Tamarah Adair, Jonathan Lawson
Baylor University, Waco, TX

Abstract

Bacteriophages are a vast, genetically diverse group of viruses that infect and lyse bacteria. Despite their prevalence, most bacteriophages remain undiscovered and uncharacterized. This study aimed to expand current knowledge by isolating, purifying, amplifying, characterizing, and sequencing an unknown bacteriophage infecting *Arthrobacter globiformis* B-2979. The phage Mardi was isolated at 30 from moist topsoil collected in Zachary, Louisiana. Through three rounds of purification, consistent plaque morphology was observed. Using electron microscopy, Mardi was identified as a Siphoviridae phage, with a capsid size of 66.3 nm and a tail length of 219 nm. Whole genome sequencing, performed by the University of Pittsburgh using the Illumina method, revealed a genome length of 55,625 bp and a GC content of 50.0%. Following an initial annotation, 93 genes were predicted; ongoing refinements suggest more genes are likely. Genes include several with strong functional assignments such as structural proteins and HNH endonuclease. Comparative analysis places Mardi in the AU6 subcluster, and shows high synteny to cluster members such as Lewando, Nan1117, and Zeina. Annotating utilizes bioinformatic programs such as PECAAN, BLAST, HHpred, and Alphafold. Nonetheless, many genes lack functional calls, demonstrating the need to broaden our understanding of bacteriophage diversity and genomic architecture. By expanding the catalog of characterized phages, this work supports ongoing efforts to develop phage-based strategies for combating bacterial infections and addressing antimicrobial resistance.

UP68

Same Host – Divergent Phage: Exploring common structural features of distinct phage that infect *Arthrobacter globiformis*

Saiah Lee, Oliver Ling, Jaxon Luddeke, Tamarah Adair, Jonathan Lawson
Baylor University, Waco, TX

Abstract

Arthrobacter globiformis is a Gram-positive species of soil bacteria found in oxygen and organic material-rich environments all across the world. As this organism lives in such a diverse array of places, it serves as a host for a diverse range of bacteriophages, and the strain used for our phage isolation, *A. globiformis* B2979, is no different. This research aimed to analyze the recently discovered phages, Nandito and Mardi, at Baylor University that infect the strain *A. globiformis* B2979 and highlight similarities despite the stark differences. The genomes of these phages were analyzed and annotated after DNA sequencing with PECAAN, GeneMark, Phamerator, Starterator, and HHPred. A comparison of phage tail proteins is being performed using Alphafold and ChimeraX. Predicted structural alignments using RMSD analysis is being performed to quantify possible conserved domains despite the low sequence similarity. Analysis of these genomes reveals numerous differences between the two phages. Nandito is a myoviridae in cluster FH with a GC content of 69.6%, similar to the GC of *A. glob* at around 65-67%, yet Mardi is a siphoviridae in cluster AU with a GC content of 50.0%. They may share this common host by having tail proteins with conserved domains that specialize in recognition and penetration of the peptidoglycan wall on *Arthrobacter globiformis*. This comparative analysis between these phages and their interactions with the common host, *Arthrobacter globiformis,* will seek to identify possible similarities in phage-host interaction despite their genomic and structural differences.

UP69

Genomic Insights into Two Newly Discovered Soil Bacteriophages

Melanie Baires[1], Olivia Kelly[1], Melissa Barnett[2], Adjia Seynabou Sy Gueye[2], Marie Blandin[1], Anna Garner[1], Cylee Karabell[1], Jaimie Kim[1], Ryleigh Kiser[1], Cooper Mau[1], Paola Sedeno Gonzalez[1], Tatum Rios[1], Cole Bridges[1], Voshita Parvathaneni[1], Katie Dale[2], Elizabeth C Williams[2], Sabrina Gregoire[2], Khushi Rathod[2], Victoria Gilchrist[2], Allie C Smith[1], Lisa M Bono[1]

[1]Texas Tech University, Lubbock, Tx. [2]University of Ottawa, Ottawa, Ontario

Abstract

Bacteriophages are viruses that infect bacteria by replicating within bacterial cells. Viruses are highly diverse and are the most abundant biological entities in the environment. The Science Education Alliance–Phage Hunters Advancing Genomics and Evolutionary Science (SEA-PHAGES) Program provides undergraduate students with hands-on research experience, including the collection, characterization, sequencing, and annotation of novel soil phages. This project focuses on the annotation of IbOuu and GoongGoong, two novel bacteriophages discovered in Ottawa, Canada, in collaboration with the University of Ottawa. The focus of this poster is the primary bioinformatic software used for annotation and analysis. DNAMaster was used to perform automated annotations with GeneMark and Glimmer, followed by manual confirmation of potential start codons for each gene. Start codons were assessed using ribosome binding site (RBS) scores, gap and overlap analysis, and open reading frame (ORF) length. Start positions were also compared to genetically similar phages within the same cluster using NCBI Protein BLAST and the Starterator database. Functional analysis of each predicted gene was conducted using NCBI BLAST, HHPred, and Phamerator, and results were compared to related phages within the same cluster. Whole-genome annotations of IbOuu and GoongGoong were completed and submitted to the Actinobacteriophage Database in PhagesDB. Both phages infect *Mycobacterium smegmatis* mc^2155 and exhibit siphoviridae morphology. IbOuu is temperate with a 70 nm capsid and 217 nm tail, while GoongGoong is lytic with a 69.4 nm capsid and 293.4 nm tail. This research contributes to publicly available genomic databases for future microbiological studies.

