Texas Branch

AMERICAN SOCIETY FOR MICROBIOLOGY

Virtual Spring 2022 Meeting
Abstract Book

Friday, March 25

9:00a-12:00p

1:00-2:00p

3:00-5:00p

Undergraduate Oral Presentations

UG O01

To kill or not to kill: Study of the lysis inhibition mechanism in Bacteriophage N4

Teresa Sullivan[1,2], Adam Tomaszewski[1,2], Cody Martin[1,2], Jolene Ramsey[1,2], Ryland Young[1,2]
[1]Texas A&M University, College Station, USA. [2]Center for Phage Technology, College Station, USA

Abstract

We are facing a crisis in which bacteria are resistant to the prescribed antibiotics designed to kill them. Because bacteriophages are natural predators of these microbes, phage therapy is an option to overcome the antibiotic resistance crisis. Bacteriophages infect target cells and use cellular resources to replicate. To complete the infection, the phage induces lysis which bursts the cell and releases the phage progeny to hunt for new hosts. Most studied bacteriophage begin lysing cells at around 60 minutes post-infection. However, a phenomenon known as lysis inhibition, or LIN, prevents lysis in certain phage infections for hours, increasing the titer of progeny released by 10-100 times. Discovering the mechanism of LIN may allow us to harness the ability to produce high titers of phage needed for therapy. LIN is a defining characteristic of bacteriophage N4, but the cellular mechanism behind LIN is unknown. The purpose of this study is to identify the molecular players behind LIN in N4. In this study, wild type N4 was subjected to screening for spontaneous mutants causing the loss of LIN, or rapid lysers. Through sequencing many rapid lyser lines, mutations in the lysis genes and noncoding genomic regions within the genome pointed to key genes involved in LIN. A cluster of mutations were found in one lysis gene, and from this, we have developed a model in which LIN regulation occurs via phosphorelay signaling. We anticipate that studying LIN will inform strategies for increasing phage production processes needed for successful phage therapy.

UG O02

Characterization of Antibacterial Mechanisms of Zinc Oxide in Staphylococcus aureus

Alexander Caron, Iman Ali, John Reeks, Dustin Johnson, Yuri Strzhemechny, Shauna McGillivray
Texas Christian University, Fort Worth, USA

Abstract

Rising precedence of antibiotic resistance has increased interest in nontraditional antibacterial agents such as zinc oxide nanoparticles (ZnO NPs). Although the anti-microbial activity of ZnO NPs is well established, the mechanism of this activity is unknown. Current literature hypothesizes that ZnO NP cytotoxicity could be mediated through one or multiple proposed mechanisms including production of reactive oxygen species (ROS), release of toxic ZnO2+ ions, and charged interactions that disrupt the cell wall and cause osmotic stress. Literature also suggests bacteria may be unable to gain resistance to ZnO because antibacterial action occurs through multiple mechanisms. To illuminate the properties of ZnO and determine which of the proposed mechanisms occur, ZnO susceptibility was assessed in Staphylococcus aureus. To determine if bacteria gain resistance to ZnO, S. aureus was passed in ZnO at sublethal doses. We find that S. aureus swiftly gain antibiotic resistance, suggesting ZnO antibacterial activity may operate through a single mechanism. To determine the predominant mechanism, susceptibility assays were performed in S. aureus mutants with deletions in KatA, a gene important to defense against H2O2, and Mprf, a gene important to cell wall charge. We find that production of H2O2 and charged interactions with the cell wall are not significant in ZnO susceptibility. Lastly, we find that media conditioned with ZnO effectively inhibits bacterial growth in the absence of ZnO particles. We conclude that physical contact with S. aureus is not necessary for ZnO activity, although the precise mechanism by which bacterial growth is inhibited is not yet elucidated.

UG O03

Determining if extracts of *Eysenhardtia polystachya*, *Pinus maritima*, and *Zingiber officinale* can combat antimicrobial resistance

Nichole Abrego, Patricia Baynham
St. Edward's University, Austin, USA

Abstract

Antimicrobial resistance (AMR) causes more than 35,000 deaths annually in the United States, so new treatments are needed. In this study, parts of three different plants were examined for their antibacterial activity: the barks of *Eysenhardtia Polystachya* (palo azul) and *Pinus maritima* (pine), and the root of *Zingiber officinale* (ginger). The plants were extracted and were then tested for antimicrobial activity using a Kirby-Bauer disk diffusion assay against *E. coli lptD4213*-. While the ginger extract displayed no zone of inhibition, the average zone of inhibition was 13mm for pine bark and 11mm for palo azul. A minimum inhibitory concentration assay (MIC) was performed, to better quantify the antimicrobial activity and showed that pine bark had an MIC of 640ug/ml while the MIC of palo azul was 600ug/ml. To investigate the mechanism of action (MOA) of the extracts, bacterial cytological profiling (BCP) was performed. BCP involves treating the bacterium with an antimicrobial substance and then visualizing the changes in shape that are different based on which part of the cell is targeted. Visualization was via confocal microscopy using FM4-64 to show the membranes and DAPI to see the DNA. The treated cells changed shape but not all cells were visualized so this will require further analysis. In the future, the cell shapes can be quantified and compared with the known MOA of known antibiotics. New treatments are crucial when AMR infections are increasing. Plant substances can be characterized and developed into possible alternative treatments to AMR microbes.

UG O04

Inhibition of autophagy in Human Microvascular Endothelial Cells during Colorado Tick Fever Virus Infection

Christian Miller, Jeremy Bechilli
Sam Houston State University, Huntsville, USA

Abstract

Colorado tick fever virus (CTFV) is the responsible agent for the acute febrile illness Colorado tick fever (CTF) can manifest mild to severe symptoms in humans including meningitis, encephalitis, and bleeding disorders. As many as 20% of diagnosed cases result in hospitalization where only supportive care is available. Despite the clinical significance, the understanding of mechanisms that induce CTFV pathology remains largely unknown. Transcriptomics analysis of CTFV infected human microvascular endothelial cells (HMEC-1's) showed increases in total mRNA expression of autophagy-associated genes including p62/SQSTM1 and BECN1, and downregulation of genes including ULK1 and WIP12, which suggested an interaction between CTFV infection and autophagy in HMEC-1's. Further investigation at the protein level through Western Blot analysis showed a reduction in the ratio of light chain 3 form II (LC3-II) to light chain 3 form 1 (LC3-I) and a significant increase in p62, highlighting an overall decrease in autophagosome formation and inhibition of degradative autophagy. Data also showed increased protein expressions of pAkt (Ser473), mTOR (Ser2448), and pp70S6K (Thr389), which highlighted a potential pathway by which CTFV downregulates autophagy through modulation of Akt/mTOR/p70S6K signaling. Cell viability analysis utilizing established modulators of autophagy rapamycin, 3-MA, and chloroquine showed significant increases in cell survival during CTFV infection in comparison to CTFV infection alone. Collectively, our data suggest that autophagy is downregulated in response to CTFV infection in HMEC-1's to subvert the cellular innate immune response and avoid degradation.

Graduate Oral Presentations

GR O01

Polymicrobial Communities in Chronic Wounds Affect Individual Antimicrobial Susceptibilities to Multiple Antibiotics

Caroline Black, Sabrina Wilson, Catherine Wakeman, Allie Clinton Smith
Texas Tech University, Lubbock, USA

Abstract

New advances in sequencing technologies have demonstrated that many chronic infections are polymicrobial in nature. Within a polymicrobial community, interactions between multiple species can allow for synergism, leading to decreased antibiotic efficacy and worse patient outcomes. Despite the knowledge that persistent infections are often polymicrobial in nature, hospital laboratories assess antimicrobial susceptibility based on monomicrobial suspensions. This project investigates the shifts in antimicrobial susceptibilities driven by the presence of the organism in a polymicrobial community. Four relevant chronic wound pathogens (*Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Acinetobacter baumannii*, and *Enterococcus faecalis*) were grown in both monomicrobial and polymicrobial conditions. When comparing species growth individually verses in a community, shifts in antimicrobial susceptibilities were observed. Using the data obtained from individual antibiotics, a combinatorial antibiotic therapy was proposed, and was shown to be effective in inhibiting bacterial growth. This demonstrates that current clinical methods for determining antimicrobial susceptibility based on the monomicrobial causative agent of disease may not fully represent the clinical environment. However, by acknowledging the impact of the community on antimicrobial susceptibilities, we can more effectively determine potent antibiotic therapies needed to treat persistent infections and improve patient outcomes.

GR O02

Arginine methylation helps SepIVA balance regulation of septation and elongation in *Mycobacterium smegmatis*

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Abstract

Growth of mycobacterial cells requires successful coordination between elongation and division of the cell wall. However, it is not clear which factors directly mediate this coordination. Here, we studied the function and post-translational modification of an essential division factor, SepIVA, in *Mycobacterium smegmatis*. We find that SepIVA is arginine methylated, and that these modifications alter both division and polar elongation of *Msmeg*. Furthermore, SepIVA impacts the localization of MurG. Polar localization of MurG correlates with polar elongation in arginine methylation mutants of *sepIVA*. We have established SepIVA as a regulator of both elongation and division, and characterize a physiological role for protein arginine methylation for the first time in mycobacteria.

GR O03

Infection of Human Endothelial Cells with Colorado Tick Fever Virus Stimulates Cyclooxygenase 2 Expression and Vascular Dysfunction

Stephanie Beane, Jeremy Bechelli
Sam Houston State University, Huntsville, USA

Abstract

Colorado tick fever virus (CTFV) is a tick-borne double-stranded RNA virus that causes Colorado tick fever (CTF). CTF is generally self-limiting, however, a more severe symptoms can develop, including hemorrhagic fever, encephalitis, and death. The mechanism of CTFV mediated pathology is currently unknown and there are no detailed studies defining the molecular mechanisms associated with vascular damage. Cyclooxygenase-2 (COX-2) is a known mediator of inflammatory response, various pathophysiological conditions, and is associated with viral replication. In this study, we demonstrate that CTFV infected human umbilical vein endothelial cells (HUVECs) have increased COX-2 and no apparent effects on COX-1 using qPCR or western blotting. Angiopoietin-1 (ANG-1) and angiopoietin-2 (ANG-2) are known biomarkers produced during dysfunction of vascular endothelium during infections. The ratio of ANG-2/ANG-1 is a known biomarker of endothelial activation and vascular damage that can be used to identify severe infections. Additionally, Tie-2 has known involvement in inflammation and vascular leakage. qPCR analysis shows an increased ANG-2/ANG-1 ratio and Tie-2 expression at 12- and 24- hours post-infection. Furthermore, Interleukin-6 (IL-6), Interleukin-8 (IL-8), and monocyte chemoattractant protein-1 (MCP-1) are recognized markers of vascular inflammation. Our data show a significant elevation of soluble IL-6, IL-8, and MCP-1 at 48-hours post-infection. This data suggests CTFV induces pathological characteristics of vascular activation and dysfunction as measured through enhanced COX-2 expression, skewed ANG-2/ANG-1 ratio, and increased levels of pro-inflammatory cytokines and chemokines. We uncover specific biomarkers for CTFV-induced vascular dysfunction and highlight future therapeutic research of this neglected tick-borne disease.

GR O04

Alteration of citrate metabolism by Vaccinia virus encoded growth factor via STAT3

[1]Lara Dsouza, Anil Pant, [2]Zhilong Yang
[1]Kansas State University, [2]Manhattan, USA. [2]Texas A&M University, College Station, USA

Abstract

Viruses are intracellular parasites that rely on host cell metabolism for their replication. Vaccinia virus (VACV) is the prototype virus of the poxvirus family, known to have members that cause diseases in animals and humans. An understanding of the metabolic requirements of VACV, the mechanisms undertaken to alter its host cell metabolism and the viral proteins involved in this reprogramming would lead to more efficient therapeutics by inhibiting these cellular metabolic pathways. Our work is focused on elucidating the role of one such VACV encoded early protein, vaccinia growth factor (VGF), in altering host cell metabolism. We have shown that infection by VACV increases cellular tricarboxylate (TCA) cycle intermediates and citrate levels. Our study explains that the increase in citrate levels is dependent on VGF. Deletion of VGF from the VACV genome, leads to a decrease in citrate levels. Further inhibition of the EGFR, MAPK and Stat3, led us to determine that VGF selectively stimulates the EGFR-MAPK-Stat3(Serine 727) pathway to increase citrate levels. We also carried forward this study to examine if VGF is sufficient to stimulate the EGFR-MAPK-Stat3(Serine 727) pathway to increase citrate levels independent of virus infection. This research opens opportunities to study the lesser-known function of Stat3 (Serine 727) and can also be applied to study fundamental cellular metabolism.

