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Abstract Book

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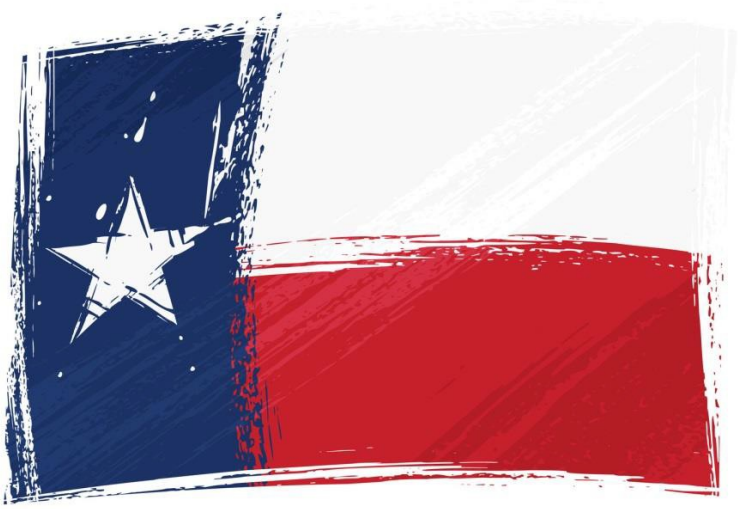

Undergraduate Student Oral Presentations

1. Role of the ClpXP protease in antibiotic resistance in *B. anthracis* and *S. aureus*

Madeline Bush, Kevin Claunch, Jacob Malmquist, and Shauna McGillivray
Texas Christian University

ClpX is a regulatory ATPase that functions along with ClpP as part of the intracellular bacterial ClpXP protease. Previous research from our group has shown that genetic loss of ClpX (ΔClpX) in *Bacillus anthracis* Sterne increases susceptibility to antimicrobial agents that target or interact with the cell wall including penicillin, daptomycin, and LL-37. In order to gain a better understanding of ClpX function in *B. anthracis* Sterne, a microarray analysis comparing WT and ΔClpX gene expression was performed in *B. anthracis*. We found that LrgAB, a negative regulator of autolysis, was significantly downregulated in the ΔClpX mutant and this finding was confirmed with QPCR. In order to determine whether LrgAB also had a role in antibiotic resistance in B. anthracis, we made a genetic deletion of LrgAB (ΔLrgAB) and found it has similar phenotypes to ΔClpX in *B. anthracis*. To see if these findings were consistent in other gram positive pathogens, we expanded our research to *Staphylococcus aureus*, the leading cause of skin and soft tissue infections. We constructed a ΔClpX mutant in the Newman strain of *S. aureus* and found it also exhibited sensitivity to cell wall active antimicrobial agents. Loss of ClpX in S. aureus also resulted in decreased expression of LrgAB by QPCR. Lastly, we examined a S. aureus ΔLrgAB mutant and observed an increase in antibiotic susceptibility. We conclude that ClpX plays a role in resistance to cell wall active antimicrobials in both *B. anthracis* and *S. aureus*, and that this is connected to its regulation of LrgAB.

2. Identification of Plant Extracts with Antimicrobial Activity

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St. Edward's University

Bacterial resistance to antibiotics has become an issue of paramount importance, with over 2 million people infected by resistant bacteria annually in the United States. Resistant bacteria are an increasing threat. Therefore, identifying and isolating new antibacterial agents is crucial to effectively combat resistant strains. The purpose of this research project is to discover and characterize new antimicrobial agents in plant extracts. Plant extracts from the National Cancer Institute were tested for bacterial inhibition by Kirby-Bauer disk diffusion using either *C. violaceum* or *S. aureus* and paper disks impregnated with plant extracts suspended in ethanol. Initially, the extracts were tested for inhibition, and the zones of inhibition ranged from 7.5 mm to 14 mm. The minimum inhibitory concentration (MIC) was measured for *E. coli lptD4213* and ranged from 0.625 mg/ml to 3.125 mg/ml. Out of hundreds of extracts tested, 43 showed activity in Kirby-Bauer tests. This project shows the various MICs measured in 4 plant extracts which are currently not used for their antimicrobial properties. The strain *E. coli lptD4213* was used for all MIC testing because of its increased outer membrane permeability that makes it susceptible to many antibiotics. In ongoing experiments, cytological profiling will be used to explore the mode of action of the extracts. This involves treating bacteria with known antibiotics or plant extracts, fluorescently staining these to view cell structures, and observing differences in morphology. The mechanism of action (MOA) will be determined based on the morphological similarity between cells treated with antibiotics of known MOA and cells treated with plant extracts. Plant extracts are a potential source of novel antimicrobial agents, and identifying these may lead to new and effective antibiotic treatments that can kill resistant bacteria.

3. Investigating the Functional Role of a Large tRNA Cassette in Novel Cluster M Mycobacteriophage 'Nanosmite'

Reavelyn M. Pray, J. Robert Hatherill, and Daisy Zhang.
Del Mar College

While ancient and genetically diverse, phages are poorly understood overall. There are over 7000 sequenced Actinobacteriophage; 1,129 of which are known to infect Mycobacterium spp, bacteria which include the causes of tuberculosis and leprosy. Mycobacteriophages are separated into clusters based on

their sequence homology and then further segregated into sub-clusters. Mycobacteriophage 'Nanosmite' is a rare Cluster M phage that was isolated from a public system waterway using standard procedures in 2015. 'Nanosmite' genome was sequenced and annotated using bioinformatics programs such as DNA Master, Phamerator, Blast, and HHPred. Cluster M phages are known for their remarkable collections of tRNA isotypes and their temperate life cycle. Like other cluster M phages investigated so far, 'Nanosmite's' genome also has a large repertoire of tRNAs. We have found that the host bacteria and cluster M phage share 17 identical codons. In lab, 'Nanosmite' lysogens were isolated and purified from mc1552 and the growth curves were developed on positive control while type *M. smegmatis* and lysogen cultures to compare effect of the prophage on host growth. These large tRNA casettes in phages may lead to a phage host benefit during lysogenic cycle by offering additional machinery held by the infecting temperate phage. By investigating the expression of tRNAs during the lysogenic cycle, as well as other novel genomic aspects of cluster M phages, we gain greater understanding of phage-host relationships. In addition, understanding molecular mechanisms in these phages may lead to novel biological approaches to medical treatment and biotechnological advancement.