GRADUATE POSTER PRESENTATIONS

Pathogenic Microbiology

GP1

Defining Contribution of Progesterone Signaling to Group B Streptococcal Interaction at the Blood Brain Barrier

Darla Delozier[1], Kathryn N Seely[1], Katelin R Stilen[1], Jacob F Wood[2], Robert E Mccullumsmith[2], Brandon J Kim[1]

[1]University of Texas at Dallas, Richardson, TX. [2]University of Toledo, Toledo, OH

Abstract

Streptococcus agalactiae (Group B Streptococcus, GBS) is a Gram-positive opportunistic pathobiont and is the leading cause of neonatal meningitis worldwide. We have previously shown that estrogen signaling can contribute to GBS interaction with the blood-brain barrier (BBB) contributing to meningitis. Examination of large deidentified datasets have revealed an additional role for the sex hormone progesterone in invasive GBS disease with an increased risk of neonatal GBS infection in from women undergoing perinatal progesterone hormone treatment. Therefore, we hypothesize that progesterone may contribute to bacterial interaction with the BBB. To model the BBB, induced pluripotent stem cell-derived brain-like endothelial cells (iBECs) and immortalized cerebral microvascular endothelial cells (hCMEC/D3s) were treated with progesterone prior to GBS infection in order to further understand the relationship between GBS infection and progesterone signaling at the BBB. Both cell lines show a trending increase in GBS adherence with an increase in progesterone concentration, suggesting that progesterone signaling could contribute to the susceptibility of the BBB to GBS infection. Further analysis of the transcriptome of progesterone-treated iBECs during GBS infection will be done using qPCR to discover which progesterone-related signaling pathways are altered during GBS infection. The results of further adherence and invasion assays and RNA collection and analysis will allow us to identify the specific pathways contributing to the weakening of the BBB and develop strategies for prevention of neonatal GBS infection as a whole.

GP2

Identification and Characterization of Repositionable Compounds with Activity Against *Clostridioides difficile* Biofilms

Cassandra G Spondike[1], Alec Solis[2], Caden Munson[1], Paola Zucchi[1], Jesús A Romo[1]
[1]University of Texas at San Antonio, San Antonio, Texas. [2]UT Health Houston, Houston, Texas

Abstract

Background: *Clostridioides difficile* is an anaerobic, toxin-producing gastrointestinal bacterial pathogen responsible for ~500,000 infections each year. Between 15-30% of patients treated with antibiotics for *C. difficile* infection (CDI) will fail initial treatment. Treatment failure increases the possibility of recurrence, with 40-60% of patients experiencing CDI relapse. While the exact mechanism leading to CDI relapse is not well understood, recent evidence suggests biofilms might be implicated.

Hypothesis/Objective: We hypothesize that targeting *C. difficile* biofilms can reduce the risk of CDI relapse. We aim to identify repositionable biofilm-active compounds.

Methods: We screened the Pandemic Response and Global Health Priority Boxes from Medicines for Malaria Venture (MMV) (640 compounds) anaerobically against mature *C. difficile* biofilms. Biofilms were formed in BHIS media supplemented with 0.1 M glucose for 24 hours followed by the addition of MMV compounds for an additional 24 hours. Biofilm metabolic activity was determined using the PrestoBlue™ metabolic assay.

Results: A total of 84 compounds reduced mature biofilms by more than 50%. Based on structural diversity and potential for novel mechanism of action, 15 candidates were prioritized. Dose response studies against the planktonic lifestyle of *C. difficile* have confirmed the activity of six compounds with MIC_{50} values ranging from 0.3188 μM through ~50 μM. Dose response assays against biofilms are in progress.

Conclusions: We have identified six repositionable candidates with activity against planktonic *C. difficile* and mature biofilms. These compounds will be down-selected further and tested in a murine model of CDI relapse to assess the efficacy of biofilm-targeted treatments.

GP4

Baseline Patterns and Determinants of *Lactobacillus*-Dominated Cervicovaginal Communities in Asymptomatic Women

Daisy C Ngetich
Stephen F Austin State University, Nacogdoches, Texas

Abstract

The cervicovaginal microbiome is essential in reproductive health and is involved in limiting pathogen colonization, and this is usually dominated by members of the *Lactobacillus* genus that confer resistance by producing lactic acid, maintaining a low vaginal pH, and secreting antimicrobial compounds (Ravel et al., 2011; Petrova et al., 2015). The disruption of this microbiome, especially in individuals who have been exposed to antibiotics, results in a decrease in *Lactobacillus* species, an increase in microbial diversity, and antimicrobial resistance (Muzny and Schwebke, 2016), while also supporting the survival of tolerant microbes and the transfer of resistance genes. The objective of this study was to characterize the cervicovaginal microbiome of asymptomatic individuals and assess the effect of various physiological and lifestyle factors, including cycle phase, body mass index, menopausal status, sexual activity, antibiotic use, and contraceptive use, on microbiome diversity and composition using 16S rRNA gene sequencing and RDP classifier. Alpha diversity indices did not show any significant variation between variables; however, beta diversity revealed the effect of the menstrual cycle. The microbiota was dominated by *Lactobacillus;* however, with higher body mass index, there was slightly higher diversity and anaerobic species. The detection of low abundance of *Escherichia* and *Shigella* species indicates that pathogenic species may be found within stable microbiota. This research has shown the stabilizing effect of *Lactobacillus*-dominated microbiota and how physiological variation impacts the microbiota; this has important implications for the effect of antibiotics on microbiota stability and the antibiotic resistance generation.

GP5

Expression of Virulence Genes in *Listeria monocytogenes* Shifts with Sub-Lethal Electron Beam Exposure.

Isabella A McGrath, Chandni Praveen, Suresh D Pillai
Texas A&M University, College Station, Texas

Abstract

Introduction

Electron beam (eBeam) and X-ray treatments are utilized by the food industry for shelf-life extension and are limited to doses up to 1 kGy. Products that have received phytosanitary doses may host pathogens that receive a sub-lethal ionizing treatment dose. Sub-lethally exposed *Listeria monocytogenes* may undergo changes related to stress response and virulence.

Methods

Three strains of *Listeria monocytogenes* from clinically relevant serotypes were selected for the study. The bacteria were grown overnight to ~ 8 Log CFU/mL, packaged in phosphate buffer solution, and exposed to 1 kGy of electron beam, an appropriate sub-lethal dose given the starting titer. RNA was extracted from the samples immediately after treatment (Day 0), Day 1, Day 5, and Day 10. The samples were stored at 4^{o}C before extraction. RT-qPCR was utilized to measure gene expression of genes related to virulence (*hly, iap, plcA*) and one gene related to stress (*sig*B). The fold change of each gene relative to the housekeeping gene (16S) was then calculated.