Post-Doctoral Oral Presentation

P/F O01

Design and sequence analysis of a synthetic macrocyclic beta-hairpin peptide library screened for antibiotic potential

Justin Randall, T. Jeffery Cole, Cory Dupai, Gillian Davidson, Renee Fleeman, Bryan Davies
University of Texas at Austin, Austin, USA

Abstract

The rapid development and spread of antibiotic resistance continues to outpace clinical antibiotic discovery, leaving doctors with fewer and fewer options to treat multidrug resistant bacterial pathogens. Natural macrocyclic beta-hairpin peptides have recently provided structural scaffolds for clinical antibiotic candidates, but strategies for their identification remain far too slow and inefficient to understand their sequence-structure-function relationships. Here we design a ribosomally encoded macrocyclic peptide library with beta-hairpin secondary structure consisting of 196,608 unique sequences. We then use antibacterial peptide display to rapidly screen ~40,000 of these peptides for antibiotic potential resulting in 1,026 hits. Biochemical characterization of 88 hits from this screen validated the in vitro antibacterial activity of 36 new synthetic peptide antibiotics with macrocyclic beta-hairpin structure functioning primarily through membrane disruption and containing little to no hemolytic activity. Machine learning then allowed us to analyze this dataset and predict sequence features impacting both antibiotic potency and hemolysis, enabling us to identify and optimize top therapeutic candidates from the entire library. Such techniques can be used to rapidly expand macrocyclic peptide sequence information to better understand their sequence-structure-function relationships and be utilized as a new tool for high-throughput synthetic antibiotic peptide discovery.

Education Session

Mama knows best: Affirming cultural knowledge using the scientific process

Karla Fuller
Stella and Charles Guttman Community College, New York City, USA

This session will explore the use of a culturally relevant pedagogical framework using backwards mapping techniques to create an integrated inquiry-based lab research project where students apply cultural and Indigenous knowledge, effectively remixing a typical antibiotic resistance microbiology lab.

Waste not want not

Davida Smyth
Texas A&M -San Antonio, San Antonio, USA

This presentation will focus on research investigating the impact of human activity on our water bodies and aquatic ecosystems. Overuse and lack of oversight has led to many of our water bodies being heavily contaminated with biological and chemical agents, particularly with sewage and run off from agricultural and industrial activities. Around the world, intrepid individuals, groups, and communities are working to improve sanitation, and to prevent contamination of our precious natural potable water resources. We'll focus on how we can leverage waste, pollution and wastewater in our research and teaching from projects on social justice, toilet access and design to microbial contamination in superfund sites and surveillance for pathogenic microbes such as SARS-CoV-2.

Manga as a fun way to teach host-pathogen interactions in microbiology

Dan Lei Zhou, Quang To, Davin Devara, Veeravenkata Garikiparthy, Jorge Cervantes
Paul L. Foster School of Medicine Texas Tech University Health Sciences Center at El Paso, El Paso, USA

There has been an increase in the diversification of backgrounds of students matriculating into medical school. We are seeing an increase in non-STEM backgrounds and students with minimal scientific background. Comics are effective in learning because the visual format effectively combines images and text, allowing readers to become engrossed in the story. Comics are popular in Japan, where they are known as *manga*, and cover a wide range of genres, including medical community settings.
To assist students in achieving a baseline level, we developed a manga detailing the pathogenesis of *Staphylococcus aureus* infection, with the aim of introducing medical microbiology concepts in a fun but informative manner.
Although we will touch upon the wide properties of all bacteria, we selected *S. aureus* as it is one of the first bacteria students learn during the first unit. Aspects covered include information such as shape, Gram-staining, and hemolytic ability. Pathogenesis covers how the bacteria enters the body, mechanism of virulence factors, and what diseases can arise from the infection. Treatment aspects were also included. A storyboard on the progression of the story and what topic we want to cover was developed in Photoshop. Symbolism was utilized to relate technical terms with everyday objects, as well as the addition of cultural references with the aim of creating associations that are easier to remember. A supplemental guide accompanying the manga was provided to not pollute the page with too much written text elements and make it more digestible to the eyes.

Undergraduate Poster Presentations

Antimicrobial Microbiology

UG P01

Survey of Antimicrobial Properties of Bacteria Isolated from Sorcerer's Cave

Evan Babb, Josh Holt, Jennifer Huddleston
Abilene Christian University, Abilene, USA

Abstract

Bacterial strains were isolated from Sorcerer's Cave, a previously unexplored cave located in Texas. These bacteria constantly compete for limited resources using naturally produced antibiotic compounds. As the medical antibiotic resistance crisis continues to grow, these cave isolates may hold the answer for novel antibiotic compounds that could treat patients with bacterial infections. We found strains that produce antimicrobial compounds that are effective growth inhibitors of *Escherichia coli* and/or *Staphylococcus aureus*. In this study, we focus on isolate 956A and investigate which of the fractions of the compound are most effective at inhibition. We also use PCR to assay all the cave isolates for the phenazine-1-carboxylic acid (PCA) gene that is important in biosynthesis of antimicrobial compounds gene in environmental bacteria.

UG P02

Identification of Bacteria from Sorcerer's Cave that Inhibit Quorum Sensing

Claire Ely[1], Caleb Rosenblad[2], Brianna Garrett[1], Sage Lauderback[1], Jennifer A. Hennigan[1]
[1]Abilene Christian University, Abilene, USA. [2]Hardin Simmons University, Abilene, USA

Abstract

Quorum sensing (QS) allows for bacteria to communicate with each other within a population using chemical messages called autoinducers. Common pathways that are under QS control include biofilm formation, bioluminescence, toxin and pigmentation production. The purpose of this study is to identify specific isolates that inhibit QS from one of the deepest caves in Texas, Sorcerer's cave in Terrell County. As with traditional antibiotics, competing species of bacteria may be a good source of novel quorum sensing inhibitors (QSIs). We completed a QSI screen utilizing two Gram-negative sensor strains to observe changes in their pigmentation due to the production of QSIs: *Serratia marcescens* and *Chromobacterium violaceum*. Altogether, we identified a total of 57 isolates that were found to have some QSI activity against *S*. *marcescens* or *C*. *violaceum*. Of those isolates, most were identified as Pseudomonas entomophilia or subspecies of *Pseudomonas chlororaphis*. Additionally, we screened these isolates against a strain of *C. violaceum* with a mutation in violecum production (CV026) to separate QSI activity from antibiotic production. We found CV026 was sensitive to several of the cave isolates despite normal growth observed in the wild-type *C. violaceum*. More research will need to be completed to determine the mechanism of pigmentation inhibition by our isolates.

UG P03

Investigating the Correlation Between Environmental Conditions and Isolated Mycobacteriophage Cluster Type

Claire P. Martinez, Hattie R. Mills, Christina E. Spencer, Angela L. Salazar, Frederick N. Baliraine
LeTourneau University, Longview, USA

Abstract

While annotating mycobacteriophage Gilberta, which belongs to subcluster A11, it was found that other subcluster A11 phages were isolated in similar environmental conditions. Phage clusters are determined by genetic similarity with other phages. Discovery notes data obtained from PhagesDB suggests a correlation between the type of bacteriophage cluster isolated and environmental conditions.

To determine the validity of this hypothesis, a systemic analysis of clusters A, L, and K discovery notes on the PhagesDB was undertaken. Discovery notes data from at least ten phages from multiple subclusters of clusters A, L, and K were analyzed. Preliminary data indicates that subclusters A5 and A9 phages were primarily collected under similar weather and soil conditions. Subclusters K1 and K6 phages were collected from comparable soil condition as subclusters A5 and A9 phages. However, isolation temperature and location differences existed between A and K phages. Many subcluster L1 and L2 phages were isolated from similar environmental conditions to each other, but the conditions were unique when compared to those of the A and K phages. Overall, the data indicate that the environmental conditions may affect the type of phage cluster isolated from environmental samples. This study discusses the possible applications of utilizing environmental condition data to increase phage cluster diversity through strategic sample collection.

UG P04

Assembly and Annotation of the Genome of an Antibiotic-Generating Soil Bacterium

Savannah Orton[1], Todd Primm[2], Aaron Lynne[2], Jeremy Bechelli[2]
[1]LeTourneau University, Longview, USA. [2]Sam Houston State University, Huntsville, USA

Abstract

The soil microbiome is rich in diversity and highly competitive. Some bacterial species produce antibiotics as secondary metabolites to inhibit or kill other bacteria competing for the same resources. These antibiotics synthesized by soil-dwelling bacteria have been the primary source of antimicrobial drugs in medicine and agriculture. A bacterial strain, A29, was isolated from soil during a CURE project at SHSU following the Small World protocols. This A29 isolate has the potential to inhibit growth of *Xanthomonas oryzae pv. oryzae*, causative agent of rice blight, using biocontrol, presumably through generating antibiotics. A29 was subjected to whole genome sequencing using the MiSeq platform. The bacterial genome reads were assembled using PATRIC 3.6.9 into 112 contigs with a total genome length of 5,881,433 base pairs. A29 was classified using a variety of different methods such as a comparison to type strains, 16S rRNA gene nucleotide BLAST, and a phylogenetic tree analysis. The isolate was confirmed to belong to the *Pseudomonas genus* with the closest relative being *Pseudomonas mosselli*. Species-level identification was not unambiguous and A29 is potentially a novel species or subspecies. Additionally, various gene clusters and biosynthetic pathways that may be responsible for antibiotic production were identified using antiSMASH and PATRIC. PATRIC-generated KEGG Maps revealed the presence of genes in pathways for synthesis of ansamycin antibiotics. Future work includes finishing the genome and confirming antibiotic production and chemical identity.

UG P06

Antibiotic Resistance in Bacterial Isolates from Sorcerer's Cave

Braden Smith, Sophia Wagle, Jennifer Huddleston
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Abstract

Antibiotic resistance is a concerning topic that has gained increasing attention over the years. This dangerous phenomenon occurs when some cells in a colony are able to survive through mutations or acquisition of genes that provide a mechanism for resisting the antibiotic (Canton, 2011). Genes conferring resistance are believed to be ancient as they have been found in DNA from permafrost, caves, and preserved pathogens from long ago (Perry, 2016). These genes are believed to have originated in Actinomycetes in order to protect them from the antibiotics they produced (Perry, 2016). It is likely that transmission by evolution and horizontal gene transfer have led to the current collection, or resistome (Aminov, 2011). As clinical use of antibiotics has increased the prevalence of these genes, it is important to consider to what degree medicine has contributed to the problem (Canton, 2011). Samples from caves may prove useful in this comparison of intrinsic versus contributed as they are isolated from impact by humans (Pawlowski, 2016). This study seeks to analyze the degree of antibiotic resistance naturally occurring in bacteria apart from human influence. Assays were created using 7 common antibiotics in 95-well plates and resistance was measured by absorbance using a spectrophotometer. DNA from resistant strains was then extracted and examined using electrophoresis for possible identification of plasmids that might indicate possible transmission of resistance.

UG P07

Testing South America Plant Extracts for Antimicrobial Activity on *Staphylococcus aureus*

Tinsae Solomon, Patricia J. Baynham
St edward's university, Austin, USA

Abstract

Every year 2.8 million people in the USA face antibiotic-resistant infections. So much research is required to find alternative treatments and medicine to cure bacterial infection. For this study, South American plant extracts were used to test if they work to inhibit the growth of *S. aureus* is a gram-positive bacterium. This bacteria can spread through the tissues, invading bone, and it can affect the bloodstream and lead to death, killing 20,000 people in the US in 2017. There is a challenge in treating these infections because many of these strains develop antimicrobial resistance (AMR). Not following the instructions of antibiotics is one activity that leads to AMR. For this research, Kirby Bauer disk diffusion was used. Eighty-eight plant extracts were tested for their ability to inhibit *S. aureus*. Using 50ug of plant extract resuspended in ethanol and 5mm paper disks were impregnated. After drying, the disks were placed on a Mueller Hinton agar plate inoculated with *S. aureus*. After 16-24 hours the plates were checked for zones of inhibition. Of 88 extracts only one showed zones of inhibition measuring 8mm. With further testing, it is possible that chemicals from these plants can be developed into a therapeutic treatment for AMR infections.