Graduate Student Oral Presentations

4. Seasonal differences in microbial succession during decomposition in southeast Texas

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The nascent effort to use metagenomics to research the microbes associated with human decay, the thanatomicrobiome, is mired by the limited availability of reference data. Trends of thanatomicrobiome succession have been observed across soil type, season, and host species – reflecting the gross physical changes in decaying tissues. We do not yet understand the influence of environmental variables on the trajectory of postmortem microbial succession, nor the spatial and temporal scales at which such causal relationships should be observed. In this work, microbial samples were collected (swabbed) from 18 defined facial regions of two cadavers placed in tandem at the Southeast Texas Applied Forensic Science (STAFS) Facility at the Center for Biological Field Studies at Sam Houston State University during Winter of 2013. Here, we expand a comparable dataset derived from two similar cadavers placed during Summer of 2012. Amplicon sequencing of the 16S rRNA genes within these samples yields a crude illustration of succession within face-associated communities during decomposition. This study benefits from the growing informatics toolbox designed to enhance to utility of 16S rRNA data. Phylogenetic Investigation of Communities by Reconstruction of Unobserved States (PiCRUST), a program that predicts the abundance of functional genes groups (Kegg Ortholog, or KO groups), within a given sample based upon the 16S rRNA data. Comparing sample diversity via unweighted unifrac, the pooled data cluster by season. Consistent with trends discussed in existing literature, human-associated bacteria appear to give rise to those associated with insects and soil. Samples from the Winter dataset, characterized here for the first time, saw an initially dominant *Firmicutes* give way to *Proteobacteria* and *Actinobacteria* as time progressed. PICRUSt identified similar abundances of KO groups across season. Considering the functional gene abundance profiles of each sample enables us to explore trends of succession that may not be apparent from a taxonomic perspective. Though this work is exploratory in nature, these community data may provide insight for future investigations – a step toward understanding the dynamics of thanatomicrobiome succession.

5. Genetic analysis of Metacaspase Type I during heat-stress induced PCD in *Chlamydomonas reinhardtii*

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Sam Houston State University

Programmed cell death (PCD) refers to the array of genetically programmed physiological processes by which a cell ceases to exist. PCD is highly conserved across living organisms, and canonically, is regulated by members of the cysteine protease (CP) family. Initially, the CP family consisted only of caspases, however, the recent discovery of metacaspases and paracaspases has significantly impacted

our understanding of PCD evolution. Metacaspases are subdivided into types I (MCA1) and II (MCA2), and are noted to be present in organisms whose PCD machinery is poorly understood, such as the unicellular green alga, *Chlamydomonas reinhardtii*. Using a genetic approach, this study sought to expand our understanding of metacaspases via analysis of an *MCA1* mutant strain of *C. reinhardtii*. We noted that in response to heat-stress-induced PCD, MCA1 absence is associated with increased disruption of cell membrane integrity, and DNA fragmentation. Furthermore, upon the onset of heat-stress, *MCA1* mutant populations were also linked to a significantly earlier display of ROS accumulation, and in the event of prolonged heat-stress exposure, MCA1 absence was also associated with a significantly increased display of ROS accumulation. Notably, absence of MCA1 did not alter rates of phosphatidylserine (PS) externalization, or cell viability, thus suggesting potential limitations to its influence in the *C. reinhardtii* response to PCD. Together, our data suggest the potential that MCA1 modulates the early cellular response to heat-stress-induced PCD in *C. reinhardtii*.

6. A genomic analysis of the white pox disease associated *Serratia marcescens*

Nicole Elledge*[1]; Lee Pinnell[1]; Ron Eytan[2]; Jeffrey Turner[1]
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Serratia marcescens has been identified as an etiological agent associated with white pox disease in the elkhorn coral, *Acropora palmata*. While this enteric bacterium has been associated with a variety of hosts including plants and animals, it can also be found in environmental ecosystems and human wastewater. If wastewater is not treated properly, *S. marcescens* can be introduced to aquatic environments, where it can contaminate coral populations. A white pox outbreak occurred in the early 2000s in the Florida Keys, resulting in the devastation of the local *A. palmata* community. Several isolates of *S. marcescens* were collected from the surface mucous layer of healthy and diseased corals during and after this outbreak. Based on pulsed-field gel electrophoresis (PFGE) patterns and differential production of the red pigment prodigiosin, fortyone of these isolates were selected for whole-genome sequencing. These genome sequences were assembled and are being analyzed to correlate genetic variation with virulent and avirulent phenotypes. Whole-genome phylogenetic analysis and average nucleotide identity calculations indicate that the majority of these isolates (N = 37) are clonal despite the temporal and spatial differences during sample collection, although intra-clonal genetic variation still persists. We will continue analyzing the results of this genomic analysis by comparing single nucleotide polymorphisms (SNPs) between the isolates to look for patterns in gene change, gene loss, and gene gain associated with the virulent phenotypes. The results of this study will provide us with a better understanding of the virulence that accompanies white pox associated *S. marcescens* infections.

7. Production of antimicrobial compounds in marine sediments

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1 Texas A&M University - Corpus Christi
2 University of Texas Marine Science Institute
3 University of Southern California

The marine deep subsurface sediment, isolated for millions of years, is an ideal location to understand how microbial populations may coexist under resource-limited conditions. Deeply buried sediment contains one of the largest biomes on the planet, with an estimated 1.3 X 1029 *Bacteria* and *Archaea* cells, but the mechanisms for competition and, therefore survival, in this extreme environment is poorly understood. Laboratory experiments have shown that microorganisms produce antimicrobial compounds to compete for limited available nutrients and carbon. Polyketide synthases (PKS) are a multi-enzyme complex that produces polyketides, which are secondary metabolites that can have antimicrobial properties. The PKS gene is composed of three essential domains including ketosynthase, acyltransferase, and acyl carrier protein, which are necessary in producing antimicrobial compounds. This study aims to evaluate the presence and diversity of genes capable of producing antimicrobials in the marine deep subsurface biosphere, contrasted with shallow marine sediments. Metagenomes extracted from sediment collected from Guaymas Basin (Gulf of California) showed that the ketosynthase domain was found among several taxa including *Bacteroidetes*, *Chloroflexi*, *Proteobacteria*, and Latescibacteria (WS3). Additionally, metatranscriptomes from Baltic Sea Basin sediment cores collected during Integrated Ocean Drilling Program (IODP) Expedition 347 contains expressed genes (e.g., transcripts) such as *accA*

and *accD* (tetracycline biosynthesis) and *rmlA*, *rmlB*, *rmlC*, and *rmlD* (polyketide sugar unit biosynthesis). Further investigation of these sites as well as metatranscriptomes planned from future sites, including the Mariana Forearc and along the Texas coast, will provide more insight to the ubiquity of antimicrobial compounds. Analyzing antimicrobial genes and transcripts can aid in determining the nature of microbial consortia living in deep subsurface and the evolution of antimicrobial genes throughout Earth's history.