Results

Relative gene expression was calculated using the ΔΔCt method, which showed significant changes in genes related to stress response and virulence after sub-lethal ionizing treatment doses. Significant differences were also seen between the three strains of *Listeria monocytogenes* selected for the study.

Conclusions

Understanding the effects of sub-lethal irradiation on *Listeria monocytogenes* is crucial for ensuring the safety of eBeam-processed foods. Ultimately, these insights will help enhance food safety protocols and improve public health outcomes.

GP6

Novel drug combinations for the treatment of *Rhodococcus equi* infection

Ricardo Munoz-Medina[1], Francesca O Agobe[1], Bhagath Chirra[1], Kush N Shah[1], Ellen R Morris[2], Angela I Bordin[2], Jaime Baeza[1], Adel M Hassan[2], Ava A Brozaovich[1], Parth N Shah[1], Qingquan Chen[1], Margeret Riebe[1], Kristina Boulos[1], László Kürti[3], Noah D Cohen[2], Carolyn L Cannon[1]

[1]Department of Microbial Pathogenesis and Immunology, Texas A&M University, Bryan, TX.
[2]Department of Large Animal Clinical Sciences, Texas A&M University, College Station, TX.
[3]Department of Chemistry, Rice University, Houston, TX

Abstract

Rhodococcus equi (*R. equi*) is a Gram-positive zoonotic pulmonary pathogen that infects the alveolar macrophages of foals and causes pyogranulomatous pneumonia. The organism can survive in the manure-enriched soil of horse farms, where transmission occurs by the inhalation of contaminated soil particles. The recommended treatment for *R. equi* infections is a combination of a macrolide with rifampicin. No commercially available vaccines exist to prevent these infections. In recent years, resistance to the recommended treatment has been increasing. Studies report the prevalence of multidrug resistant (MDR) *R. equi* strains as high as 40% in isolates cultured from infected foals. To address the need for effective antimicrobial therapies to treat *R. equi* infections, we evaluated the efficacy of C58, a natural compound with potent antimicrobial activity against other resistant Gram-positive pathogens, both alone and in combination with gallium maltolate (GaM), which has previously shown activity against *R. equi* both *in vitro* and in infected foals. The combination of C58/GaM demonstrated synergy against *R. equi* based on the fractional inhibitory concentration and low toxicity as demonstrated by an 82% normalized cell viability at C58/GaM concentrations 8X the MIC. We observed a greater than 2-log reduction of intracellular *R. equi* isolates treated with C58/GaM. To enhance localized delivery into the lungs, we encapsulated C58 into nanoparticles fabricated with poly(lactide-co-glycolide) (PLGA) and polyethylene glycol (PEG) (PLGA-PEG; C58-NP). Combination treatment with C58-NP and GaM demonstrated antimicrobial efficacy against *R. equi*, as well as *Mycobacterium smegmatis*, a nonpathogenic species in the same genus as *Mycobacterium tuberculosis*.

GP7

Polymicrobial Interactions of the Food-Borne Pathogens *C. jejuni* and *S. enterica*

Samuel R Cannon, Todd P Primm, Anaand B Karki
Sam Houston State University, Huntsville, Texas

Abstract

Foodborne gastroenteritis is intestinal inflammation spurred by infection. In the United States, over two million annual cases of gastroenteritis are caused by *Campylobacter*. The most common species of this genus for human infections, *C. jejuni*, is surprisingly fragile in laboratory monoculture. *C. jejuni* requires a microaerobic environment, a small temperature range (37-42° C), and supplementation with blood for growth. The narrowness of *C. jejuni's* permissible conditions is due in large part to the simplicity of *C. jejuni*'s genome (only ~1.8 Mb) with very little in regard to nutrient acquisition and stress resistance. However, *C. jejuni* is often isolated from meats, factories, and even soil and water. This gap between epidemiological prevalence versus lab brittleness, sometimes termed the 'Campy paradox,' has puzzled the field, however; a potential explanation has been proposed of cooperation between bacteria. In the past several years, polymicrobial interactions have been of increasing interest, and more evidence has shown that *C. jejuni*'s virulence factors are enhanced when cocultured with select microbes, i.e. *S. aureus*, *A. polyphaga*, and *Pseudomonas* spp. I propose that a common co-contaminate of meats, *Salmonella enterica* subsp. *enterica*, enhances the survival and growth of *C. jejuni*. This is being examined both *in vitro* and *in vivo*.

GP8

Coxsackievirus B utilizes host chaperones to manipulate mitochondrial interactions for vesicle-mediated egress

Jayden H Aleman, Jon Sin
University of Texas at Dallas, Richardson, Texas

Abstract

Coxsackievirus B (CVB) is a non-enveloped, positive-sense RNA virus that causes mild respiratory illness but can also lead to severe diseases including myocarditis and pancreatitis. During infection, CVB manipulates host membrane pathways to enable viral replication and non-lytic dissemination. Recent studies demonstrate that CVB infection induces mitochondrial depolarization and fragmentation, activating mitophagy and promoting the release of infectious extracellular vesicles (EVs). However, the mechanism by which viral material is incorporated into EVs remains unknown.

Preliminary studies from our laboratory demonstrate that viral capsid protein VP1 is detectable within purified mitochondrial sub-compartments following infection, suggesting that viral proteins may gain access to internal mitochondrial compartments. Concurrently, we observe that the host molecular chaperone heat shock protein 70 (HSP70) is enriched both in mitochondrial fractions and in EVs released from infected cells. Co-immunoprecipitation experiments further demonstrate a direct interaction between HSP70 and VP1, implicating host chaperone machinery in viral trafficking. Canonically, HSP70 maintains protein folding and regulates stress responses, but its role in mitochondrial viral localization and vesicle-mediated viral egress has not been defined.

We hypothesize that CVB exploits HSP70 to facilitate trafficking of viral proteins to mitochondria and promote mitophagy-dependent incorporation of virions into EVs. We aim to define how HSP70 regulates mitochondrial viral localization, mitophagy initiation, and viral cargo loading into vesicles. These studies will establish a novel role for host chaperones in coordinating mitochondrial quality-control pathways with viral dissemination, providing new mechanistic insight into viral egress.