UG P08

A Novel Antimicrobial Susceptibility Testing Assay to Examine Polymicrobial Communities Reveals Potential Adverse Changes to Drug Efficacy

Sabrina Wilson, Caroline Black, Allie Smith
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Abstract

New discoveries in sequencing technologies show that most chronic infections are colonized by more than a single microorganism, implying that these types of infections are typically polymicrobial in nature. The ability for species in polymicrobial communities to interact and synergize activities leads to increased intermicrobial cooperation, resulting in a decrease in the effectiveness of antibiotics and worsening patient prognosis. Despite the knowledge that chronic wounds are highly associated with polymicrobial biofilm infections, most hospital laboratory assessment of antimicrobial susceptibility is still conducted on monomicrobial suspensions. The purpose of this research was to investigate the role polymicrobial communities have in influencing an individual species' survival when faced with multiple antibiotics. New methodology allowed for the determination of antimicrobial susceptibilities via both visible turbidity and viable colony counts. Four clinically relevant wound pathogens, Staphylococcus aureus, Pseudomonas aeruginosa, Acinetobacter baumannii, and Enterococcus faecalis, were grown individually as well as in a polymicrobial community. The antibiotic susceptibilities of the monomicrobial and polymicrobial conditions were compared and changes to the antimicrobial efficacies were seen. Notably, P. aeruginosa showed a decreased susceptibility to ceftazidime in the polymicrobial condition, which is concerning given this drug is a recommended treatment option for Pseudomonas infections. This shows that the presence of a community has a major influence on whether antibiotic treatment is successful or not, even if the treatment is combinatorial in nature. Accounting for the roles of polymicrobial communities in infections is vital when determining antimicrobial susceptibilities, which overall allows for more effective treatment of chronic infections.

Bacteriophage Microbiology

UG P09

Bacteriophage Grapple, Isolated from a Microbacterium foliorum Culture

Megan Ashcraft, Ashley Fannin, Marlee Goppert, Dustin Edwards
Tarleton State University, Stephenville, USA

Abstract

Within the last decades, there is a renewed interest in bacteriophages as therapeutic agents for antibiotic-resistant infections. As part of ongoing research on bacteriophage evolution and applications, institutions including Tarleton State University are working to broaden the knowledge of bacteriophages through the Howard Hughes Medical Institute SEA-PHAGE program. Bacteriophage Grapple was isolated from a soil sample that was collected under tree cover and less than 10 cm under the surface in Cedar Park, Texas. It was incubated with Microbacterium foliorum SEA B-24224 in peptone-yeast extract-calcium media for 48 hours at 29°C for viral replication. Following two rounds of serial dilutions assays, Grapple formed lytic plaques about 2 mm in diameter. A Siphoviridae morphology was revealed through negative-staining transmission electron microscopy, showing a 65 nm diameter capsid and a 151 nm tail. Bacteriophage DNA was extracted with a modified zinc chloride precipitation method and was sent to the Pittsburgh Bacteriophage Institute for next-generation sequencing. Restriction enzyme digestion HaeIII, NspI, and SalI were unable to digest bacteriophage Grapple DNA. Grapple was archived at Tarleton State University and the Pittsburgh Bacteriophage Institute.

UG P10

Bacteriophage LemonZest, Isolated from a *Microbacterium foliorum* Culture

Dasire Brawley, Marlee Goppert, Dustin Edwards
Tarleton State University, Stephenville, USA

Abstract

Bacteriophages, viruses that target a specific bacteria, are a treatment strategy used to combat antibiotic-resistant bacterial infections without infecting eukaryotic cells. Investigation of environmental bacteriophages can help to better understand bacteriophage evolution and dynamics to improve therapeutic applications. Bacteriophage LemonZest was isolated from dry soil under a tree in Dublin, Texas (32.0492406, -98.3859251). LemonZest was incubated with the host *Microbacterium foliorum* NRRL B-24224 in peptone-yeast extract-calcium media for 48 hours at 29°C. Following two rounds of serial dilutions and plaque assays with soft agar overlay, the bacteriophage formed lytic plaques less than 0.5 cm in diameter. Negative-staining transmission electron microscopy showed LemonZest to have Siphoviridae morphology with a 50 nm in diameter capsid and a 109 nm in length tail. LemonZest DNA, extracted by a zinc chloride precipitation method, was unable to be digested by restriction enzymes HaeIII, NspI, SacII, and SalI. The DNA of LemonZest was sent to the University of Pittsburgh for next-generation sequencing.

UG P11

Isolation of Bacteriophage Enchi from a *Microbacterium foliorum* Culture

Grace Clements, Sydney Keyon, Marlee Goppert, Dustin Edwards
Tarleton State University, Stephenville, USA

Abstract

As certain infections become resistant to antibiotics, phage therapy is being considered as an alternative treatment. Bacteriophage Enchi was found in a soil sample collected along the Bosque River trail access point in Stephenville, Texas. The soil was collected at a depth of approximately 4 cm at the base of a tree. Bacteriophage Enchi was isolated through direct isolation and incubated in PYCa media with the host *Microbacterium foliorum* NRRL-24224 SEA for 24 hours at 29°C. After two rounds of serial dilutions, purified bacteriophage Enchi formed small-to-medium-sized round, lytic plaques in soft agar overlay. Transmission electron microscopy determined Enchi to be of *Siphoviridae* morphology with a capsid diameter of 35 nm and tail length of 95 nm. A zinc chloride precipitation method was used to extract bacteriophage DNA, which was determined by Nanodrop to be 407.4 ng/µL. Gel electrophoresis showed that the viral genome was approximately 23,130 bp and incubation of viral DNA with restriction enzymes SacII, NspI, SalI, and HaeIII did not digest the genome. Bacteriophage Enchi was archived at both Tarleton State University and the Pittsburgh Bacteriophage Institute and added to the Actinobacteriophage Database.

UG P12

Bacteriophage BlueJean, Isolated from a Microbacterium foliorum Culture

Alexis Gastin, Kara Wiggam, Marlee Goppert, Dustin Edwards
Tarleton State University, Stephenville, USA.

Abstract

Bacteriophages are viruses that infect bacteria and reproduce through either a lytic or lysogenic cycle. Bacteriophages reproduce through a lytic cycle the phage will infect and replicate within the bacterial host causing lysis at the end of the cycle, forming a plaque in cultured cells, and releasing progeny virus particles. Temperate bacteriophages reproduce through the lysogenic cycle, where the bacteriophage genome is integrated into the host bacterial genome to persist as a prophage and be replicated in daughter cells. Soil samples were collected from an anthill in Heritage Park in Stephenville Texas and incubated with the Microbacterium *foliorum* NRRL-24224 in PYCa media at 29°C for 48 hours. Bacteriophage BlueJean was isolated following two rounds of serial dilutions. The plaques that formed were small, clear, and pinpoint-like. Transmission electron microscopy was performed and revealed that BlueJean had *Siphoviridae* morphology with a tail length of 921 nm and a capsid diameter of 126 nm. DNA concentration was found to be 21.6 ng/µL and had heavy salt (0.07 A260/A230) and mRNA contamination (2.76 A260/A280). Bacteriophage BlueJean was achieved at Pittsburgh Bacteriophage Institute and Tarleton State University.

UG P13

Comparative Analysis of Minor Protein Sequences Among Actinobacteriophages in Subcluster A11

Jordan N Angeles, Myah B Beylotte, Christina E Spencer, Angela L Salazar, Frederick N Baliraine
LeTourneau University, Longview, USA

Abstract

Actinobacteriophages are viruses that infect bacterial hosts in the phylum Actinobacteria. These viruses are grouped in “clusters” based on their overall nucleotide sequence similarity, “subclusters” based on average nucleotide identity values, and “singletons” if there is no significant homology to previous clusters. Currently, there are 717 cluster A members, 22 of which belong to subcluster A11.

Minor tail proteins (MTPs) are involved in host cell recognition and infection. Therefore, they are expected to play a role in phage host range, thus sequence diversity in MTPs could be useful for phage phylogenetic analyses. To examine this possibility, a comparative analysis of all currently fully annotated MTPs from subcluster A11 were analyzed using PhagesDB, NCBI BLAST, Phamerator, HHPred, MultAlin, GC Content Calculator, and SplitsTree. Members of a subcluster should be expected to have high sequence homology. However, Phamerator and MultAlin analysis of the MTP upstream of the ParA-like dsDNA partitioning protein revealed a consistently notable lack of homology in its left arm among various members of subcluster A11.

Variations in the MTP sequence length, GC content, and BLAST data were observed. HHPred showed no significant hits to phage or bacterial MTPs, but significant hits to collagen-like proteins in eukaryotic organisms were observed. Interestingly, SplitsTree data showed sub-clustering within the A11 subcluster. Possible implications of observed lacking homology in MTPs on host range and Actinobacteriophage phylogenetics will be discussed.

UG P14

Comparative Analysis of the Reliability of DNABIND and DNAbinder Software for Identifying Bacteriophage DNA-binding proteins

Christina A. Holder, Christina E. Spencer, Angela L. Salazar, Frederick N. Baliraine
LeTourneau University, Longview, USA

Abstract

DNA-binding proteins (DNA-BPs) are an important class of proteins that target specific DNA sequences and play key roles in relation to DNA, such as transcription regulation and host defense. Therefore, identifying DNA-BPs is important in genome annotation. Many annotated phage genomes in NCBI GenBank have open reading frames with unknown functions, called hypothetical proteins (HPs). HPs may be unidentified DNA-BPs. The purpose of this study was to test the reliability of the DNABIND and DNAbinder programs for determining DNA-BP gene function.

While annotating mycobacteriophage Dynamo, these two DNA-BPs detection software were used to check HPs for DNA-binding activity. Dynamo gp 52 had a strong DNA-binding prediction in both programs and strong alignments in HHpred to a TATA-box binding protein. However, a function was unable to be confidently determined due to no significant alignments with known functions in NCBI BLASTp.

DNABIND and DNAbinder were investigated for their sensitivity and specificity by utilizing published phages from various clusters. Seven non-DNA-BPs and eight DNA-BPs were analyzed from each phage. DNABIND was run at a false positive rate of 15% and DNAbinder was run using an amino acid composition with main, realistic, and alternate datasets. Preliminary results suggest DNABIND is fairly reliable while DNAbinder's reliability depends on which of the three datasets was used in the analysis. This study describes the application of the collected data to elucidate the reliability, sensitivity, and specificities of the programs for calling DNA-BPs.

UG P15

Investigating the Presence and Implications of a Potential Wrap-Around Gene in P1 Mycobacterium Phage Dynamo

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Abstract

While annotating the P1 Mycobacterium phage Dynamo, an HNH endonuclease was called at the end of the genome while a terminase was located at the beginning. HNH or homing endonucleases are required for the activity of terminases and are indispensable components of the bacteriophage DNA packaging machine. The location of the HNH endonuclease gene in phage genomes is highly conserved near the terminase gene. A possible explanation for the observed locations of HNH endonuclease and terminase genes in Dynamo's genome would be that there is a wrap-around gene overlapping the beginning and end of the genome. This fact highlights the circularization of phages in their host. To investigate the possibility of a wrap-around gene, various bioinformatics tools were utilized: DNA Master, HHpred, Glimmer, GeneMark, NCBI BLASTp, Phamerator, DNABIND, DNAbinder, and PHASTER.

Glimmer, GeneMark, HHpred, and BLASTp did not support the hypothesis for a gene in this region. However, DNABIND and DNAbinder indicated a high probability that this region contains a DNA-binding protein. Also, it was noted that phages with a confirmed wrap-around gene were circularly permuted, whereas Dynamo has a 3' sticky overhang. Therefore, the bioinformatic evidence against the presence of a wrap-around gene in Dynamo outweighed the evidence for its presence. Further research should be conducted to determine whether wrap-around genes are present in phages with an overhang as well as the significance of a phage being circularly permuted. This study investigates bioinformatically the possibility and implications of the presence of a wrap-around gene in Mycobacterium phage Dynamo.