8. A tale of two nurdles: plastic-microbe interactions in Texas' coastal waters

Lee J. Pinnell & Jeffrey W. Turner
Department of Life Sciences, Texas A&M University – Corpus Christi

In coastal areas plastic can represent up to 95% of anthropogenic debris, yet our understanding of how microbes interact with it is limited. Broadly speaking, there are two types: petroleum-based plastics, and bioplastics. While petroleum-based plastics such as polyethylene terephthalate (PET) are largely resistant to biodegradation, bioplastics like polyhydroxyalkanoate (PHA) are designed to rapidly biodegrade. With a greater public awareness about plastic pollution and fluctuating oil prices, the demand for bioplastics has increased recently. However, they still only represent 4% of all plastic production due to higher manufacturing costs. Here, we investigate which microbes are colonizing petroleum-based (PET) and a bioplastic (PHA) in Texas' coastal waters, and determine whether there is a difference in biodegradation rates between the two. We deployed PET and PHA nurdles, along with ceramic pellets as a biofilm formation control, within 315µm Nitex mesh in the Upper Laguna Madre, TX on October 18, 2016. Subsamples are being collected at 4-week intervals over a 60-week period. For each subsample DNA was isolated from the biofilms fouling the nurdles, prepared using an Illumina Nextera preparation kit, and sequenced using paired-end (2x151bp) chemistry with an Illumina HiSeq 2500. Identification of 16S rRNA gene sequences was performed with rRNASelector, and taxonomic analyses were conducted using QIIME and MEGAN6. Following the digestion of organic matter fouling the pellets, biodegradation rates were calculated by comparing the pre- and postexposure mass. Scanning electron microscopy (SEM) was used to visualize both the microorganisms forming biofilms, and any signs of degradation. Preliminary results show that after 142 days' exposure PHA samples have decreased by almost 400mg, representing a drop of approximately 13% from the pre-exposure mass. In contrast, the masses of both PET and ceramic samples have not changed. Visual analysis with SEM supports this disparity in biodegradation. Taxonomic analyses have revealed unique bacterial communities associated with PHA versus PET and ceramic. Ongoing research is currently focused on identifying which algal species are present in the plastic-associated biofilms, and determining which microbial species and genes are responsible for biodegradation.

9. A Novel Biaryl Amide Compound with Inhibitory Activity against *Candida albicans* Filamentation and Biofilm Formation as an Anti-virulence Agent for the Treatment of Candidiasis.

Jesus A. Romo[1], Christopher G. Pierce[1], Ashok K. Chaturvedi[1], Anna Lazzell[1], Stephen P. Saville[1], and Jose L. Lopez-Ribot[1]

Candida albicans is the most common fungal pathogen and candidiasis represents the third most frequent nosocomial infection in the U.S. Targeting functions essential for virulence constitutes an attractive, yet clinically unexploited alternative for the development of new antifungal agents. The yeast-to-hyphae transition and biofilm formation, two virulence factors vital to *C. albicans* pathogenicity, have not yet been exploited as potential drug targets. We have performed high content screenings in search for inhibitors of *C. albicans* filamentation and biofilm formation. A total of 30,000 small molecule compounds from Chembridge's DIVERSet™ chemical library were evaluated for their inhibitory activity using two different 96-well microtiter plate-based methods for filamentation and biofilm formation. A battery of *in vitro* and *in vivo* tests was used to further characterize some of the leading compounds identified. Results from the screenings identified several hit compounds with inhibitory activity against filamentation, biofilm formation, or both. Of these, a series of compounds with a common biaryl amide core demonstrated potent inhibitory activity against both filamentation and biofilm formation at low micromolar concentrations. The leading compound of this series, N-[3-(allyloxy)-phenyl]-4-methoxybenzamide, was able to prevent filamentation under all liquid and solid media conditions tested, suggesting that it impacts a common, core component of the cellular machinery mediating hypha formation under different environmental conditions. It demonstrated *in vivo* activity in three different clinically-relevant murine

models of invasive candidiasis, oral candidiasis and catheter-related candidemia. This compound also displayed activity against a panel of azole resistant clinical isolates. Furthermore, it showed a safe profile based on CEREP Safety Panel and CC_{50} (toxicity) studies, underscoring its excellent "drug-like" characteristics. RNA-Sequencing data revealed differential expression of key filamentation genes. Current efforts are aimed at identifying its target at the molecular level. We have also embarked in a medicinal chemistry campaign to identify analogues with improved pharmacodynamic and pharmacokinetic properties. Based on its *in vitro* and *in vivo* activities, this leading compound represents a promising candidate for the development of novel anti-virulence strategies against *C. albicans* infections

10. Growth Characteristics of Two Newly Discovered Fungi from the Oligotrophic Marine Deep Subsurface

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Texas A&M University – Corpus Christi, Department of Life Sciences
Japan Agency for Marine-Earth Science and Technology – Kochi Core Center

Microbial communities thriving in marine deep subsurface sediments are known to be extremely diverse and contribute to global biogeochemical cycles. These marine sediments are poorly understood, which provides the potential to discover novel organisms. Only recently have fungi been discovered in marine sediments, living alongside prokaryotic communities. It is hypothesized that these fungal communities contribute to the deep subsurface carbon cycle by degrading recalcitrant carbon and remineralizing it to labile carbon. Few studies have been able to culture and characterize marine subsurface fungi until now. This study sampled sediment from whole round cores collected during the Integrated Ocean Drilling Program Expedition 329 to the South Pacific Gyre on board the D/V JOIDES Resolution in November-December 2010. Fungal isolates from 12 and 124 meters below seafloor were found to be most closely related to *Penicillium chrysogenum* and *Penicillium brevicompactum*, respectively. These findings have been independently verified through *in situ* 18S rRNA gene sequencing from the sediment. This study aims to fully characterize both isolates by sequencing the entire genome of both species to understand their metabolic potential. *P. chrysogenum* grew faster than *P. brevicompactum* under oxic and anoxic conditions at 5 ℃ as well as oxically at 26 ℃. Growth will also be assessed in different salinities, nitrate, sulfate and hydrocarbon sources. This will allow us to further analyze their role in global biogeochemical cycles and understand how these organisms have adapted to extreme environments.