GP9

Female sex hormones in bronchiectasis and *Mycobacterium chimaera* lung disease

Christine R Ponce[1], Kristin T Dean[2], Rachel N Wilsey[2], Pamela J McShane[3], Jennifer R Honda[2]

[1]Biotechnology Master of Science Program, Department of Cellular and Molecular Biology, School of Medicine, University of Texas Health Science Center at Tyler, Tyler, Tx.

[2]Department of Cellular and Molecular Biology, School of Medicine, University of Texas Health Science Center at Tyler, Tyler, Tx. [3]Critical Care Medicine and Pulmonary Branch, National Heart, Lung and Blood Institute, National Institutes of Health, Bethesda, MD

Abstract

Background: Female sex hormones influence lung disease (LD) progression, but their role in nontuberculous mycobacterial (NTM), *e.g.*, *Mycobacteria chimaera* LD, is ill-described. NTMLD primarily affects slender, post-menopausal women with bronchiectasis, questioning the role of sex hormones in disease susceptibility. We hypothesize bronchiectatic female patients show lower levels of the sex hormone 17β-estradiol (E2), reduced capacity to control *M. chimaera* replication, and produce higher proinflammatory cytokine levels than healthy, age-sex matched donors. **Methods:** E2, TNFα, and IL-1β were quantified from plasma collected from bronchiectatic patients (n=9) and healthy female donors (n=37) by ELISA. Monocyte derived macrophages (MDM) from three bronchiectatic and three age-sex matched healthy donors were infected with 10:1 *M. chimaera* MOI. 1, 24, 48 and 96hr CFU were tabulated and TNFα and IL-1β quantified from cell culture supernatants. **Results**: TNFα levels were similar between patient and healthy donor plasma. Conversely, E2 levels were significantly reduced (p=0.0099) and IL-1β levels were significantly (p=0.0309) increased in bronchiectatic compared to healthy donor plasma. Matched MDM from bronchiectatic and healthy donors show equal capacity to control *M. chimaera* infection. TNFα and IL-1β levels quantified from cell culture supernatants were similar at each timepoint tested. **Conclusions**: While *M. chimaera* was similarly controlled by MDM from bronchiectatic and healthy matched donors, differential hormone and pro-inflammatory cytokine plasma level suggest an underlying systemic interplay between post-menopausal hormones and inflammation. Complementary plasma hormone dehydroepiandrosterone is also being quantified. *Ex vivo* studies to elucidate the role of hormone exposure for airway epithelial organoid function are ongoing.

GP10

Propranolol Restricts Coxsackievirus B3 Replication Through an IFIT1-Dependent Mechanism.

Vaishnavi V Nair, Ebenezer Senu, Jon J Sin
The University of Texas at Dallas, Richardson, TX

Abstract

Coxsackievirus B3 (CVB3) is a non-enveloped, positive-sense single-stranded RNA virus belonging to the genus *Enterovirus* within the family *Picornaviridae*. It is the most extensively studied and clinically prevalent viral pathogen responsible for myocarditis and pancreatitis. However, there is no approved virus-specific antiviral therapy for CVB3 infection. Propranolol is a non-selective β-adrenergic receptor (β-AR) antagonist with well-established clinical use in cardiovascular conditions. This study therefore evaluated propranolol as a means of repurposing a drug for CVB3 infection.

In this study, we found that propranolol exerts a dose and time-dependent antiviral effect against CVB3 in vitro. Viral replication assays demonstrated significant reductions in viral protein levels and infectious titers following propranolol treatment at a concentration of 100μM. Notably, western blot analysis revealed minimal induction of IFN-β1 and ISG15 following propranolol treatment, indicating that its antiviral activity does not depend on robust activation of canonical type I interferon responses. Further mechanistic studies identified IFIT1 as an essential cytokine for propranolol-mediated viral restriction. Genetic depletion of IFIT1 abolished the antiviral effect, despite the absence of strong upstream interferon signaling. These findings suggest that propranolol may require basal IFIT1-dependent antiviral restriction through a non-canonical pathway.

This study identifies propranolol as a potential host-directed antiviral against CVB3 and uncovers a previously unrecognized IFIT1-dependent mechanism that operates independently of overt interferon induction. These findings expand our understanding of adrenergic signaling in intrinsic antiviral immunity and provide a foundation for the mechanistic dissection of β-adrenergic modulation in enteroviral infection.

GP11

Microplastic Bead Pre-Exposure Alters Coxsackievirus B3 Infection and Host Trafficking Responses

Zeenat F Alishah, Ramina Kordbacheh, Jon J Sin
University of Texas at Dallas, Richardson, Texas

Abstract

Coxsackievirus B3 (CVB3) is a positive-sense RNA virus that causes myocarditis, pancreatitis, and meningoencephalitis, it relies on host autophagic and vesicular trafficking pathways to support replication and non-lytic release. Environmental plastic associated pollutants, such as di(2-ethylhexyl) phthalate (DEHP), have been shown to increase viral release by altering host stress and increasing viral release. However, whether particulate microplastics similarly modify host–virus interactions remains' unclear. To address this question, we investigated whether pre-exposure to polystyrene microplastic beads alters CVB3 infection and host trafficking responses. HeLa cells were pre-treated with polystyrene beads (20–500 nm) prepared from 2% solid stocks at 100 µg/mL for 48 hours. Beads were removed prior to 24-hour CVB3 infection. Viral protein expression and host trafficking markers were analyzed by Western blotting, and infectious viral release was quantified by plaque assay. Microplastic uptake was strongly size-dependent, with larger particles forming intracellular aggregates. Under these conditions, pre-exposure enhanced infectious viral release. This was accompanied by modulation of viral capsid protein VP1 and coordinated changes in host trafficking markers: microtubule-associated protein 1 light chain 3 (LC3) decreased with increasing bead size, most prominently at 500 nm, while lysosomal-associated membrane protein 1 (LAMP1) increased during infection. Together, these findings suggest that microplastic pre-exposure alters autophagosome–lysosome trafficking during viral replication rather than causing generalized cellular collapse. Future studies will evaluate autophagic flux and extracellular vesicle cargo and extend these analyses to 266-6 pancreatic acinar cells to better understand how environmental microplastic exposure may alter viral susceptibility and disease outcomes.