UG P16

Bacteriophage DopeGoat, Isolated from a *Microbacterium* Culture

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Abstract

As part of the Howard Hughes Medical Institute (HHMI) SEA-PHAGES program, Tarleton State University collected environmental samples to isolate and characterize bacteriophages. Genomic data from these bacteriophage libraries further our knowledge of bacteriophage evolution and genetics. Soil samples collected from an anthill about 3 cm in depth next to a trashcan on Tarleton State University campus in Stephenville, Texas were washed in phage buffer with calcium and incubated with the host *Microbacterium foliorum* NRRL-24224 SEA in PYCa media for 48 hours at 29°C. Two rounds of serial dilutions isolated the bacteriophage, which formed 3 mm lytic plaques. Negative-staining electron transmission microscopy showed that DopeGoat had siphoviral morphology with a tail length of 110 nm and a capsid diameter of 39 nm. Following DNA extraction by a zin chloride precipitation method, restriction enzymes HaeIII, NspI, SalI, and SacII were unable to digest the bacteriophage DNA. Bacteriophage DopeGoat was archived at Pittsburgh Bacteriophage Institute and Tarleton State University.

UG P17

Isolation of BenitoVP from *Microbacterium foliorum*

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Abstract

As part of the Howard Hughes Medical Institute SEA-PHAGES program to further characterize bacteriophage genetics, bacteriophage BenitoVP was extracted from soil samples collected under a rock on a farm outside of Glen Rose, Texas (32°18′21.79 ″N, -97°70′40.97 ″W). The samples were washed in phage buffer with calcium and the supernatant containing virus particles was filtered through a 0.22 µm filter before plating with host *Microbacterium foliorum*. Bacteriophage BenitioVP was isolated following two rounds of serial dilution. High-titer lysate was prepared for uranyl-acetate stained transmission electron microscopy grids and for archiving at -80°C. Negative-staining transmission electron microscopy of isolated microbacteriophage BenitoVP displayed *Sipoviridae* morphology with a capsid diameter of 61 nm and a tail length of 143 nm.

UG P18

The Impact of Shiga toxin Bacteriophages on Acid Resistance in *Escherichia coli* O157:H7 Isogens

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Abstract

Background: Phage-borne Shiga toxin (Stx) production is a virulence hallmark of Stx-producing *Escherichia coli* (STEC), however atypical non-shigatoxigenic isolates are found that either never acquired Stx-phages or secondarily lost stx during the course of infection, isolation, or routine subculture. In this study, we examine the impact of Stx-phage carriage in two isogens of the O157:H7 serotype, TT12A (Stx+) and TT12B (Stx-), isolated from a patient in Japan in 1994 by assessing growth under controlled pH conditions.

Methods: To determine the cell growth under different metabolic conditions, phenotypic analyses for the stx+/- isogens were performed using BIOLOG MicroArray plates for a total of 48 hours with reads collected in 15 minute intervals at an Optical Density (OD) of 595nm. Media with pH ranging from 3.5 to 10 were tested with the addition of amino acids that play a role in the different *E. coli* acid resistance mechanisms. Resulting phenotypes were analyzed in context of the isogens' global transcriptomes and identified differentially expressed loci confirmed by quantitative RT-qPCR[ME1] .

Results and Conclusions: We determined the viable pH-range that allows culture growth. The recorded BIOLOG growth curves specifically suggested a role for both glutamate- and arginine-dependent pathways in conferring increased acid resistance in TT12A. Overall, our data indicate that Stx-phages associated with this serotype are interconnected with acid resistance, which is critical for stomach passage during human infection.

UG P19

Isolation and Annotation of Cluster EE Bacteriophage Loca

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Abstract

Bacteriophages are viruses that infect bacteria and are a potential novel treatment for antibiotic-resistant infections. Bacteriophage Loca was isolated from a shopping cart handle swab in Stephenville, Texas. The swab was suspended in peptone-yeast extract-calcium media and the supernatant was filtered through a 0.22µm filter and incubated with host Microbacterium foliorum NRRL-24224 SEA. Following two rounds of 10-fold serial dilutions and plaque assays with a soft agar overlay, Loca formed small, defined lytic plaques less than 1cm in diameter. Negative-staining transmission electron microscopy revealed Siphoviridae morphology with an approximate tail length of 102 nm and capsid diameter of 43 nm. Bacteriophage DNA was extracted with a modified zinc chloride precipitation method and sequenced to 73-fold genome coverage by the Pittsburgh Bacteriophage Institute using Illumina next-generation sequencing. Loca has a double-stranded DNA genome that is 17,475 base pairs in length with a 9 base 3' sticky overhang (CCCGCCCCA) and 68.7% G+C content. Loca is the eighth-largest member of cluster EE, and is most closely related to Microbacteriophages Leafy, Quaker, Livingwater, and Hulk, with nucleotide sequence identity >96%. Whole-genome sequence analysis using PECAAN, PhagesDB, NCBI BLASTn, and BLASTp, HHPRED, and tRNAscan-SE software determined 25 protein-coding genes, including DNA-binding and bridging proteins, one HNH endonuclease, and no predicted tRNAs. This whole-genome sequence contributes to our understanding of microbacteriophage genetics.

UG P20

Bacteriophage EnderDragon, Isolated from a *Microbacterium foliorum* Culture

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Abstract

Bacteriophages are viruses that infect and replicate within bacteria. Tarleton State University, as part of the Howard Hughes Medical Institute SEA-PHAGES Program, collected liquid and soil samples to isolate and characterize novel bacteriophages with the purpose of further characterizing their genetics and evolution. Bacteriophage EnderDragon was isolated from a grassy environment under a rock next to a tree from surface to 1 cm depth in Glen Rose, Texas, and was directly isolated and incubated with the host *Microbacterium foliorum* NRRL-24224. Following two rounds of serial dilution, isolated bacteriophage EnderDragon formed small lytic plaques approximately 2 mm in width. High volume lysate with a titer of 1.5×10^9 pfu/mL was used for negative-staining transmission electron microscopy and showed that EnderDragon had a *Siphoviridae* morphology with an approximate tail length of 150 nm and capsid diameter of 60 nm. Bacteriophage DNA was extracted by a modified zinc chloride precipitation. Restriction enzyme digest and gel electrophoresis showed restriction enzymes HaeIII, NspI, SacII, and SalI were unable to digest bacteriophage EnderDragon DNA. The bacteriophage EnderDragon was archived at the Pittsburgh Bacteriophage Institute and Tarleton State University.

UG P21

Isolation of Bacteriophage Interrobang from *Microbacterium foliorum*

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Abstract

Bacteriophages are viruses that infect and undergo viral reproduction in the cytoplasm of bacteria. Tarleton State University participates in the Howard Hughes Medical Institute SEA-PHAGES program with the goal of increasing our understanding of bacteriophage genetics and evolution. Bacteriophage Interrobang was discovered 10 cm underneath a rock on a farm in Glen Rose, Texas. Soil samples were suspended in phage buffer containing calcium, and the supernatant was filtered through a 0.22 µm filter and incubated with isolation host *Microbaterium foliorum* NRRL B-24224 in PYCa media for 48 hours at 29°C. Bacteriophage Interrobang was isolated following two rounds of serial dilution. A high volume lysate with a titer of 2.5×10^7 pfu/mL was used for transmission electron microscopy, which determined that Interrobang had a *Siphoviridae* morphology with a capsid diameter of 62 nm and a tail length of 137 nm. DNA was extracted by a zinc chloride precipitation method. Restriction enzymes NspI, HaeII, SalI, and SacII were unable to digest Interrobang DNA.

Basic and Environmental Microbiology

UG P22

Survey of Coronaviruses in Texas Bats

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Abstract

Coronaviruses (CoV) are positive-sense single-stranded RNA viruses known to cause disease in avian and mammalian species. CoV genomes from bats have been sequenced but mostly from captured animals, which involves inherent risks to the bats and researchers. Due to the size and genetic diversity of CoVs, full-length genome sequencing is expensive, and time and labor-intensive. We proposed to develop and demonstrate a protocol that reduces biohazards, uses rapid and non-invasive sample collection, and can be used to search for and characterize known and novel coronaviruses by inexpensive and simplified genome sequencing. The proposed protocol uses easily acquired guano samples for complete genome sequencing, removing the need to capture or handle bats, which is safer for both the bats and the researchers and could lead to more full-length genomes sequenced from wildlife by increasing available samples. Further, we added a step to inactivate biological agents present in guano while still in the field, which will permit the use of this sample type in BSL-1 and BSL-2 laboratories. The species source of guano was verified by cytochrome c oxidase I (COI) DNA mini-barcode assays using PCR to amplify extracted DNA with Chiroptera order-wide COI primers and thermocycler program, followed by Sanger sequencing at Texas A&M University-Corpus Christi Genomics Core. To detect CoVs, we attempted to amplify viral RNA by reverse transcription-PCR using conserved pan-coronavirus primers that target a 440-bp region of the RNA-dependent RNA polymerase (RdRp). We have not yet detected CoV RdRp cDNA products in any of the samples.

UG P23

Transcriptional regulation of LD-transpeptidases in the nosocomial pathogen, *Acinetobacter baumannii*

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Abstract

Acinetobacter baumannii is an emerging nosocomial pathogen that can rapidly develop resistance to overcome antimicrobial treatment. Carbapenem-resistant *Acinetobacter baumannii* has been classified as a high-level threat to public health by the Center for Disease Control (CDC). Typically, polymyxin E (colistin) is prescribed to treat carbapenem-resistant *A. baumannii* infections, but unfortunately, resistance has emerged. Colistin resistance in *A. baumannii* can occur through the inactivation of lipooligosaccharide (LOS) biosynthesis, which is unique because LOS was thought to be essential for the viability of Gram-negative bacteria. While the underlying molecular mechanisms that enable *A. baumannii* viability without LOS are not yet understood, it has been previously found that two LD-transpeptidases (Ldts), LdtJ and LdtK, are essential for *A. baumannii* survival without LOS. Here, we constructed *ldtJ* and *ldtK* transcriptional reporters to measure gene expression, which will enable us to screen for transcriptional regulatory elements. We describe cloning each promoter to create a *lacZ* fusion, which serves as a reporter to visualize differential transcriptional activation at different growth phases and stress conditions. This reporter will also be used to create a transposon library to identify the pathways *ldtJ* and *ldtK* interact with and which genes regulate their transcription. A better understanding of how *ldtJ* and *ldtK* contribute to this colistin resistance mechanism may help identify new strategies to address this issue.

UG P24

Putative Viral Induction in a Common Bacterial Symbiont of Coral

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Abstract

Stony corals and other marine invertebrates are often characterized in terms of their core microbiota, and Endozoicomonas bacteria tend to dominate these communities in some host species. Given that Endozoicomonas genomes are populated by multiple prophage, when these viruses enter a lytic cycle, they may influence the composition of their host's microbiome. However, few studies have tested whether prophage are viable. To explore prophage viability in a coral bacterial symbiont, we exposed Endozoicomonas montiporae CL-33, a strain encoding 8 prophage sequences, to physicochemical stressors (i.e. elevated temperature, ultraviolet radiation, and mitomycin C) known to induce temperate viruses in other systems. Mitomycin C resulted in early signs of culture lysis 5-6 hours after treatment and accumulated up to a 43±2% decrease (n=3) in absorbance values by 30 hours compared to controls, whereas elevated temperature and ultraviolet radiation lacked a similar response. Epifluorescence microscopy of filtered, SYBR Gold stained lysate revealed the presence of virus-like particles (VLPs), which resolved in Transmission Electron Microscopy (TEM) images as non-tailed VLPs ~ 25 nm in diameter. Prophage gene expression was monitored using transcriptome sequencing of cultures before mitomycin C treatment and at 2 and 6 hours post-treatment. Final results will include differential gene expression analyses between mitomycin C-treated and untreated cultures to confirm prophage activity, as well as host mechanisms associated with viral induction (e.g. SOS response). Ultimately, these findings will provide insights into virus co-evolution with an important symbiont of corals and with implications for marine holobiont health.

UG P25

Microbiome Composition and Derived Metagenome Analysis from Gold Mining Sites of the Mazaruni River, South America

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Abstract

Mercury (Hg) contamination from gold mining causes health and environmental issues worldwide. When inorganic Hg is introduced into an environment, it can be transformed into an organic form, methyl mercury (MeHg) via the Acetyl CoA pathways within microorganisms, specifically by Sulfate Reducing Bacteria (SRB) and Iron Reducing Bacteria (IRB). The health effects, bioaccumulation, and biomagnification of MeHg are well documented, however, the effects of mercury contamination on the microbiome composition and metagenome based metabolic predictions are not well studied. This study tested the following hypotheses: 1) There will be a difference in the microbiome composition between gold-mined and non-mined sites. 2) There will be a difference in the metabolic capabilities of microbiome derived metagenome between gold-mined and non-mined sites.