Eugene and Millicent Goldschmidt Graduate Student Award

Streptococus mitis and *Streptococcus oralis* mutate an 'essential' gene upon exposure to daptomycin

Hannah Adams[1], Luke Joyce[1], Ziqiang Guan[2], Ronda Akins[1,3], and Kelli L. Palmer[1]
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Viridans Group Streptococci (VGS) are Gram-positive opportunistic pathogens which are part of the normal gastrointestinal and oropharyngeal flora. Clinically, VGS are causative agents of infective endocarditis and bacteremia in immuno-suppressed and neutropenic patients. Multidrug resistance (MDR) is on the rise within the VGS, as many isolates are now resistant to ampicillin, fluoroquinolones and macrolides. As with most pathogens, novel treatment methods are needed to combat VGS infections. Daptomycin (DAP) is a lipopeptide antibiotic with bactericidal activity against Gram-positive bacteria. DAP has been shown to be effective against MDR infections, including methicillin-resistant *Staphylococcus aureus* (MRSA) and vancomycin-resistant enterococci (VRE). Previously, Akins et al obtained DAP-resistant VGS strains after overnight exposure to DAP in a simulated endocardial vegetation model. Using two *Streptococcus oralis* strains (1647 and 1648) and one *Streptococcus mitis* strain (1643), we demonstrated that DAP resistance up to 256 µg/mL could be obtained overnight in standard laboratory conditions with a single 4 µg/mL DAP exposure. Whole genome sequencing identified loss-of-function mutations in the gene *cdsA* for all three DAP non-susceptible VGS strains. These mutations reverted when the resistant strains were serially passaged until susceptibility was observed. *cdsA* is essential in better studied streptococci such as *S. pneumoniae* and *S. pyogenes*. CdsA is responsible for catalyzing the conversion of phosphatidic acid (PA) into cytidine diphosphate-diacylglycerol (CDP-DAG), which is a key intermediate in the biosynthesis of major phospholipids found in bacterial membranes, including phosphatidylglycerol (PG) and cardiolipin (CL). Using liquid chromatography/mass spectrometry (LC/MS), we confirmed CdsA loss-of-function mutations in each of the DAP-resistant strains, as evidenced by the absence of PG and CL, and the accumulation of PA in these strains. In addition, we have identified a new glycolipid in our *S. mitis* strain. Revertant strains possess PG levels similar to those of the susceptible parental strains. Interestingly, one *S. oralis* revertant did not possess restored levels of CL, indicating that DAP does not require the presence of CL in order to carry out its bactericidal activity. Here we show that DAP resistance proceeds through a novel adaptive pathway in *S. mitis* and *S. oralis* as compared to MRSA and VRE; that *cdsA* is non-essential in these VGS and that adaptation to DAP results in altered membrane lipid composition in these organisms. These data combined point to *S. mitis* and *S. oralis* possessing unique physiological characteristics that allow them to adapt in ways not previously seen in better studied organisms.

Undergraduate Student Posters

U1. Occurrence of Antibiotic Resistant Bacteria in Soil Samples Near the Gulf of Mexico in Port Aransas, Texas

Tara L. Clancy, Erica L. Duncan, John F. Ramirez, Alexa Raney, and J. Robert Hatherill

The Prevalence of Antibiotic Resistance in the Environment (PARE) project allows for a “crowdsourcing" approach to study antibiotic resistance in the environment. Soil was collected from the Animal Rehabilitation Keep (ARK), where injured and sick sea turtles are given antibiotics to help improve their condition prior to being released back into the Gulf of Mexico. The PARE project’s focus is centered on tetracycline resistance, which has not been given to the turtles at the ARK for approximately five years. Following PARE protocol, the soil sample was diluted and plated on MacConkey agar and tetracycline (Tet 3 µg per mL/Tet 30 µg per mL) plates. The plates were incubated for 96 hours at 28°C and demonstrated 815,000 colony forming units (CFU) per gram of soil on the MacConkey plates. Tet 3 showed 40,500 CFU per gram of soil, while Tet 30 showed 100,000 CFU per gram of soil. Future studies include investigating the environment for resistant bacteria by testing the current regimen of antibiotics given to the turtles (enrofloxacin/ceftazidime). The current study shows bacterial isolates that are resistance to tetracycline, which could result from lateral gene transfer from commensal bacteria. While sea turtles are known carriers of antibiotic resistant genes, this study shows the effect of encountering multiple antibiotics in their environment, along with the prevalence of antibiotic resistance within a coastal community.

U2. Apollo 16 Micro-fungi Isolates: Viability and phenotypic examination of lyophilized cell lines *Sacchromyces cervisiae* and *Rhodoturla rubra*, exposed to space flight conditions 40 years ago.

Aaron Cristan, Rea Pray, Alexa Raney and J. Robert Hatherill.

In 1972, Apollo 16 spaceship took a stock of yeast cells that were medically significant into deep-space. The experiment called “Microbial Ecology Evaluating Devise” (MEED), coordinated by the National Aeronautics and Space Administration (NASA), placed several species of fungi in an apparatus to accumulate exposure scenarios. Amongst those species selected, *Rhodotorula rubra* and *Saccharomyces cervisiae* had exposure to cosmic-rays known to be present in deep-space. Once the isolates returned to Earth, continuous cultures of spaceflight yeast were lyophilized for studying viability and phenotypic changes due to the exposure of deep-space conditions. Among those yeast cells, we obtained forty-year-old freeze dried cell-line samples *S. cervisiae* (Sc-6997-1) and *R. rubra* (Rr-6861-1). We successfully re-cultured both strains for viability analysis and phenotypic observation. We hypothesized that after forty years of being inactive; we can still detect the phenotypic change due to the space exposure in both strains compared to non-exposed controls. A greater understanding of the effects of outer-space environment on microbes is needed because of an ongoing space-race mission to Mars and advancements in space exploration. The effects of deep-space exposure on microbial yeast can help us understand light- radiation on cellular organism’s genomic DNA.

U3. Bacterial Composition During Human Cadaver Decomposition in Southeast Texas

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The microbiome of a decomposing human body is diverse and continuously changing throughout decomposition. By sampling the core microbiome of decomposing bodies continuously over a period of time, patterns of bacterial compositions can be established. These patterns can be combined to create models that represent the presence of certain bacteria during each stage of decomposition- fresh, bloat, purge, active, advanced, and dry decay. Over three years, 24 donated human bodies, two bodies in every season, were all placed unclothed and in standardized positions outside in a fenced area. They were consistently swabbed at the same sites at the same time every day for one month, every other day for another month, then in decreasing amounts as decomposition slowed. The operational taxonomic units (OTUs), or the genetic sequence that can be used to categorize a bacterium, were taxonomically identified typically down to the genus level for each bacterium found on the bodies. A model

demonstrating the fraction of samples that a particular OTU was observed in the core microbiome was generated. It was found that as decomposition progressed, the number of different OTUs (and thus different bacteria) present dramatically decreased. This data was also separated by season since the temperature affects the rate of decomposition. This generated model of bacterial composition could possibly be used as evidence in homicides that occur in similar environments. This can be accomplished by using the same sampling techniques, then using the models of bacterial colonization to work backwards to estimate time since death.