GP12

Discovery of a Novel Virulence Factor Contributing to *Streptococcus agalactiae* Interaction with Brain-Like Endothelial Cells

Bryan J Estrada, William D Cutts, Brandon J Kim
University of Texas at Dallas, Dallas, Texas

Abstract

Streptococcus agalactiae (GBS) is a Gram-positive pathobiont that asymptomatically colonizes the vaginal tract of up to 30% of women. GBS is the leading cause of neonatal meningitis, a life-threatening illness characterized by inflammation of the meninges that occurs when GBS interacts with brain endothelial cells (BECs) and disrupts the blood brain barrier. Therefore, understanding the mechanisms of infection is necessary for the development of therapeutics. Discovery of GBS factors that contribute to bacterial meningitis has often relied on transposon mutant screens that are complicated by insertion site bias. To overcome this, we have performed an RNA-seq analysis of the GBS transcriptome during interaction with induced brain endothelial cells (iBECs), revealing factors that are significantly upregulated compared to the non-interacting GBS, enabling the discovery of novel virulence factors. One such upregulated transcript, Potential Virulence Factor (PVF), encodes an uncharacterized protein containing a surface anchoring LPXTG motif, indicating a possible role in interaction with host cells. We hypothesize that as PVF is upregulated during GBS interaction with BECs, that PVF contributes to GBS-BEC interaction. For preliminary studies using CRISPR interference, PVF knockdowns (K/Ds) were generated and adherence and invasion assays were conducted on BECs in vitro. Compared to control, PVF K/Ds had significantly reduced invasion and adherence in hCMEC/D3s and adherence to iBECs. We recently generated a ΔPVF mutant using CRISPR-Cas12 technologies and further characterization of ΔPVF and PVF's contribution to GBS-BEC interaction is underway. Here represents a novel method for discovery of GBS virulence factors and their contribution to disease progression.

Molecular Microbiology

GP13

Harnessing the Power of 10 Mev Electron Beam (eBeam) Technology to Reduce Ara h 1 and Ara h 2 Allergen Levels in Peanuts

Tonali S Lara-Ramos[1,2], Chandni Praveen[1], Kyung-Min Lee[3], Wei Li[3], Timothy J Herrman[3,1], Suresh D Pillai[1,2]

[1]Texas A&M University, College Station, Texas. [2]National Center for Electron Beam Research, College Station, Texas. [3]Office of the Texas State Chemist, College Station, Texas

Abstract

Introduction: Peanut allergy is one of the most prevalent and serious food allergies, with no established processing method that effectively reduces peanut protein sensitization. This study focuses on the major allergens Ara h 1 and Ara h 2, which are clinically significant due to their resistance to heat and digestion. eBeam technology is a non-thermal, environmentally friendly processing method that generates ionization and reactive species capable of modifying molecular structures. We hypothesized that eBeam treatment induces structural changes in peanut allergen epitopes, reducing IgE binding.

Methods: Pure allergens and total protein extracted from whole peanuts were treated at six eBeam doses (0, 5, 10, 15, 20, and 30 kGy) in triplicate. Structural and immunological changes were assessed using ELISA, Raman spectroscopy, and MALDI-TOF.

Results: eBeam treatment produced a dose-dependent decrease in antibody binding for pure Ara h 1. Similarly, treated total peanut protein showed reduced IgE binding with increasing dose. Raman spectroscopy revealed structural changes, including modifications to disulfide bonds, aromatic residues, and secondary structure. MALDI-TOF confirmed protein modification and fragmentation, with reduced high-molecular weight peptides and shifts in mass peaks consistent with cleavage and oxidative changes.

Conclusion: eBeam treatment resulted in dose-dependent reductions in Ara h 1 detection and measurable structural alterations, supporting its potential to reduce peanut allergenicity. Ongoing studies include mechanistic evaluation, Ara h 2 analysis, and sensory analysis.

GP14

Arsenic Tolerance and the Landscape of Adaptation via RNA Regulatory mechanism; Nonsense-Mediated mRNA Decay (NMD) Pathway.

Stephnie Ogechi Nwaiwu, Bessie Kebaara
Baylor Uniersity, Waco, Texas

Abstract

The nonsense-mediated mRNA decay (NMD) pathway is a conserved, translation-dependent RNA regulatory mechanism that degrades transcripts that undergo premature translation termination including fully functional "natural" mRNAs [1,2]. By targeting natural mRNAs possessing specific architectural features, such as upstream open reading frames (uORFs) or long 3′ untranslated regions (UTRs), NMD directly modulates the *Saccharomyces cerevisiae* transcriptome. NMD indirectly influences the expression of many other transcripts lacking canonical NMD-targeting features, through upstream regulatory cascades and feedback mechanisms, thereby broadly shaping gene expression programs. Although NMD regulates transcripts involved in bio-metal homeostasis [3 14]; its role in toxic metal detoxification remains undefined. Arsenic is a global environmental toxin that disrupts genome stability and inhibits translation through eIF2α phosphorylation, raising important questions about how NMD functions during arsenic stress. Arsenic exposure triggers a complex landscape of adaptation in eukaryotic cells, primarily characterized by the induction of detoxification and antioxidant pathways [7, 8]. However, the role of RNA surveillance in orchestrating this response remains a significant gap in the field. We hypothesize that arsenic exposure induces RNA modifications that alter NMD-mediated gene regulation, thereby promoting environmental adaptation . Furthermore, recent work indicates that NMD mutant S. cerevisiae strains from different genetic backgrounds exhibit differential survival and uptake responses when exposed to metal toxins [2, 9, 10].This project explores arsenic tolerance and adaptation through RNA regulation; specifically how the NMD pathway fine-tunes gene expression. This work is essential to understanding post-transcriptional mechanisms that guard cells against heavy-metal toxicity [2,8].

GP15

Transcriptome Based Detection and Characterization of DrERV in New World Phyllostomidae Bats.