81 soil sediment samples were isolated from 27 mined and non-mined sites. Samples were first analyzed for mercury concentrations. 15 soil samples (3 replicates from 3 mined and 2 non-mined sites) were chosen for further analysis of methyl mercury concentrations and the remainder of procedures. Microbiome analysis was performed through DNA Extraction, PCR amplification of V3 and V4 16S rDNA, library preparation, and MiSeq Sequencing. QIIME 2.0 software processed sequences, assigned taxonomy, and established an Operational Taxonomic Units (OTUs) for further identification of bacterial genera, families, and phyla. Derived metagenome analysis was computed by filtering and analyzing OTU table with PICRUST 2.0. producing predicted KEGG orthologs. Results revealed significant differences of the microbiome and derived metagenome compositions between gold-mined and nonmined sites.

UG P26

Developing tools to genetically modify cockroach-infecting Gregarines

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Abstract

Gregarines are a subclass of apicomplexan parasites found to infect nearly every clade of invertebrates. They are some of the most abundant and diverse organisms on the planet, with the number of species estimated in the millions. Despite this, Gregarines tend to be understudied and relatively little is known about their cell and molecular biology. Gregarines also have the potential to serve as a model for their close relative, *Cryptosporidium spp.*, parasites determined by the WHO to be a leading source of global diarrheal illness and infant mortality. A key goal in developing Gegarines as an experimental model is to genetically manipulate them by developing transgenic and CRISPR tools. We chose to focus on *Blabericola migrator*, the only gregarine known to infect Madagascar Hissing Cockroaches (*Gromphadorhina portentosa*). Expression constructs are being generated with reporter genes that will allow *B. migrator* to express nanoluciferase and GFP and to express CRISPR/Cas9. Putative *cis*-regulatory sequences for *beta-actin* and *enolase* have been identified in the genome of *B. migrator* and molecularly cloned to drive gene expression in vector constructs. Once the vectors are fully assembled, the next steps will be to develop a method to electroporate the vector into the parasite and surgically implant the modified parasite into the cockroach intestine. Our ultimate goal is to better understand the genetic and molecular functions of this abundant yet understudied organism.

UG P27

Isolation of Genomic DNA from the Gregarine *Blabericola migrator* to Perform Long-Read, Whole-Genome Sequencing

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Abstract

It has been estimated that all animals can be infected by at least one parasitic protist from the phylum Apicomplexa. Important human and veterinary disease-causing apicomplexans include the causative agents of malaria (*Plasmodium spp.*) and cryptosporidiosis (*Cryptosporidium spp.*). Gregarines are a sub-class of invertebrate-infecting apicomplexans believed to be closely related to *Cryptosporidium spp.* and share many of their specialized lifestyle characteristics. Our goal was to isolate genomic DNA from the parasite utilizing the external and environmentally resistant gametocyst stage of the life cycle of the gregarine parasite of the Madagascar Hissing Cockroach, *B. migrator*, using an optimized DNA extraction protocol. The purity and genomic integrity of the isolated samples was assessed by spectrophotometry and agarose gel electrophoresis. The specificity of the parasite versus the host DNA was determined by PCR reactions with DNA primers specific to each organism along with primers against bacterial contaminants. Whole-genome sequencing of *B. migrator* will enable us to shed light on the molecular mechanisms of the parasite and further understand its genetic makeup.

UG P28

Describing nitrogen-fixation like sequences within the Asgard archaea superphylum

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Abstract

In the past five years several genomes have been reconstructed from several environments around the world using metagenomics belonging to new lineages of Archaea that are related to eukaryotes (2,3,4). Metagenomic approaches have also identified unique microbial pathways that have altered our understanding of global sulfur and carbon cycling and the evolution of nitrogenases, including previously undescribed nitrogen fixation-like sequences. In Bacteria, these novel nitrogenases are proposed to synthesize methionine and byproducts (ethylene and methane) from volatile organic sulfur compounds (1). Here we describe the first Asgard archaea genomes coding nitrogen fixation-like sequences, nifD/H, within these updated nitrogenase families. Scaffolds from more than 400 Asgard metagenomically-assembled genomes were extracted and aligned using nifD/H prokaryotic references. We used these alignments to generate maximum-likelihood gene trees with iqtree. Asgard phyla, Vidararchaeota and Helarchaeota, encode group IV-B nitrogenase-like enzymes, CfbD (nifD) and CfbC (nifH). These enzymes enable the synthesis of F430, a cofactor for methyl coenzyme M reductase (MCR), which catalyzes the final step in methanogenesis (1). Uncovering CfbC/D expands the known physiological activities of the archaeal lineages most closely related to the eukaryotic host. Further analysis is necessary to determine the function of these genes in Archaea and their role in the evolution of nitrogenases.

References: [1] North et al., 2020. [2] Baker et al., 2021. [3] Zaremba-Niedzwiedzka et al., 2017. [4] Spang et al., 2015. [5] Imachi et al., 2020.

UG P29

Standardization of *Saccharomyces cerevisiae* microplate reader growth conditions

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Abstract

Plate-reader-derived growth curves are commonly used in various fields of research, but researchers often fail to report critical experimental settings which can be associated with inconsistencies and reproducibility issues. In order to increase reproducibility in future experiments and generate a set of standard parameters for 96-well plates, we determined the optimal shaking conditions and plate covering for growth curve optimization. Yeast growth at 37 C was measured at OD_{600} every 15 minutes for 24 hours using a BioTek Synergy H1 microplate reader. Of the seven shaking conditions tested, continuous and intermittent linear shakings have the lowest generational time, even though cells clump around the well edges, and all the shaking conditions have similar OD600 at 24 hours. However, the growth rates for both linear shakings are lower than what is typically observed in yeast while other shaking conditions had rates that are more consistent with previous research. Four lid coverings were tested for evaporation under the same growth conditions with no shaking. Of the four lid coverings tested, the sticker covering was the most effective at reducing evaporation. Not using a lid resulted in high levels of evaporation with nearly 100% evaporation seen on the bottom edge of the plate. These results display that it is crucial to standardize procedures and methods to obtain consistent results for future research.

UG P30

PlrA is a novel polar growth regulator in Mycobacteria

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Abstract

Mycobacteria elongate their cell wall through polar expansion. The enzymes that expand the cell wall are well known but how they are regulated to control polar growth is not well understood. In this study, we introduce an essential protein involved in regulating polar growth, PlrA. This protein localizes to the poles and a bit at the septum. When this protein is depleted, cells are bulgy and unable to elongate.

UG P31

Isolating the cell-invasive stage of a cockroach-infecting Gregarine for gene expression analysis

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Abstract

Gregarines are parasites from the phylum Apicomplexa which parasitize the intestinal epithelia of nearly all invertebrate clades. They are most closely related to the parasite, *Cryptosporidium spp*, which infects vertebrates and causes cryptosporidiosis in humans. The similar lifestyles and life cycles of these two parasites allow the largely understudied Gregarines to serve as a model for Cryptosporidium. Like Cryptosporidium, Gregarines that infect arthropods infect their host through an oral-fecal pathway via infectious oocysts. When ingested oocysts encounter the host midgut, intestinal epithelial cell-invasive sporozoites are released from the oocyst. In order to develop a better understanding of the Gregarine-host intestine molecular interactions, we plan to sequence the transcriptome of the invasive sporozoite stage. To this end, we are developing a method to isolate sporozoites in vitro via a combination of differential centrifugation and the application of roach bile. Our goal is to optimize the specific isolation of this stage in vitro, purify and sequence total RNA, and, in the process, shed light on the genes expressed at this invasive stage.

UG P32

A comparative study on the effects of cryopreservative agents on culture-dependent analysis of aquatic microbiomes.

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Abstract

Advances in microbial cultivation techniques have renewed interest in isolating microbes from natural and host-associated systems. Obtaining libraries of isolates is particularly important for aquatic systems, where culturable bacteria are used to assess fecal contamination, and libraries of isolates can be used for tracking the emergence of antibiotic-resistant bacteria. Generally, fresh samples are required when cultivating and isolating bacteria, as freezing can kill many species of bacteria. This limits the scope of research. Cryoprotective agents (CPAs) can preserve fecal microbiome samples, but these agents have not been evaluated for use with aquatic samples. In this study, two CPAs - dimethyl sulfoxide and glycerol- were evaluated by comparing libraries generated from fresh and cryopreserved samples in terms of number and types of bacteria isolated. Samples treated with the two CPAs were stored at -80°C for durations of 14-days and 100-days. The type of bacterial colonies isolated post thawing the samples was identified with matrix-assisted laser desorption ionization-time of flight (MALDI-TOF) and further identification of some isolates was done using 16s rRNA gene sequencing. While freezing with different CPAs did not show a dramatic change in the number of bacterial strains isolated, we saw a profound change, between libraries generated from samples preserved in different CPAs, in the type of bacteria isolated. This suggests that CPAs can create a bias in culture-dependent studies of aquatic microbiomes.

UG P33

Study of a lanthanide-dependent methanol dehydrogenase in *Paracoccus denitrificans*

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Abstract

The *xoxF* gene of the bacterium *Paracoccus denitrificans* encodes a predicted lanthanide-dependent methanol dehydrogenase, XoxF. The presence of lanthanum is suggested to inhibit expression of a calcium-dependent methanol dehydrogenase, Mdh, expressed from the *mxaF* gene. To assess promoter function in response to lanthanide compounds, I inserted the *xoxF* or *mxaF* promoter into the multiple cloning site in front of the lacZ gene in the reporter plasmid, pMP220. *lacZ* is responsible for the production of β-galactosidase. Then, this plasmid was conjugated into the *P. denitrificans* wild type strain. Log-phase cultures growing in minimal media containing methanol in the absence or presence of lanthanum were collected to perform β-galactosidase assays. The assays showed that the addition of lanthanum to methanol-containing media abolishes *mxaF* promoter activity. Under these conditions, methylotrophic growth appears to slow down. My next immediate step is to perform a β-galactosidase assay to examine how the *xoxF* promoter responds to lanthanum-containing media.

Future research involves three interacting regulatory proteins: FlhS, FlhR, and FlhT, on which methylotrophic growth depends. These proteins respond to formaldehyde generation. I will conjugate *xoxF* and *mxaF* promoter fusions into mutant strains that each lack one of the regulatory proteins; the conjugation will also be performed in the respective complemented strains. I will measure promoter activities in these mutants and complemented strains to determine the roles of the regulatory proteins in lanthanide-mediated alterations in gene expression.

UG P34

Growth Characteristics and Synthesis of Silver and Gold Nanoparticles in *Rhodobacter sphaeroides* under Microgravity Conditions

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Abstract

Over the years, heavy-metal nanoparticles have been synthesized in various microorganisms and plants. The heavy-metal nanoparticles have been utilized as an antimicrobial and other nanotechnological applications in numerous disciplines, including medical research. *R. sphaeroides* has been shown to enzymatically detoxify heavy metal salts and accumulate metal nanoparticles intracellularly and/or extracellularly. The objective of the current research is to synthesize silver and gold bio-nanoparticles using Rhodobacter sphaeroides under both microgravity and normal gravity growth conditions. We tested two hypotheses. (1) *R. sphaeroides* grows more rapidly under microgravity conditions compared to normal gravity conditions. (2) Bacterial cells grown under microgravity conditions synthesize and accumulate less silver- and gold-bio-nanoparticles compared to gravity conditions. To understand growth characteristics, bacterial cells were grown and sampled at 24, 48, 72, and 96 hours; Cell growth was estimated by measuring the optical density at 600 nm (OD600). CFU were determined and growth characteristics were investigated by plating the cells at various dilutions on the SIS-Agar plates. Cell size was estimated using light microscopy and ImageJ software. Accumulation of silver and gold elements in bacterial cells was confirmed by EDS analysis. Results revealed that R. sphaeroides grew more rapidly under microgravity conditions. However, the cells isolated were smaller in size compared to normal gravity conditions. Preliminary results suggest higher accumulation of silver and gold in bacterial cells grown under normal gravity conditions compared to the microgravity conditions. The physical, chemical and biological properties of bio-nanoparticles will be further analyzed using TEM, SEM, FTIR, and antibiotic sensitivity tests.