U4. Diana Desai, Alyssa Wilder, Samantha Studvick, Josh Brokaw, Diana Flanagan
Characterization of the bacterial flora of the Sorcerer's Cave, deepest cave in Texas

Microorganisms are ubiquitous, living in abundance throughout all types of environments. With antibiotic resistance on the rise, bacteria are becoming a common topic of interest for researchers. While their significance in our everyday lives has been noted by many, little studies have been done to identify the interspecific relationships between various bacteria. This research aimed to construct a phylogenetic tree using bacterial specimens obtained from a cave. At 558 feet, Sorcerer's Cave is the deepest in Texas, and this unique and isolated habitat is the source of bacteria in this study. The samples were collected aseptically from the cave wall and inoculated on TSA plates. The samples were kept cold until incubation and subsequently incubated at room temperature until growth appeared. Various methods of DNA extraction and PCR protocol were implemented using the 27f and 1492r primers in order to successfully amplify the DNA. Results were visualized using gel electrophoresis, and the DNA was purified and sent to Yale University for sequencing. Coinciding with previous research and preliminary identification of 16S sequences through comparisons with accessions from Genbank and the Ribosomal Database Project, the specimens were determined to be from the genera *Pseudomonas* and *Bacillus.* This project not only aims to identify and characterize the microbes of Sorcerer's Cave but also serves as a precursor to explore antibiotic resistance.

U5. Characterization of Antibacterial Resistance of Possible Enteric Soil Bacteria Collected in the Coastal Bend of Texas
Breanna N. Edinger, Anastasia R. Guseva, John F. Ramirez, Reavelyn Pray, Alexa Raney, and J. Robert Hatherill

There is an increasing prevalence of bacteria developing antibiotic resistance. Bacteria survive in the presence of antibiotics through methods such as efflux pumps or ribosomal binding. Antibiotic resistance leads to a global health threat. Two soil samples from different locations, a backyard and a local pond, were serially diluted and then plated on tetracycline (Tet) MacConkey agar plates. A pure culture isolate of bacteria was taken from each sample and streaked on 3 and 30 µg/ml Tet plates. EnteroPluri tests and a disk diffusion assay were performed with both isolates to determine if the bacteria are enteric and if they possessed resistance to a single or multiple antibiotics. The backyard soil sample showed strong resistance to Tet while the pond sample presented a moderate resistance. A strong resistance was exhibited when the growth of bacterial colonies occurred in all three Tet plated dilutions while the bacterial growth covered a majority of the plate. Moderate resistance, in comparison, is when there was growth in at least two of the plated Tet dilutions with a minimal amount of colonies present. A single EnteroPluri test was performed on both samples, but the results obtained were unremarkable and no specific identification was made leading to the possibility that the samples are more complex than originally thought. Future studies include the isolation of pure bacterial colonies and testing for antibiotic sensitivity. We hope to document the presence of antibiotic-resistant bacteria in our community and, furthermore, identify the antibiotic resistant bacteria present in our samples.

U6. Assessing the Emergence of Antibiotic Resistant Bacteria in Corpus Christi: An Undergraduate Crowdsourcing Approach
James T. Gonzalez, John Ramirez, Alexa Raney, and J. Robert. Hatherill

The increasing emergence of antibiotic resistant bacteria (ARB) is effectuating one of the most pressing crises currently being imposed on multiple fields of healthcare worldwide. Both anthropogenic and natural factors contribute to this phenomenon including the inappropriate prescription and overuse of antibiotics

as well as their dissemination in the environment via improper disposal, partial metabolism, and ubiquitous use throughout the agricultural industry. These factors provide selective evolutionary pressures which may exacerbate mechanisms by which bacteria acquire antibiotic resistant genes (ARGs) in nature. Natural mechanisms contributing to ARB include random genetic mutations, which select for resistance and horizontal gene transfer (mediated by mobile genetic elements and bacteriophages). As anthropogenic and natural factors producing ARB become increasingly complimentary, the rate of emerging ARB will continue to escalate. The PARE assessment aims to develop a profile of ARB in the environment surrounding Corpus Christi, TX. During this project, soil samples were collected from various locations potentially harboring ARB throughout the city. These samples were then subjected to serial dilutions prior to being plated on 3 series of plates, each with differing antibiotic concentrations. To attain statistically significant CFU counts, serial dilutions were conducted in triplicates prior to incubation. The CFU counts were compiled for differing experimental conditions and conducting a student's T-test. It is proposed that crowdsourcing the collection of data forming a profile of ARB present in Corpus Christi will be critical in developing an understanding of this emerging threat. Furthermore, we postulate that these data will serve to aid in developing a more detailed understanding of emerging ARB dynamics on a nationwide scale as it is added to the national PARE dataset.

U7. Fresh and Frozen Cadavers and Their Impact on Forensic Science
Hathaway A
Sam Houston State University

Decomposition is a unique and small ecosystem involving bacteria, cadavers themselves, insects, and scavenging animals. If there is a predictable sequence of bacteria seen over time during decomposition this can aid investigators in determining when a victim died. To perform this research many cadavers will need to be studied in the field. It can be difficult to obtain non-frozen cadavers and we wanted to determine if frozen cadavers can be used and have similar results as fresh cadavers. Human cadavers were placed outdoors at the Southeast Texas Applied Forensic Science facility and allowed to decompose naturally, some had previously been frozen and others had not. Samples were taken daily for thirty days and then sequenced. Our research shows that there is no distinction in bacterial sequences in the initial stages of decomposition, but over time there is a difference in fresh and frozen cadavers, particularly in the later stages of decomposition. These results indicate that fresh cadavers are ideal for future studies and for the development of a protocol that would use bacteria to determine the post mortem interval.