Enoch Ghosh, Austen Rowell, Lisa M Bono
Texas Tech University, Lubbock, Texas

Abstract

The Phyllostomidae family, commonly known as New World leaf-nosed bats, represents one of the most morphologically diverse bat families with a broad geographical distribution and an extraordinary ecological diversity. In 2015, scientists discovered a new endogenous retrovirus in Desmodus rotundus, a sanguivorous member of the Phyllostomidae family, which they named Desmodus rotundus endogenous betaretrovirus (DrERV). While initially found in D.
rotundus, we detected DrERV sequences serendipitously in RNA-seq data. Upon further inspection of the SRA database, we found DrERV in a large portion of the Phyllostomidae species, suggesting that this ERV is widespread in the family. Due to the unexpected ubiquity of DrERV in the family, we characterized the abundance of the virus across species. We then used the sequences for the envelope and polymerase genes, as they are most likely to be complete, to infer a phylogenetic tree. From this tree, we tested whether the virus was found in multiple monophyletic groups, suggesting multiple endogenization events or if it is fixed to a single independent event. Furthermore, using a timescale phylogeny of the host species, we can predict the timeline for endogenization. In the future, we would like to test if we can detect a phylogenetic signal for DrERV evolution within specific hosts.

GP16

Adaptation in acidic environment potentiates pathogenicity in bacteria

Kingsley Kwaku Amoateng, Wei-Chin Ho
The University of Texas at Tyler, Tyler, Texas

Abstract

Microbes frequently adapt to altering environmental stress with molecular and phenotypic changes favored by natural selection. For example, acidic stress appears in various natural and anthropogenic contexts. However, the rate, trade-offs, and genetic mechanisms of adaptation under persistent acidic stress remains poorly understood. More importantly, whether the adaptive results can alter the opportunity of host infection or difficulty of host treatment remains largely unexplored. To address these questions, we experimentally evolved *Escherichia coli* to acidic stress as a model system. Specifically, we propagated 12 replicate populations of E. coli for ~1,900 generations in Luria-Bertani media with two pH values (acidic: 4.5; neutral: 7.0) with daily transfers and shaking at 37°C. The evolved populations in the acidic environment adapted rapidly, sustaining a significant improvement in competitive fitness by 55.8 ± 7.3 %, while no significant difference was found in the neutral environment (-0.9 ± 1.5 %). Whole-metagenomic sequencing of evolved populations revealed nonsynonymous and structural mutations enriched in the genes *rho*, *rlmE, rpoC, and yfcD* for acidic environments. This result suggests the adaptation to acidic stress may involve the change of transcription and translation machineries or the enzymatic ability of dephosphorylation. Strikingly, adaptation in the acidic environment uniquely resulted in the emergence of population-level biofilm formation and collateral resistance to the aminoglycoside class of antibiotics. Together, our results demonstrate that environments can strongly shape the speed of microbial adaptation and reveal how pathogenicity can evolve as a byproduct of new adaptation to stressful environments.

GP17

CRISPR/Cas12-Mediated Chromosomal Knock-In of a Stable Fluorescent Marker in *Streptococcus agalactiae*

Aryah L Lee
University of Texas at Dallas, Richardson, TX

Abstract

Streptococcus agalactiae (Group B Streptococcus, GBS) is a Gram-positive opportunistic pathogen and the leading cause of neonatal meningitis worldwide. There is currently no vaccine available, therefore understanding the mechanisms behind GBS pathogenesis is crucial for developing targeted therapies. Recently, we have employed a CRISPR/Cas12-based genome editing strategy to introduce targeted chromosomal modifications. Using Cas12 and sequence-specific guide RNAs, we generated a homology-directed repair construct to knock-in the gene encoding Green Fluorescent Protein (GFP) into the GBS chromosome. Successful genomic integration was confirmed by molecular screening. This approach produced a fluorescent GBS strain for visualization of bacterial migration during infection. GBS-GFP will be characterized to ensure there is no confounding phenotype by conducting growth curves in multiple media conditions, and cell-based adherence/invasion assays relative to wild-type GBS. Inserting GFP as a stable genomic knock-in eliminates the risk of plasmid loss during replication, making the fluorescent GBS strain more robust and scalable. This Cas12-based editing system broadens genetic manipulation strategies in GBS. It enables the chromosomal integration of additional fluorescent reporters, such as mScarlet, a synthetic construct derived from the Red Fluorescent Protein (RFP) lineage. This approach will enable co-visualization with host cell markers and expanded deletion or complementation of functional genes to better understand the role of virulence factors in bacterial infection.

GP18

When Viruses Return Home: Exploring Viral Spillback Events

Akiko Kaitlin Balitactac[1], Lynley G Sombito[1,2,3], Austen Rowell[1], Enoch Ghosh[1], Lisa M Bono[1]

[1]Texas Tech University Department of Biological Sciences, Lubbock, TX. [2]Honors College, Texas Tech University, Lubbock, TX. [3]TrUE Scholars Undergraduate Research Program, Texas Tech University, Lubbock, TX

Abstract

Viruses exist in a world where they are constantly exposed to potential hosts—giving viruses the opportunity to shift to novel hosts (spillover) and to re-encounter their ancestral host (spillback). During host range evolution, it is possible that a virus may incur a fitness cost on their ancestral host. Alternatively, evolution in another host may allow the population to explore sequence space inaccessible in the ancestral host. Many studies focus on spillover, but little is known about how a virus responds to a spillback event. Here, we aim to evaluate the fitness consequences of spillback. Previously, the host range of the bacteriophage phi6 was expanded across two novel hosts: *P. syringae* pv. tomato and *P. pseudoalcaligenes* ERA. The initial mutations that enabled host range expansion were costly (antagonistic pleiotropy). These mutants were then evolved on either one novel host or alternating between the two novel hosts for 150 generations. Here, we isolated a single plaque from the end of these evolution experiments and passaged them on their ancestral host *P. syringae* pv. *phaseolicola* (Pp) for 100 generations. We found that populations that experienced spillback maintained a broad host range and had fitness effects that were idiosyncratic compared to the wild-type, indicating that phage experienced weak selection during spillback despite long-term adaptation to novel hosts. Currently, we are working on identifying the type of mutations that arose during spillback. Our results will allow us to assess the impact of a spillback event on the evolutionary trajectory of host range expansion.