Pathogenic Microbiology

UG P35

Identifying Novel Genes with Increased Susceptibility to Reactive Oxygen Species in *Bacillus anthracis* Sterne

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Abstract

Bacillus anthracis is a gram-positive bacterial pathogen that causes the deadly infectious disease anthrax. *Bacillus anthracis* contains two plasmids, pX01, and pX02. These plasmids were found to be necessary for the virulence of *B. anthracis*. However, *Bacillus anthracis* contains over 5,000 chromosomal genes and we believe that there are additional virulence genes that have yet to be discovered. Our lab constructed a transposon mutant library with random disruptions in the *B. anthracis* Sterne genome to screen for novel virulence factors. This library has been successfully used to identify the chromosomal genes *clpX* and *yceGH* and show their importance for *B. anthracis* virulence. To find additional novel virulence genes, we used the same transposon library and screened around 1,000 mutants using hydrogen peroxide, a reactive oxygen species (ROS). ROS are involved in the immune defense and the mutants that are attenuated in its presence may have a disrupted gene that contributes to the pathogenicity of *B. anthracis*. We obtained two mutants that were repeatedly susceptible to hydrogen peroxide *in vitro*. To determine the virulence of these mutants in an animal model, we will be performing an *in vivo* assay using the waxworm, *Galleria Mellonella*. Mutants that have reduced virulence in *G. mellonella* will then be further tested to determine the location of the transposon in the genome to find out which genes are disrupted. The findings of this research could be used as potential therapeutic drug targets and could offer insight into the mechanisms that *B. anthracis* uses for its pathogenesis.

UG P36

Modulation of Bacterial Host Phenotypes by Mycobacteriophage Pixie Gene Products

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Abstract

Bacteriophage genes are being studied for their potential clinical use in phage therapy for antibiotic-resistant infections. With the support of the Howard Hughes Medical Institute, as part of the SEA-GENES network, we have amplified all 100 genes from Mycobacteriophage Pixie and cloned 88 genes for study in cytotoxicity assays. Genes were amplified from Pixie high titer lysate by PCR amplification, and the products were purified and ligated into a pExTra plasmid by isothermal assembly. Plasmids were transformed into 5-alpha F'I^q *Escherichia coli*, and the extracted plasmid DNA was electroporated into *Mycobacterium smegmatis* mc^2155. Phenotypic assays were conducted by plating transformed *M. smegmatis* on agar containing anhydrotetracycline to induce the production of gene inserts. Cytotoxicity was determined by spotting serially diluted transformed *M. smegmatis* growth versus controls. Superinfection assays were conducted by inoculation of transformed or controlled *M. smegmatis* lawns with serially diluted bacteriophages to determine the efficiency of plating. Our study revealed 5 potentially cytotoxic genes: a putative major capsid protein, an uncharacterized gene downstream of the lysin A/B proteins, putative tyrosine integrase, and an uncharacterized gene adjacent to the immunity repressor. This information broadens our understanding of bacteriophage-bacteria interactions and the potential clinical use of cytotoxic genes to treat bacterial infections.

UG P37

Investigating the Contribution of Fungal Extracellular Matrix on the Autofluorescent Properties of Biofilm

Elise Bolin, Kaitlyn Bui, Isaiah K. George, Eleanna Carris, Rachel C. Diaz, Allie C. Smith
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Abstract

Biofilms are microbial communities contained within an extracellular matrix (EM), which acts as a mechanical barrier to protect against the immune system and antimicrobial treatment. The formation of biofilms within chronic wounds contributes to increased severity of infection and adverse patient outcomes due to decreased treatment efficacy. The MolecuLight i:X is a hand-held bacterial imaging device that detects the autofluorescent properties of most clinically relevant species of bacteria, both planktonically (free-living) and within biofilm. Currently, there is interest in expanding the capabilities of the device by determining if the biofilm-associated extracellular matrix also exhibits any unique fluorescence markers that are detectable with the device. Additionally, there is interest in determining how the contribution of fungal species affects fluorescence detection with the i:X device. To explore these questions, we investigated the fungal pathogen Candida albicans both planktonically and as a biofilm and performed a full emission spectral scan to identify any unique fluorescence peaks associated with biofilm EM. This work could inform a specific setting within the MolecuLight i:X device for detection of fungal-associated biofilm within chronic wound infections. This could allow physicians to visualize and diagnose biofilm-associated infections in real-time, improving treatment and patient care.

UG P38

Bacteriophage-containing Biodegradable Microsphere Technology to Treat Osteomyelitis

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Abstract

The rise in antimicrobial resistant (AMR) infections is a growing concern bacteriophage (phage) provide a unique biological approach to the AMR problem. A major issue in delivery of active bacteriophages using poly(lactic-co-glycolic acid) (PLGA) microspheres, a degradable drug delivery system, is possible phage inactivation when they are in contact with the organic solvent dichloromethane during the manufacturing process. We investigated two protocols for microsphere manufacture, utilizing our previously developed microsphere technology for antibiotic delivery. We used bacteriophage K, which we found lysed the clinical Staphylococcus aureus isolate UAMS-1, obtained from an osteomyelitis infection, when grown on agar plates, in liquid culture, and in biofilms. In method one $2.5\text{-x-}10^{10}$ phage/ml were added directly to PLGA-dichloromethane. In method two $2.5\text{-x-}10^{10}$ phage/ml solution were added to polyvinyl alcohol (PVA) before addition to PLGA-dichloromethane. Both methods were evaluated using a 1-ml elution assay on 1-, 3-, and 7-day samples. The eluents were spotted onto lawns of UAMS-1 S. aureus, and the resulting plaque forming units (PFUs) were counted. The total phage eluted over 7-days and the average entrapment efficiency for the 250 mg microsphere batches were calculated. For Method one $2.0\text{-x-}10^{7}$ phage eluted after 7 days yielding a 0.6% entrapment efficiency. For Method two $5.1\text{-x-}10^{5}$ phage eluted after 7 days yielding a 0.02% entrapment efficiency. Thus, method one eluted more active phage and had a greater entrapment efficiency. We are currently studying the effectiveness of phage-containing PLGA microspheres against S. aureus in in vitro biofilms and plan to examine in vivo animal models.

UG P39

Amino Acid Changes in the Spike (S) Protein in SARS-CoV-2 Variants (B.1.1.7 SARS-CoV-2)

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Abstract

This project focuses on characterizing the composition of sequenced SARS-CoV-2 samples taken at Abilene Christian University. In designing primers targeting the spike protein region of the SARS-CoV-2 variants, we continue to gain a better understanding of the introduction and subsequent circulation of various strains infecting the population of ACU. RNA samples were acquired from the ACU COVID-19 PCR testing program in Abilene, TX. The viral RNA samples were then converted into cDNA, and primer pairs 1210F/CR2 and CF1418/2150R were used to isolate a segment of the cDNA associated with the spike protein. We then used PCR and gel electrophoresis to isolate workable samples before purifying the products and obtaining sequences of these products. Finally, we compared the sequences of our samples with known COVID variants of interest in order to determine their identities. Our preliminary results show consistency with an early arrival of the ancestral B.1 variant on the ACU campus, followed by a transition to the more contagious variant of concern B.1.1.7 (Alpha). Further testing of more recent samples will be essential for tracking ongoing changes in the B.1.1.7 variant in response to shifting and changing public opinion and behaviors, as well as increasing natural and vaccine-induced immunity.

UG P40

Exploring the Auto-fluorescent Properties of Biofilm to Improve Diagnostics of Biofilm-Associated Infections

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Abstract

Chronic wounds are those that remain open for six weeks or longer and are often driven by polymicrobial biofilm-associated infections. Biofilm-associated bacteria can secrete an extracellular polymeric substance (EPS) which acts as a mechanical barrier to both the immune system and antimicrobial treatment. The absence of a reliable diagnostic criterion in assessing biofilm-associated infections impacts effective treatment, which leads to adverse patient outcomes. The MolecuLight *i:X* handheld imaging device detects autofluorescent properties of bacteria in real-time via the exoproduct porphyrin to aid in chronic wound diagnostics. The device has previously demonstrated detection of bacteria both planktonically (free-living) and within biofilm, and there is interest to determine if the bacterial-derived EPS matrix exhibits any unique fluorescence signature that could be indicative of biofilm. To investigate this, a polymicrobial mixture of chronic wound pathogens *Staphylococcus aureus*, *Escherichia coli*, and *Enterobacter cloacae* were evaluated as both planktonic polymicrobial suspensions and biofilms. In a full emission spectral scan, a panel of excitation and emission wavelengths from different bacterial growth environments were evaluated to determine if there was a specific fluorescence peak associated with bacterial EPS. This could inform a specific setting within the MolecuLight *i:X* device for detection of a biofilm within chronic wound infections, allowing clinicians to diagnose not only bacterial infections but also the presence of a biofilm in real-time. The MolecuLight *i:X* imaging device has the potential to alter how biofilm-associated chronic wounds are diagnosed and treated in a clinical setting, which can significantly reduce patient morbidity and mortality.

UG P41

Identification of Novel Virulence Genes in *Bacillus anthracis* using invertebrate models of infection

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Abstract

Bacillus anthracis is the causative agent of anthrax disease, a serious disease that presents itself in the form of skin ulcers and systemic infections with a high mortality rate. This bacterium is dangerous to humans due to its virulence factors that help it infect a host organism. Much research in the field has focused on the importance of the plasmid-encoded anthrax toxins and capsule, both are critical for virulence, however, our research goal is to identify new chromosomal genes that also contribute to virulence in *B. anthracis*. We use the Sterne strain of *B. anthracis* in our research, which lacks the capsule and can be safely used in a biosafety lab. Previous screening of a transposon library identified 11 *B. anthracis* mutants attenuated in *Caenorhabditis elegans*, an invertebrate worm model. To further validate virulence phenotypes and prioritize transposon mutants for follow-up, we used a *Galleria mellonella* invertebrate infection model to assess survival of the 11 transposon mutants originally identified in *C. elegans*. One mutant, TN2, showed virulence attenuation in both models. We know that TN2 has a disruption in a promoter region that we hypothesize controls two genes, a putative BNR repeat domain protein (TN2A) and a glycosyl-like 2 transferase family protein (TN2B). Through the creation of insertional mutants and their comparison to wild-type B. anthracis, we are characterizing the function of the genes above to confirm their importance in virulence. This research will provide insight into bacterial virulence mechanisms and may identify potential novel targets for antibiotics.

UG P42

Investigation of Cyan Fluorescence in Pseudomonas aeruginosa for Point of Care Detection

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Abstract

Chronic wound infections contribute to high rates of mortality and morbidity and are of significant clinical concern. One of the most significant contributors to the pathogenicity of chronic wounds is the bacterium *Pseudomonas aeruginosa*. The handheld bacterial imaging device MolecuLight *i:X* is designed to detect the auto-fluorescent characteristics of most clinically relevant species of bacteria; most clinically relevant species of bacteria exhibit red fluorescence with the *i:X* device due to production of the exoproduct porphyrins. Interestingly, *P. aeruginosa* exhibits a unique, cyan fluorescence signature; clinically, when a chronic wound exhibits cyan fluorescence with the *i:X* device, there is over a 90% positive predictive value that the wound will harbor *P. aeruginosa*. This could allow for the real-time detection and visualization of *P.aeruginosa*, improving treatment outcomes in the clinical setting. This work describes the determination of the source of the unique cyan fluorescent signature exhibited by *P.aeruginosa*, by utilizing genetic knockout mutants to isolate the exoproduct responsible for the cyan phenotype. Investigations regarding fluorescence is needed to understand the detection of these signatures in chronic wounds through utilization of the MolecuLight *i:X* device.

UG P43

Uncovering the Mechanism Behind Metronidazole Degradation in *Enterococcus faecalis*

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Abstract

Enterococcus faecalis are gram-positive bacteria that normally colonize the gastrointestinal tract of humans. *E. faecalis* is also a problematic causative agent of several nosocomial infections, including urinary tract infections, bacteremia, surgical site infections, and endocarditis. *E. faecalis* are intrinsically resistant to metronidazole, a nitroimidazole antibiotic that is used to treat infections caused by anaerobic intestinal bacteria such as *Clostridioides difficile*. Previous literature reported that some enterococcal species can degrade or otherwise inactivate metronidazole, thereby protecting anaerobic bacteria from the antibiotic. The main goal of this study is to underpin the elusive molecular mechanism behind enterococcal degradation of metronidazole. Using a simple and efficient spectrophotometric assay that we developed, we are able to obtain reliable and valid measurements of metronidazole in culture supernatants. The results from our assays indicate that the degradation of metronidazole by *E. faecalis* is dependent upon the concentration of iron in the growth medium. We additionally established that genes encoding various enzymes in the chorismate biosynthesis pathway and in *eetAB* are crucial for metronidazole degradation, as transposon mutations in these genes resulted in a loss of the wild-type phenotype in *E. faecalis*. In current work, we are investigating the roles of extracellular electron transfer mechanisms and nitroreductase enzymes in the *E. faecalis* metronidazole degradation mechanism.