U8. Prevalence of Pathogenic Bacterial Isolates in Migratory Birds and Transfer to Local Populations
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Midwestern State University
Like humans, birds serve as hosts for microorganisms. In some cases, these microorganisms can cause diseases in humans. Migratory birds, such as seagulls, can transport these potentially pathogenic microorganisms across long distances and introduce them into new communities by contaminating local water supplies. The introduced pathogens may become a permanent part of these communities if taken up by local waterfowl. The goal of this research project is to determine if the annual appearance of seagulls in Wichita Falls affects the composition of existing bacterial populations in local waterfowl. Fecal samples were collected from seagulls and resident waterfowl at a small lake on the university campus. Selective media were used to enrich for *Escherichia coli* and *Staphylococcus aureus*. Thus far, we have collected samples from 78 birds including Ring-billed Gulls, Mallards, Canada Geese, Double-crested Cormorants and Common Coots. From these samples we obtained seventeen *E.coli* isolates and one *S. aureus* isolate. Susceptibility to four antibiotics (oxacillin, imipenem, cefotaxime, and tetracycline) was determined for each isolate. We identified one *E. coli* isolate resistant to tetracycline. Four other *E. coli* isolates showed intermediate resistance to tetracycline. The single *S. aureus* isolate was susceptible to all antibiotics tested. Our current results suggest that antibiotic resistance is not widespread in these bird populations. Current work is focused on strain typing the *E.coli* isolates by analyzing their protein and DNA profiles. Preliminary results comparing cell lysate proteins suggest that the *E. coli* isolates are not a homogeneous population. The results will show how closely related the strains in the gulls are to the ducks, geese and other resident birds. This work will provide information about bacterial transfer and

antibiotic resistance spread in our local environment.

U9. Identification of a *Vibrio* isolate with crude oil degredation properties
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Bioremediation uses microorganisms to restore a polluted environment to its previous quality after an ecologically unfriendly event occurs. Hydraulic fracturing is used by the oil industry to break loose subterranean pockets of oil by shooting chemicals into the ground at high pressures. This can cause uncontrolled spills to occur and often harms the ecosystem around the well, and additionally creates a high salt environment polluted with crude oil. We seek to restore these ecosystems using microorganisms to degrade the oil present and allow for natural regrowth and population of the land's flora and fauna. Truscott lake is a unique man-made high salt environment in West Texas and its biodiversity has yet to be fully explored. Bacteria in the lake may possess the qualities required to survive in a high salt environment and degrade crude oil. After obtaining water samples from Truscott lake, we analyzed and determined the identities of four isolates that could survive in at least 10% salt. We identified species of *Salinivibrio*, *Vibrio,* and *Bacillus*. Preliminary testing of the *Vibrio* isolate shows promising results on its ability to degrade oil as determined by gas chromatography. We determined that *Vibrio* reduced crude oil by 36.19% in the presence of 5% NaCl, while *Pseudomonas aeruginosa*, which has known crude oil degradation properties, only reduced crude oil by 14.83%. These results indicate that the *Vibrio* strain could be a good candidate for bioremediation.

U10. Isolating *Aeromonas veronii* from Flood Water
Motaharah Geneva Jahan-Tigh, M. Gabriela Bowden
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Severe and flash flooding have become more common in the Houston area in just the past few years. As a direct result, people residing in the Houston area have had an increased amount of contact with flood water, either by having to walk through it, their residence becoming flooded by it, or children choosing to play in it. This project considered the possible public health concern posed by bacteria living in the flood water. This research analyzed samples of flood water from the Houston area to see if it contained any pathogenic bacteria that are known to cause illness in humans. The results of the study determined that the human pathogen, *Aeromonas veronii,* was successfully isolated from a flood water sample. After the water sample was collected, vacuum water filtration was performed to isolate the bacteria. The bacteria was quadrant streaked on nutrient agar for single colony isolation. The single colonies then underwent DNA extraction. After polymerase chain reaction (PCR) of the 16S ribosomal RNA segment was performed, the sample was sequenced by an independent laboratory. This sequence was entered into the Basic Local Alignment Search Tool (BLAST) search program which confirmed the identity of the pathogenic bacteria, *Aeromonas veronii*.

U11. Microbial Degradation of Plastics in Galveston Bay
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Annually, 25 million tons of synthetic plastics accumulate along the coastal seas and terrestrial environments. More than a million different marine animals die by choking or by entanglement in the debris. Recent studies have confirmed the presence of microbial biofilms on plastics that appear to be degrading. The purpose of this project was to identify the microorganisms that can form biofilms on marine plastic debris and to assess their role in plastic degradation. Biochemical testing on plastic samples placed in Galveston Bay have identified three dominant bacterial species - *Staphylococcus aureus*, *Pseudomonas aeruginosa*, and *Micrococcus luteus*. DNA analysis is being done to identify the bacteria in the biofilm. An artificial environment in the lab is currently being tested. Marine water from Galveston Bay has been placed in a tank, and four types of plastic - PE, HDPE, LDPE, and Styrene have been placed in it. Microbes growing on the plastic are swabbed biweekly and plated on various media.

One isolated organism is being analyzed using biochemical tests. In addition, the plastics will be tested for changes over time in marine water.

U12. Detection of Antibiotic Resistant Bacteria Using Soil Samples Collected from Livestock Environment

Lorie Leyva, Joanna Frontera, Alexa Raney, Daiyuan Zhang, and J. Robert Hatherill

Antibiotics that have been used for decades as a method to treat bacterial infections are now becoming obsolete due to the emergence of antibiotic resistant bacteria. Scientists are trying to come up with new ways to manipulate the current antibiotics so that they are still effective. However, this is not an easy task due to the mutation rate of the antibiotic resistant bacteria. In Texas, livestock industry has been using antibiotics as preventative measures to treat or to prevent any illness from starting and spreading to other animals for years. We hypothesize that the overuse of antibiotic drug in farming has increased the bacteria resistance to these drugs drastically and also decreased the ability to treat these types of bacteria. During this project, we were able to test soil samples from local areas with livestock to statistically calculate the rise in antibiotic resistant bacteria by using different types of medium. After confirming the presence of the antibiotic resistant bacteria using tetracycline, several different methods were used to analyze and characterize these bacteria. A total of fourteen other antibiotics with different concentrations were used to test two strains. EnteroPluri-Tests were used for classification and bacteriophage isolated from the same area was used to see if phage could be used as an alternative to antibiotics. Our results will be uploaded into the PARE database which could help extend the use of current antibiotics or find new ways to defend us against antibiotic resistant bacteria. With future research, bench work and collaboration, we may be able to treat or eliminate the pandemic spread of antibiotic resistant bacteria.