GP19

The ecology and evolution of bacteriophage phi6 under the effects of relative humidity

Laurissa N Miller, Abigail Frank, Lisa M Bono
Texas Tech University, Lubbock, Texas

Abstract

During transmission between hosts, viruses can experience radically different abiotic conditions than during infection within a host. These factors can include changes in temperature and relative humidity. Respiratory droplets are a common vehicle for viral transmission, and these droplets evaporate during exposure to conditions outside of the host. Enveloped viruses are especially sensitive to changes in temperature and humidity due to their lipid composition, which in turn affects viral stability. In this study, we used bacteriophage phi6 as a model enveloped virus to test viral survival across a range of abiotic stressors in pre-conditioned humidity chambers. We tested this by measuring 1) the evaporation of droplets and 2) viral survival across a range of relative humidity and temperature conditions. First, we measured the evaporation of droplets of ultra-pure water or phage lysate on stainless steel coupons. We did not detect a difference in evaporation between the two liquids. While we found no difference in evaporation across humidities after 15 minutes, we did detect a negative relationship between evaporation and relative humidity after 2 hours. Second, we measured viral survival (decrease in viral titer) after exposure to the same conditions for 15 minutes only. We found a U-shaped relationship between viral survival and relative humidity. Furthermore, viral survival was lower at higher temperatures. We are currently investigating the impact of low relative humidity on the evolution of phi6 and its ability to expand its host range.

GP20

Anthropogenic Impact of Land Development on Soil Microbial Diversity and Natural Product Production

Robert J Garcia, Dennis G Castro
Texas A&M San Antonio, San Antonio, Texas

Abstract

This study examines the abiotic, physicochemical, and hydrological impact of land development on microbial diversity and the abundance of antimicrobial-producing organisms. Building on previous research conducted at VIDA, a 600-acre residential development situated near Texas A&M University in San Antonio, which includes site comparisons of Undisturbed, Residential, and Greenway (LID site). Soil samples were collected monthly for a year, with additional samples taken from secondary LID, undisturbed, and residential sites to reduce bias and evaluate if patterns observed at VIDA sites were consistent with broader LID and undisturbed sites. Preliminary findings suggest greater similarity among the Greenway Undisturbed sites, with similar abundances of antimicrobial-producing microbes and overall colony-forming units (CFUs) in VIDA. Eight Isolates exhibiting the largest reproducible zones of inhibition were confirmed as bacterial by electrophoresis of PCR products amplified with *tuf* primers and submitted for 16S rRNA sequencing for species identification and secondary metabolite characterization. Soil physicochemical properties will be analyzed to determine their influence on microbial patterns. The study aims to identify which environmental variables have the most significant impact on soil microecology.

GP21

Not A Wild Goose Chase - Effect of Western Cattle Egrets in San Antonio

Uriel Eddy[1], Daniel Guerra[2,1], Joseph Plappert[3], Clay Green[2], Davida Smyth[1]
[1]Texas A&M University - San Antonio, San Antonio, Texas. [2]Texas State University, San Marcos, Texas. [3]Texas Parks & Wildlife Department, San Anotnio, Texas

Abstract

Western Cattle Egrets (*Ardea ibis*, hereafter WECE) in the San Antonio metropolitan area have become a nuisance species in mixed-species urban rookeries and could pose a health risk to the nearby human and bird populations. Egrets could be carriers or distributors of pathogens (e.g. E. coli or influenza) in high density rookeries. WECE are migratory birds that have adapted well to urbanized environments, often finding themselves in close proximity with humans. We hypothesized that WECE affect water quality through high frequency of guano deposits from nesting birds, and that WECE are carriers of harmful pathogens to nearby humans in these dense urban environments. We surveyed known, local rookery sites that were either active or historical–determined through city officials or local word of mouth–near bodies of water and took water samples from under the rookery and from an unoccupied location as a control. Coliform counts and total nucleic acid (TNA) data were obtained through IDEXX and qPCR processes, then compared to established gene banks.

GP23

The Dark Side of the Medicine Cabinet: Impact of Medications on *Candida* Resistance & Fitness

Weerakkody S Ranasinghe[1,2], Madhvi Bhakta[1], Diana M Proctor[1,2]

[1]The University of Texas MD Anderson Cancer Center UTHealth Graduate School of Biomedical Sciences, Houston, Texas. [2]Department of Microbiology and Molecular Genetics, UTHealth Houston McGovern Medical School, Houston, Texas

Abstract

Large-scale drug screens have identified medications that exert collateral effects on *Candida* antifungal resistance, though the mechanism remains unknown. Since many drugs are known to induce mitochondrial dysfunction in human cells, we hypothesize that these medications, including antibacterials, increase antifungal resistance by inducing mitochondrial dysfunction in *Candida*. To test this hypothesis, we screened a panel of 22 drugs, including antibacterials, against *C. albicans* for minimum inhibitory concentration (MIC) using broth microdilution tests, interactions with fluconazole using checkerboard assays, and effects on mitochondrial function using assays such as MitoROS. Similar to all antifungals (4/4), we found that 1/4 of cardiac meds and 2/2 statins inhibited *Candida* growth in a dose-dependent manner. The checkerboard assay for vancomycin (Van) showed increased *Candida* growth (30-600%) compared to Van-free controls in a medium- and dose-dependent manner, but only in the presence of fluconazole. Despite vancomycin-induced pH changes (4–6.5), permutation testing showed its effect on *C. albicans* growth with fluconazole was independent of acidification. Several drugs (36%) induced mitochondrial superoxide production (4/5 antifungals, 2/2 antidepressants, 1/1 statin, and 1/4 cardiac medications), a hallmark of mitochondrial dysfunction. We are currently probing other axes of mitochondrial function that may explain these altered phenotypes while expanding our dataset to include over 1000 FDA-approved drugs.