Graduate Poster Presentations

Basic and Pathogenic Microbiology

GR P01

Outer membrane lipoproteins remodel the LOS deficient *Acinetobacter baumannii* cell envelope to provide homeostasis in stress

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Abstract

The LPS/LOS moiety was canonically thought to be essential for Gram-negative survival and has been targeted by antimicrobial therapeutics. *Acinetobacter baumannii* (*Ab*), a critical threat nosocomial pathogen, can shut down LOS biosynthesis (LOS-) to gain resistance to the last-resort antibiotic colistin, and other clinically important antimicrobials. We do not have a comprehensive understanding of how *Ab* survives without LOS to develop colistin resistance but found that specific lipoproteins redecorate LOS- outer membrane (OM). Proteomic analysis of peptidoglycan attached proteins showed two putative outer membrane lipoproteins (denoted as Lpp1 & Lpp2) were covalently attached to peptidoglycan. Notably, both Lpp1 & Lpp2 encode signature C-terminal lysine residues, a motif essential for LD-transpeptidase-dependent covalent attachment of the lipoprotein to the meso-diaminopimelic acid (meso-DAP) residue in peptidoglycan stem peptides. Using transposon mutagenesis, we determined that a putative LD-transpeptidase, LdtK is also essential for LOS- Ab fitness. Covalent tethering of the outer membrane to the peptidoglycan via lipoproteins likely stabilizes the cell envelope when LOS is not produced. Mutational analysis of Lpp1 and Lpp2 illustrates increased outer membrane vesicle production relative to wild type, which supports a model where lipoprotein attachment stabilizes the outer membrane. We have found that Lpp1 is constitutively expressed whereas Lpp2 is only expressed in stasis, suggesting separate roles for the lipoproteins in outer membrane assembly. Together, our studies show that *A. baumannii* encodes two lipoproteins that physically link the outer membrane to the cell wall to increase cell envelope stability in response to outer membrane defects.

GR P02

Development of a SYBR Green-Based RT-qPCR for the Detection and Quantification of Lone Star Virus

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Abstract

Lone Star virus (LSV) is a newly characterized tick-borne bandavirus with pathogenic potential as indicated by infection and cytopathic effect in human and non-human primate cell cultures. However, there are no detection methods available to identify and monitor LSV *in vitro*. Here we describe the development of a SYBR green-based RT-qPCR for the detection and quantification of LSV. Primers were developed for the M segment of the tri-segmented genome and were initially tested for amplicon formation and non-specific binding. Portions of the LSV genome were cloned into a plasmid and propagated in competent *Escherichia coli* to obtain the template for a standard curve. Amplicon formation of the developed primers indicated that a single product was formed of the expected size of 152 base pairs with a consistent melting temperature (Tm) of 82°C. The limit of detection for the assay was fewer than 10 copies/µl of the viral genome. Specificity testing revealed slight cross-reactivity with four related viruses (Heartland virus, La Cross virus, Jamestown virus, Crimean-Congo Hemorrhagic Fever virus); however, the Tm for the related viruses was either below the threshold or dissimilar to the previously indicated Tm for LSV. Standard curve analysis showed the efficiency of the primers was 96.3%-102% with an R2 value of 0.992-0.996 and a slope of 3.276-3.363. For reproducibility analysis, the interassay coefficient of variation (CV) was 0.471%-1.108%, and the intra-assay CV was 0.110%-4.203%. This data suggests the SYBR green-based RT-qPCR assay for the M segment of LSV is highly sensitive, specific, and reproducible.

GR P03

Investigating The Role of ClpATPase Family Members in Regulation of Stress Responses in Bacillus anthracis Sterne

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Abstract

Anthrax is an infectious disease caused by Bacillus anthracis, which is a spore forming bacterium. Even though the anthrax toxins and capsule, encoded on 2 plasmids pXO1 and pXO2, play crucial role in the pathogenesis of anthrax infection, evidence suggests that chromosomal genes also play a role. The ClpX ATPase was discovered to be crucial for B. anthracis virulence via protection against host antimicrobial peptides. In this study, we want to investigate the role of clpX in regulation of other stressors including acidic stress, temperature stress, salt stress, and non-cell envelope active antibiotics. We found that clpX is necessary for survival in an acidic environment and growth under heat stress. We demonstrate that acidic stress resistance is mediated by the formation of the ClpXP protease using a ClpX complementation plasmid that is incapable of interacting with ClpP. There is no association between clpX with other stressors. Additionally, we genetically disrupted another Clp ATPase in B. anthracis, ClpB, to study its role in the regulation of stress responses. We discovered that clpB is necessary for growth under heat stress. Acidic stress, salt stress, antibiotics have no association with clpB. We conclude that the ClpX ATPase is required for B. anthracis pathogenicity via defenses against host antimicrobial peptides and for survival in an acidic environment. Understanding the role of members of the Clp ATPase family in the regulation of stress responses will ultimately infer us with more targets for either directly combating infection or improving the efficacy of already available medicines.

GR P04

A polar network protein undergoes changes in homo-oligomeric topology, and controls both activation and inhibition of cell wall synthesis

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Abstract

Mycobacterial cell elongation occurs at the cell poles; however, it is not clear how cell wall insertion is restricted to the pole and regulated. Wag31 is a pole-localized cytoplasmic protein that is essential for polar growth, but its molecular function has not been described. Wag31 homo-oligomerizes in a network at the poles, but it is not known how the structure of this network affects Wag31 function. In this study we used a protein fragment complementation assay to identify Wag31 residues involved in homo-oligomeric interactions, and found that amino acids all along the length of the protein mediate these interactions. We then used both N-terminal and C-terminal splitGFP fusions to probe Wag31 network topology at different sites in the cell, and found that Wag31 N-terminal-C-terminal interactions predominate at sites of active elongation, but C-terminal-C-terminal interactions predominate at inactive sites. We then dissected Wag31's functional roles by phenotyping a series of wag31 alanine mutants; these data show that Wag31 has separate functions in not only new and old pole elongation, but also inhibition of both septation and new pole elongation. This work establishes new functions for Wag31, and indicates that changes in Wag31 homo-oligomeric network topology may contribute to cell wall regulation in mycobacteria.

GR P05

Phylogenetic Analysis of Colorado Tick Fever Virus

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Abstract

The genus Coltivirus, consists of Colorado tick fever virus (CTFV), a double-stranded RNA virus with a genome of twelve segments. CTFV is the second most transmitted arbovirus in the United States and causes a biphasic fever, body aches, and leukopenia. Typically, CTFV is transmitted by a Rocky Mountain tick. Previous research has shown genomic variation between CTFV isolates within the Bitterroot Valley of Montana. More recently, the complete genomes of five CTFV strains (ranging in locations from Colorado, Idaho, Wyoming, and California) showed genetic assortment and a potential new species, California Hare coltivirus (S6-14-03), formally known as CTFV-Ca. Currently, it is unknown if there are recombination events or genomic and sequence variation between the Bitterroot Valley of Montana CTFV partial isolates and the full-length five strains isolates. First, we focused on the complete five whole genomes and conducted sequence analyses. In VP4, we found potential myristoylation by NMT in all strains except S6-14-03, suggesting that S6-14-03's VP4 is not lipid-modified. Furthermore, a guanylyltransferase motif in VP3 was found between residues 272 to 293 for all five strains, suggesting that S6-14-03 encodes the same VP3 as the other sequences. In addition, we focused on segments nine to twelve of the full and partial isolates and conducted a phylogenetic analysis. Bayesian inference was used to construct phylogenies, and the Bayesian topology trees were verified by Maximum Likelihood trees. Collectively, these results further validate the possibility that S6-14-03 is a new species with some homology to CTFV strains.

GR P07

In *Mycobacterium abscessus*, the Stringent Factor Rel Regulates Metabolism but Is Not the Only (p)ppGpp Synthase

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Abstract

The stringent response is a broadly conserved stress response system that exhibits functional variability across bacterial clades. Here, we characterize the role of the stringent factor Rel in the nontuberculous mycobacterial pathogen, Mycobacterium abscessus (Mab). We found that deletion of rel does not ablate (p)ppGpp synthesis and that rel does not provide a survival advantage in several stress conditions or in antibiotic treatment. Transcriptional data show that RelMab is involved in regulating expression of anabolism and growth genes in the stationary phase. However, it does not activate transcription of stress response or antibiotic resistance genes and actually represses transcription of many antibiotic resistance genes. This work shows that there is an unannotated (p)ppGpp synthetase in Mab

GR P08

Carbapenem tolerance in *Acinetobacter baumannii* is largely dependent on peptidoglycan recycling

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Abstract

The Gram-negative cell envelope works as a critical barrier and protects the cells from external environment. Typically, cell wall acting antibiotics, ß-lactam impedes cell envelope essentiality by inhibiting peptidoglycan synthesis, resulting rapid cell lysis. However, several Gram-negative pathogens exhibit ß-lactam tolerance (ability of the bacterial populations to survive for an extended period of time in the presence of bactericidal drugs) by forming a cell wall deficient like structure called spheroplast & involved in treatment failure. Despite, tolerance is considered as a steppingstone in acquisition of true resistance, the molecular factors that promote intrinsic tolerance are poorly understood.

Acinetobacter baumannii (Ab) is a critical-threat nosocomial pathogen, rapidly develop resistance to conventional antibiotics. Carbpenem antibiotics (i.e., meropenem) are first-line prescriptions to treat *Ab* infections. Since, tolerance is a prerequisite for true resistance, we reasoned factors promoting carbapenem tolerance may be widespread among susceptible *Ab*. Therefore, identifying molecular factors involved in tolerance may provide fundamental insight into how resistance rapidly spread among populations & provide new anti-microbial therapeutics.

Based on transcriptome and transposon sequencing, we characterized several novel factors associated with OM integrity maintenance genes, periplasmic & cytoplasmic cell wall acting enzymes & several efflux genes promotes bacterial survivability. Moreover, peptidoglycan recycling genes play a major role to the barrier, disruption of cytoplasmic peptidoglycan maintenance enzymes compromise OM integrity. Furthermore, we defined the enzymatic activities of *PBP7* (encoded by *pbpG*) and *LdtK*, which are tolerance determinants in *Ab*.

Lastly, this study suggest that outer membrane integrity and peptidoglycan recycling working together and promotes meropenem tolerance in *Ab*.

GR P09

Colorado Tick Fever Virus Mediated Apoptosis in Human Endothelial Cells

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Abstract

Colorado tick fever virus (CTFV), the causative agent of Colorado tick fever (CTF), is a member of the Family Reoviridae and genus Coltivirus. Symptoms of CTF include sudden biphasic fever, headache, myalgia, and petechial rash. Severe forms of the disease can include meningoencephalitis, hemorrhagic fever, and death in children. However, the mechanisms underlying CTFV induced pathology remain unknown. Our previous work indicated that CTFV induces apoptosis in HMEC-1 cells. To gain a better understanding of CTFV-induced apoptosis, we investigated the mechanisms of apoptosis initiation in HMECs during CTFV infection. We first analyzed the expression of key death receptors and ligands during CTFV infection in HMECs by qPCR and observed significant increases in gene expression for TRAIL and its receptors, DR4 and DR5, at 24 hours post infection (hpi). We then analyzed the protein expression of TRAIL in infected cells by western blot and observed a significant increase of protein expression at 24 hpi. We also analyzed the protein expression of BID and observed a decrease in full length BID at 24 hpi, indicating BID activation. This data suggests that the extrinsic pathway is activated during CTFV infection in HMEC-1 cells. Next, we analyzed the protein expression of caspase-9 by western blot and observed a decrease in full length caspase-9 at 48 hpi, indicating caspase-9 activation. Overall, our data suggests that both pathways are activated to initiate apoptosis during CTFV infection in HMEC-1 cells. Further studies will examine if inhibition of these pathways will reduce CTFV-induced apoptosis in HMEC-1 cells.