U13. The Identification and Characterization of Antimicrobial Plant Extracts

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Multidrug-resistant bacteria are a current crisis including cases of colistin-resistant infections detected in China, Europe and United States. Conservative projections indicate that, if no action is taken, these resistant infections will result in the loss of 10 million lives each year by 2050 with a cumulative cost of 100 trillion US dollars. In this study, plant extracts from different countries were analyzed to determine if any of these had antimicrobial activity. Using the Kirby Bauer disk diffusion method, five plant extracts were identified that showed antimicrobial activity with zones of inhibition between 8mm and 14mm. The majority of extracts that exhibited antimicrobial properties were geographically located in tropical climates. For instance, *Dissochaeta gracilis* and *Miconia pilgeriana*, which have not been previously shown to display antimicrobial properties, were collected from Thailand. The minimum inhibitory concentration (MIC) of the extracts was then determined and these varied from 0.26 to 3.1 mg/mL. Ongoing studies seek to determine the mechanism of action of these extracts using cytological profiling. In this procedure, bacteria are treated with each of the plant extracts and stained with fluorescent dyes to identify changes in the cell structure indicative of the target of action. It is possible that this will result in potential therapies for known cell targets of in the identification of substances with new mechanisms of action. These plant extracts may be further developed as novel treatments for bacterial infections and give physicians more treatment options.

U14. Isolation and Characterization of a Unique Soil Isolate

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Southwestern University

While antibiotic resistance in clinical settings is a serious concern and an intense area of research, less is known about levels of antibiotic resistance in environmental settings. To survey antibiotic resistance in the environment, soil samples from the Southwestern University campus were analyzed for the presence of tetracycline resistant bacteria. One soil isolate taken from the practice football field was found to be resistant to 30 µg/ml tetracycline, and was notable for its bright red pigment and rugose colony morphology. To identify this unknown organism, the sample was cultured on a nutrient agar

plate for biochemical identification tests, including: a catalase test, gram stain, MIC test, oxidase test, and motility test. After isolating the 16S rRNA sequence, it was determined that the unknown soil isolate belongs to the genus *Serratia* and is likely of the *marcescens* species. Interestingly, the organism's rugose colony morphology and production of the red pigment at 37º C are traits that deviate from standard laboratory strains of *S. marcescens*. Future experiments will include genetic analyses and additional antibiotic resistance tests to further characterize this unique strain of *Serratia*.

U15. Factors affecting the abundance of enterococci in two urbanized bays

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Enterococcus is a genus of Gram-positive bacteria consisting of at least 50 species. The crude quantitation of enterococci is an established indicator of fecal pollution in marine waters, as some species such as *E. faecalis* and *E. faecium* are a common component of the human gastrointestinal microbiota. In response to a history of elevated enterococci concentrations in Oso Bay and Corpus Christ Bay, the Islander Stream Team monitored concentrations of enterococci and other water quality indices over the course of several months. Monitoring took place between May 2016 and January 2017 and was conducted using EPA-approved water quality methods. Of the eight sampling sites, four were located in Oso Bay and the remaining four in Corpus Christi Bay. All sites exhibited elevated enterococci in the months of May, June, July, and December. Data indicate that elevated concentrations in May, June, and July were correlated with increases in precipitation and temperature. However, enterococci were also elevated in December, when precipitation was minimal (0.69 inches) and water temperatures were low (14°C), suggesting that other factors were involved. We hypothesized that seasonal changes in population could be correlated with elevated enterococci. To test this hypothesis, we collected occupancy data from the Omni Hotel in Corpus Christi. Occupancy increased during warmer months (May, June, and July) and peaked in July (95% occupancy). We conclude that enterococci concentrations were correlated with temperature, precipitation, and seasonal population change. The correlation with hotel occupancy raises questions about how future tourism and population growth will impact water quality in the Coastal Bend.

U16. Comparison of effects of broad vs narrow spectrum antibiotics on the microbiome

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The mucosal linings of the human body contain the highest counts of bacteria which have an intimate interaction with the immune system and on human health. The skin microbiome of the western mosquitofish (*Gambusia affinis*) is a good model system for the mucosal microbiome, allowing easy experimental manipulation and repetition. Past experiments have shown a rapid gain of antimicrobial resistance (AMR) in bacterial community members following exposure to the single broad-spectrum antibiotic, rifampicin. Further, phenotypic resistance persisted in the skin microbiome community for at least two weeks after treatment cessation. AMR may be problematic in infectious clinical settings due to horizontal transfer of resistance genes between normal microbiota and invading pathogens. With drug resistance rising, treatment protocols must be altered in an effort to reduce AMR among the host's bacterial community. Comparison experiments have suggested a reduced amount of AMR when using multiple antibiotics in sequence. Multiple treatment regiments using both narrow and broad-spectrum antibiotics such as tetracycline, vancomycin, and erythromycin, are being compared for their effects on the selection of and persistence of AMR in normal microbiota using bacterial cultures extracted from the mucosal layers of *G. affinis*, with the long-term goal of uncovering ways to minimize AMR spread in the microbiome.

U17. The marine environment is a reservoir for highly virulent *Vibrio parahaemolyticus*

Marci Parks, Lee Pinnell, James Tallman, Rohinee Paranjpye and Jeffrey W. Turner

Vibrio parahaemolyticus (*Vp*) is a Gram-negative bacterium and a natural inhabitant of coastal marine ecosystems worldwide. *Vp* is also an opportunistic pathogen of humans. Virulent strains of *Vp* often possess hemolysin genes: the thermostable direct (*tdh*) and the *tdh*-related (*trh*) hemolysins. These two genes are commonly used to determine if a strain of *Vp* is virulent, but virulence is multifaceted and many virulent *Vp* isolates do not carry *tdh* or *trh.* Here, we report the discovery of a novel environmental isolate (805) that is *tdh*- and *trh*negative, but was shown to be more virulent than the pandemic type strain RIMD2210633 in zebrafish. Multilocus sequence typing indicates that 805 belongs to a rare sequence type (ST323), which has been isolated from environmental samples collected from the United States' Pacific and Atlantic coasts. This discovery demonstrates that the environment is a reservoir for virulent strains, and it raises questions about hitherto unknown mechanisms of virulence. To further investigate, the isolate was sequenced using Illumina paired-end chemistry (2 x 300), and a draft genome was assembled with velvet. This initial draft genome was comprised of 75 contigs totaling 5,303,204 bp. A search for novel coding sequences revealed the presence of an IncF conjugative pilus (1.3 Kb), a colicin V plasmid (1.49 Kb), and five toxin-antitoxin (TA) modules. Currently, we are attempting to improve the draft genome by integrating SOLiD mate-paired (2 x 35) sequences. The improved assembly is expected to provide additional details per the isolate's mechanisms of virulence. Future research will be focused on the construction of mutants for further virulence testing in zebrafish.