POST-DOCTORAL POSTER PRESENTATIONS

PP1

Rhesus macaques (*Macaca mulatta*) as a comparable gut microbiome model

Kaelyn M Dobson, Carolyn L Hodo
UT MD Anderson Michale E. Keeling for Comparative Medicine and Research, Bastrop, Texas

Abstract

The observed similarities in morphology, immunology, genetics, behavior and sociality between humans and non-human primates (NHP) support the use of NHPs in translational biomedical research. Though NHPs have been utilized for decades, current research advancements and ethical concerns are suggesting a shift away from the use of NHPs biomedical research. The 3 Rs (refinement, reduction and replacement) are currently beneficially applied to any projects involving NHPs. Although the 3 Rs are important in NHP research, the replacement of all NHPs could be a concern for the translation of research findings to human health as it is not possible to replicate certain primate systems in organoid or murine models. Many scientific articles have previously presented data as to the importance and applicability of NHP models, providing supporting evidence for the continued use of NHPs. As an example of the direct translational ability of NHPs, we present the gut microbiome of an adult female rhesus macaque from the University of Texas MD Anderson Michale E. Keeling Center for Comparative Medicine and Research. The composition of the gut microbiome is directly comparable to the microbial signature of a human with chronic diarrhea with high abundance of the phyla Lactobacillus, Streptococcus, Faecalibacterium and Prevotella, highlighting the availability of a highly comparable model for study. The microbiome also matches at the genus level with a composition of *Clostridioides, Faecalibacterium, Roseburia* and *Collinsella*. This case study emphasizes how maintaining and utilizing NHP in ethical manners for biomedical research is still a crucial component of translational research.

PP2

Developing Phage Cocktails for Treatment of AMR-*Staphylococcus aureus* Orthopaedic Device-Related Infections

Cora Kosnik, Raquel C Luna, Violeta Chavez, Catherine G Ambrose, Heidi B Kaplan
UTHealth Houston, Houston, TX

Abstract

Orthopedic device-related infections (ODRIs) affect ~200,000 Americans annually and are a complication in approximately 3% of orthopedic device-related surgeries. The primary causative agent is *Staphylococcus aureus*, a challenging pathogen to treat due to its high incidence of antimicrobial resistance (AMR) and ability to form biofilms. This task is further complicated by low blood flow to ODRI sites. One alternative to antibiotics is to use bacteriophages (phage), which are viruses that selectively lyse bacterial cells. To address these issues, we are designing a phage therapy treatment for AMR *S. aureus* ODRIs using biodegradable microspheres for localized delivery.

Bacteria can encode or evolve resistance to phage similar to other antimicrobial treatments. As a result, many phage therapies employ a mixture of multiple phage, termed a phage cocktail, to reduce resistance and improve efficacy. We designed a preliminary anti-staphylococcal phage cocktail by analyzing the host ranges of ten lytic phage and selecting those with the broadest host range and minimal overlap. Each phage's host range was characterized based on 63 *S. aureus* clinical isolates from our biorepository of bacteria from deep musculoskeletal infections. From these data, four phage were selected that together successfully lysed 56 (87.5%) of the 63 clinical isolates and our reference strain *S. aureus* UAMS-1 (ATCC 49230). I am currently testing this preliminary cocktail for increased efficacy and potential synergy in both planktonic and biofilm growth conditions.

We hypothesize that our phage cocktail will increase treatment efficiency as more *S. aureus* strains will be susceptible and resistance should not develop.

PP3

Deletion mutants of an alternative ribosome rescue factor in oxidative stress response

Julia D Meredith, Ken C Keiler
University of Texas at Austin, Austin, TX

Abstract

Ribosome stalling occurs when a ribosome becomes trapped on a piece of mRNA during translation and is unable proceed with elongation and termination, and therefore essential protein synthesis. It is a hurdle that all bacteria must overcome to survive, as accumulation of stalled ribosomes will lead to cell death. Nearly all bacteria utilize a process known as *trans*-translation to free stalled ribosomes and degrade nascent protein products, keeping protein synthesis moving. A subset of bacterial species, including *Escherichia coli*, possess additional ribosome rescue methods beyond *trans*-translation known as Alternative Ribosome-rescue Factors ArfA and ArfB. While the mechanism of action behind ArfB to free stalled ribosomes is well-characterized, the advantage of encoding *arfB* is unknown. Conditions where *arfB* is upregulated have not been identified and typical expression of ArfB alone is not enough to rescue *E. coli* when both *arfA* and *trans*-translation are lost. In fact, there has never been a growth difference observed in a strain of *E. coli* lacking solely *arfB*. However, our preliminary data suggest a previously unidentified relationship between *arfB* and oxidative stress. We have surprisingly found that *arfB* knockouts recover from stress cause by hydrogen peroxide much faster than wild-type cells. Furthermore, when the uncharacterized gene located just upstream of *arfB, yaeQ* is knocked out, there is also a difference in growth and recovery from H_2O_2 stress. These data suggest a potentially redundant role of these two genes in bacterial regulation against oxidative stress, and a role for *arfB* that extends beyond ribosome rescue alone.

PP4

Building a Pipeline for Predicting E. coli Protein Interactions Using AlphaPulldown on an Academic HPC Cluster

Sarah A Vastani, Joseph Carr, Angela M Mitchell
Texas A&M University, College Station, TX

Abstract

Computational tools such as AlphaFold have made it possible to predict protein structures with remarkable accuracy, but using these tools to study protein–protein interactions (PPIs) at scale can be challenging on shared high performance computing (HPC) systems. AlphaPulldown is designed to screen many possible interaction partners for proteins of experimental interest. We have developed an AlphaPulldown workflow tailored to the Texas A&M HPRC environment to investigate potential interaction partners of the poorly characterized protein SanA. SanA is an *E. coli* cell envelope protein involved in maintaining envelope integrity and has been experimentally linked to cell division. Using over a thousand pairwise predictions, the workflow produced structural models and interaction scores that enabled identification of promising candidates for experimental follow up.

The candidate protein, DamX, has emerged as a potential SanA interactor. Early experimental tests support this prediction: efficiency of plating assays on vancomycin and SDS EDTA indicate that DamX may phenocopy the resistance phenotype associated with the *ftsI* V98E mutation. This mutation arises spontaneously in strains lacking both *sanA* and *wecA*, restoring membrane barrier function and detergent resistance. The observation that DamX overexpression produces a similar phenotype suggests a possible functional connection between DamX, envelope stress responses, and SanA associated pathways.

Overall, this work provides a practical and accessible framework for researchers who want to use deep learning structure prediction tools for PPI discovery. By focusing on reliability and ease of use, the pipeline lowers technical barriers and supports broader adoption of computational approaches in microbial systems research.