GR P10

Assessing a continuous-flow culture model for vaginal microbial communities

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Abstract

In continuous-flow microbial cultivation, mechanical pumps continuously introduce fresh media into the growth culture and remove used media. This technique allows us to study dynamic behaviors of microbial communities in vitro and has led to novel discoveries in the world of gut microbiota. Such a model for cultivation of vaginal microbiota has never been published. To fill this gap, we assessed a high-throughput, small volume continuous flow culture system termed Mini-Bioreactor Arrays (MBRAs) for the cultivation of vaginal microbes. The original MBRA protocol is designed for gut microbiota, therefore we optimized some MBRA system parameters for the vaginal microbiota. The gut is often rich in nutrients, whereas the vagina contains a less complex and less abundant array of nutrients, so we developed Simulated Vaginal Fluid growth medium to mimic the nutrient profile in the vaginal lumen. Vaginal fluid secretion is slower than gut transit time, so we reduced the flow rate of growth media. The vagina has more oxygen than the colon, so we changed conditions from anaerobic (50ppm) to micro-oxic (5%). On each day of cultivation, we calculated the number of colony-forming units (CFU) and measured pH as a marker of community health. Communities achieved stable growth by Day 3, as well as stable pH within the normal acidic physiological range. Overall, the results suggest that MBRAs are a feasible and promising model for the in vitro cultivation of vaginal microbiota. Using MBRAs will allow us to study microbe-microbe and microbe-environment interactions efficiently on a long-term time scale.

Environmental Microbiology

GR P14

Comparing the microbiomes of a native and invasive Texas grass to determine potential biocontrol methods

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Abstract

Microbiomes constitute the collection of microorganisms inside of every living being that assist in survival and reproduction. The microbiomes of plants are less well understood compared to those of animals. This project sought to identify the specific compositions of the microbiomes of two grass species: little bluestem (LBS; *Schizachyrium scoparium*), a common high-value Texas native bunchgrass, and King Ranch bluestem (KR; *Bothriochloa ischaemum* var. *ischaemum*), a virulently invasive Eurasian bunchgrass that threatens native prairie ecosystems across Texas. During the summer of 2021, the student collected tissue samples from both grass species, as well as composite samples of the soil surrounding their roots, from sites across Texas where the two species coexist together. Those samples were added to previous collections. The student isolated and sequenced the 16S and ITS regions of the microbial DNA present to identify which bacteria and fungi, respectively, make up each plant species' microbiomes and soil microbiomes. The student will use QIIME 2.0 and other bioinformatics software to analyze the sequencing data to investigate the microbial ecology associated with successful or unsuccessful competitive relationships between LBS and KR. Understanding the microbiomes of these two grass species and how they shift based on the host plant's proximity to the other species could help us to understand how KR invades native prairie ecosystems so readily, and it could lead to a natural remediation strategy that inhibits invasive plant growth with selected microbial inoculants.

GR P15

Application of Matrix-Assisted Laser Desorption/Ionization Time-of-Flight Mass Spectrometry (MALDI-TOF MS) for Tracking *Escherichia coli* from Sewage and Septic tanks

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Abstract

The frequency of extreme flooding events, driven by tropical cyclones and sea-level rise, may increase dramatically this century. Flooding can spread untreated sewage from wastewater treatment plants and onsite sewage treatments (septic tanks), which creates the possibility of outbreaks of water-borne diseases associated with fecal contamination. To assess the risk of disease spread, managers need tools to track the source of this contamination. *Escherichia coli*(E.coli) is widely used to as fecal indicative bacteria (FIB); however, microbial source tracking tools used to identify the source of E. coli are time-consuming and expensive. Matrix-Assisted Laser Desorption/Ionization Time-of-Flight Mass Spectrometry (MALDI-TOF MS) is a time- and cost-effective way to identify bacteria. This mass spectrometry method can distinguish strains of bacterial species but has not yet been widely used for microbial source tracking. In this study, E. coli strains were isolated from wastewater treatment plant and a sample composited from septic tanks. For comparison, a library of isolates was also generated from seal scat and dog feces. Isolates were then identified by MALDI-TOF, and cluster analysis was performed of mass spectra to determine if MALDI-TOF could differentiate the sources of these FIB. Clustering patterns were compared to phylogenetic classification determined by whole genome sequencing. MALDI-TOF distinguished E. coli isolated from sewage and septic tanks. Sewage isolates were also clustered separately from bacteria isolated from animal sources. This suggests that MALDI-TOF MS can be applied to track the sources of fecal contamination of floodwater. This could improve risk assessment and point to mitigation strategies.

GR P16

Monitoring Surface Cleanliness at a Dental School Simulation Lab. A Pilot Study.

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Abstract

Clinical contact surfaces can be directly contaminated from patient materials either by direct spray, spatter generated during dental procedures, or contact with gloved hands of dental health care personnel. These surfaces can subsequently contaminate other instruments, devices, hands, and gloves. We aimed to evaluate the contamination of surfaces at a Texas Tech University Dental Clinic as well as the effectiveness of sanitization methods routinely used. Three surfaces, i.e. computer keyboard, operatory light handle, and lab door handle were swabbed and then cultured in brain-heart-infusion, and blood agar plates. Cultures were inspected every 24 hours for 72 hours. Appearance of colonies was evaluated and a Gram stain was performed. The majority of contamination observed was due to Gram positive cocci. A noticeable decrease in the number of colony forming units was observed after wiping the surfaces with disinfecting towelettes. Our results support the use of surface disinfectant in high-touch surfaces and instrumentation to decrease bacterial contamination in a dental clinical setting.

GR P17

Identification and Characterization of Triclosan Tolerant Onion Rhizobacteria Isolated from South Texas Commercial Onion Farms

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Abstract

Bioremediation using native degraders can circumvent crop accumulation of antimicrobials in contaminated systems (via biosolids or recycled water irrigation). In South Texas, the Rio Grande serves as a primary source of irrigation with levels of triclosan higher than the national median. This research aims to develop pre-harvest interventions using triclosan-degrading plant growth-promoting rhizobacteria isolated from south Texas commercial onion farms (irrigated indirectly with recycled water) and assess the potential for transfer of resistance to the native community via horizontal gene transfer. In total, 75 morphologically unique triclosan-tolerant onion rhizobacteria (1,500 µg L^{-1}) were isolated and screened initially for biodegradation potential by assessing triclosan utilization using a minimal salts medium containing 2 g L^{-1} of triclosan (MSMT). Plant growth activities such as iron siderophore production (iron-deficient chromazurol S plates) and phosphate solubilization (Pikovskaya medium with 0.38% calcium phosphate tribasic) were assessed. Nineteen isolates tested positive for all three assays of which nine unique bacterial isolates were identified as *Pseudomonas spp*. via 16S rRNA gene amplification. Potential for horizontal transfer of resistance with triclosan exposure will be determined using a culture-based assay using three triclosan-degrading PGPRs as donors of mobile genetic elements to a traceable recipient, *E.coli* GFP. To observe changes in baseline recipient resistance, minimum inhibitory assays will be conducted. The effects of triclosan-contaminated irrigation waters on antibiotic resistance are understudied, and the use of biofertilizers for bioremediation is promising; however, inadvertent effects, such as transfer of antibiotic resistance, using inocula requires risk assessment prior to its implementation.

GR P18

Bacterial Community Profiling of *Ixodes scapularis* ticks from Western New York, USA.

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Abstract

The microbial community composition of disease vectors, including ticks, is an area of growing interest due to its ability to transmit a diverse array of human pathogens resulting in Lyme disease, anaplasmosis, and ehrlichiosis. We examined the diversity of bacteria associated with the blacklegged tick (Ixodes scapularis) by sequencing the hypervariable region three (V3) and four (V4) of the bacterial 16S rRNA gene originating from ticks collected from Cattaraugus County, New York across life stages (larvae, nymphs, and adult males and adult females). Sequencing generated 598 ESVs (exact sequence variants) that were assigned to 195 taxa. The microbiome across all life stages was dominated by gram-negative proteobacteria, specifically Rickettsia species closely matching Ixodes Rickettsial endosymbionts. Rickettsial abundance decreased as ticks matured, and adult females had significantly more Rickettsia than adult males (81.3% and 32.8% respectively). We detected Anaplasma species in adults (16.67%) and nymphs (75%) by 16s sequencing and confirmed *A. phagocytophilum* in 62% of nymphs and 14.58% of adults using primers for msp2. The findings of our study confirm previous data about the *I. scapularis* microbiome conducted in other geographical regions and provide insight into the microbial diversity and pathogen burden of *I. scapularis* in Western New York that is applicable for a One-health approach for monitoring and prevention of tick-borne disease transmission.

GR P19

Soil Microbiome Responses to Stormwater Infiltration Berms in Urban Land Management

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Abstract

San Antonio, Texas is the seventh largest city in the United States with approximately 1.5 million residents located over the Edwards Aquifer, one of the most significant water resources in Texas. Due to extreme precipitation and drought events reflective of South-Central Texas, and increasing with climate change, land management techniques are being created in green spaces to manage flooding while retaining surface water during dry periods. This project seeks to understand the impact of stormwater infiltration berms on microbial community structure and function within the Edwards Aquifer region. Topsoil (top 10-cm) from 10 2x2m replicate plots within a berm-managed and an unmanaged sub-watershed along Cibolo Creek were collected in March, June and November 2021 and February 2022. Sampling will continue throughout the growing season of 2023. Soil bacterial, archaeal and fungal microbiomes are being targeted with high throughput amplicon sequencing of 16s rRNA and ITS gene regions for identification of microbial taxon responses, microbial biomass carbon (MBC) and total microbial activity as measured by soil dehydrogenase activity to link both microbial structural and functional differences that occur with these important land management practices. These data will inform managers how stormwater berms may impact carbon sequestration potential and the microbial communities which mediate this important ecosystem service.

Post-doctoral and Faculty Poster Presentations

P/F P01

Studies on the Variability of Class B (Metallo) *beta*-Lactamases

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Abstract

The Class B family of b*eta*-lactamases contains over 500 enzymes whose three-dimensional structures have been solved either by X-ray crystallography or nuclear magnetic resonance. We have examined the enzymes structurally related to the NDM-1 as presented in the VAST structure alignment system. For those enzymes with over 200 residues aligned with those of NDM-1, the variability of the sequence identity to NDM-1 ranges from 25% or higher, and the root mean square deviation of the structure is 1.7 Å or lower. Thus, at least 75% of the amino acid residues of NDM-1 can be changed in a single enzyme, without a significant change in the three dimensional structure of the enzyme. This presentation shows the locations and effects for some of this variability.

P/F P02

Microbial contamination and antimicrobial resistance in the Mississippi Gulf Coast

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Abstract

Background: Microbial contamination and spread of antimicrobial resistance is a global emerging public health concern. The Mississippi Gulf Coast serves as a critical resource for recreational, travel, and economic purposes. Understanding the occurrence and distribution of pathogens and drug resistance across the coastal waters will help improve the state's environmental and public health control measures serving as an important dataset to keep the coast clean and sustainable.

Methods: In this study, we collected and analyzed surface ocean water samples from ten sites across the Mississippi Gulf Coast over a period of 10 months to -

1) *Enumerate* pathogenic bacteria using the EPA methods 1603 (*E. coli*) and 1600 (Enterococcus)
2) *Identify* the patterns of antimicrobial drug-resistance using Kirby-Bauer disc diffusion method

Results:

Data collected suggest the prevalence of highly concerning levels of indicator bacteria (both Enterococcus and *E. coli*) in coastal waters within all sites sampled across the Mississippi Gulf Coast. The *E. coli* numbers ranged between 120 – 990 cfu/ 100 ml, mostly falling well beyond the water quality standards set by EPA for recreational waters (126 *E. coli*/ 100 ml). A significantly high resistance to the antibiotics Ampicillin, Tetracycline, Cephalothin and Amoxicillin was also observed. Moreover, many of the isolates were positive for a concerning high number of multidrug resistance (resistant to ≥2, ≥3, ≥4, ≥5, ≥6 antimicrobial agents) on all sites across all sampling events.

Future Directions

- qPCR analysis of antibiotic resistance genes.
- Finding the sources of contamination across the coast – using molecular methods of microbial source tracking