U18. Antibiotic Production of Microbes Isolated from Sorcerer's Cave, Texas

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In the wake of medical advancements, caves give potential for discovering groundbreaking micro-biodiversity and may harbor the next clinically-applicable antibiotics. Microorganisms were isolated from samples collected from Sorcerer's cave, Texas, and were studied for their potential to produce antibiotics. Cave isolates were grown in tryptic soy broth and then added to wells in tryptic soy agar plates inoculated with lawns of Staphylococcus aureus and Escherichia coli. Zones of inhibition were measured, showing possible antibiotic producers in twelve of the 225 studied microbial isolates. Four cave isolates inhibited the growth of E. coli and six inhibited S. aureus. Two isolates inhibited both strains which indicates broad spectrum antibiotic activity. This data supports the hypothesis that producers of antibiotics can be isolated from the cave samples. More data should be collected to determine if previously unknown antibiotics are being produced by these unique cave isolates. Although in its infancy, this study merely touches the possibilities and uniqueness of cave environments to help develop biomedical promises in new medicine and research opportunities.

U19. *In vitro* Selection of *Clostridium difficile* Resistance Mutants

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Clostridium difficile is a gram-positive anaerobic bacteria that causes approximately five hundred thousand infections each year in the US. Current treatment for *C. difficile* infection (CDI) include metronidazole, vancomycin and fidaxomicin. Ridinilazole is a novel antibiotic currently initiating phase III studies but the exact mechanism of action is still unknown. To understand the effects of ridinilazole on resistance development and shed light on its mechanism of action, *C. difficile* was passaged serially with ridinilazole or comparator antibiotics to develop genetically resistant strains. *C. difficile* strains 630 (ribotype 012) and R20291 (ribotype 027) were subjected to 15 passages in BHIS supplemented with metronidazole, vancomycin, fidaxomicin and ridinilazole at multiple concentrations. Bacterial populations growing at >8xMIC values for three passages were subjected to 3 extra passages in BHIS only and then re-exposed to a range of concentrations of the specific antibiotic. A phenotypic analysis was also made on these specific mutants by scanning electron microscopy. No mutants, as demonstrated by a >8xMIC, were observed with the 630 strain exposed to any of the antibiotics tested. The R20291 strain developed resistance (>8xMIC) with fidaxomicin and ridinilazole at passages 10 and 11 respectively. The R20291 mutant exposed to fidaxomicin and ridinilazole were stable at 128xMIC and 8xMIC after 3 passages without antibiotic pressure respectively. Fidaxomicin mutant strains presented a phenotype similar to the negative control (without antibiotic) and the ridinilazole mutant strains presented a filamentous phenotype already observed in sub-MIC treated population. Resistance development occurred with the R20291

strain exposed to fidaxomicin or ridinilazole after multiple sub-MIC passages. These strains will be used to help elucidate the mechanism of action of ridinilazole and possible resistance pathways. Texas Department of State Health Services (#2015-045577) and NIH (NIAID 1UO1 AI24290-01)

U20. Media Plays a Role in Antimicrobial Silver Nanoparticle Efficacy

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In light of rising antibiotic resistance, the use of silver nanoparticles as antimicrobial agents is gaining interest. They are a promising approach for the future as they could be used in different ways. However, they vary widely in activity, and information is still needed to understand how silver nanoparticles react to different conditions. In this experiment, six bacterial culture media were used to test the antimicrobial efficacy of silver nanoparticles for the same bacterium, *E. coli*.

The data was collected using LB, SOC, SOB, PDC, TDC, and TSA as the culture medias and then analyzed. The results indicated that the inhibitory efficacy of the negatively charged silver nanoparticles used in this experiment was dependent upon the media, with little activity seen in most conditions. Upon closer inspection, inhibition was only detected when the assay was done using LB which had relatively high salt concentration compared to the other medias.

The rest of the medias had a salt concentration around 0.4% compared to 1% in LB. This led to the next step, which was varying the salt concentration in the media with the greatest inhibitory activity. For this experiment, culture medias of LB using 0.4, 0.5, and 1% of salt concentration were used and the inhibitory efficacy of negatively charged silver nanoparticles was once again tested. The results using different salt concentrations confirmed that higher salt concentrations promote the inhibitory activity of negatively charged silver nanoparticles.

Our findings, contrast with previous work in which other negatively charged silver nanoparticles had reduced activity with increasing salt. This underlines the lack of basic understanding of how silver nanoparticles function and the need for further study.

U21. Employing CRISPR-Cas9 Technology to Study the Essential Role of Cyclic GMP-AMP Synthase (cGAS) in Recognition of Microbial DNA in Human Cells

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To prove the significance of cyclic GMP-AMP synthase (cGAS) in recognition of DNA in host cells, cGAS -/- knockout mice were recently generated. Lung fibroblasts derived from these mice were infected with the herpes simplex virus 1 (HSV1), vaccinia virus (VACV), and a mutant strain of HSV1 known as HSV d109, all of which are DNA viruses. The control group was infected with the Sendai virus, an RNA virus. IFN-β production in the cGAS -/- cells was largely abolished in response to all DNA viruses. The response to the RNA virus, known to activate the RIG-I pathway, was not affected. These studies conducted in mice concluded that cGAS is required for IRF3 activation and cytokine induction solely in response to viral DNA, but not viral RNA, in lung fibroblasts. In order to replicate this finding in human cells, we will use HT29 human colorectal adenocarcinoma cells and the CRISPR-Cas9 technology for genome engineering as outlined by Ran et al. A cGAS knockout cell line will be created by using 20-nt RNA, which specifically targets the human HT29 cGAS gene locus. We will then use Polymerase Chain Reaction (PCR) and restriction enzymatic analysis to verify the successful inactivation of cGAS protein expression. Lastly, we will challenge HT29 knockout cells with HSV1, VACV, HSV d109 and Sendai viruses. We expect viral DNA will go undetected in HT29 knockout cells as in the cGAS -/- mouse lung fibroblast.

U22. Evaluation of the antibacterial mechanisms of novel silver nanoparticles (AgNPs)

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Recently, the use and production of silver nanoparticles (AgNPs) for a variety of industrial and therapeutic

objectives has increased. However, little is known about how different characteristics of the AgNPs influence their antibacterial activity. The goal of this project is to examine how the surface characteristics of a panel of novel AgNPs, photochemically-synthesized by the Omary group at UNT, play a role in the inhibition of bacterial growth. *Escherichia coli* along with *Bacillus megaterium* were tested initially as well characterized models of human pathogens to both deduce the minimum inhibitory concentration (M.I.C) of AgNPs and evaluate their mechanism of action. The AgNPs given consisted of some positively charged nanoparticles while others were negatively charged. In order to determine the growth of the bacteria and note whether the AgNPs played a role in inhibition, samples, which consisted of LB media, the bacteria and AgNPs, were diluted to different concentrations and observed using spectrophotometry after having set an overnight before evaluating them hourly for three hours the next day. After several trials were performed to ensure the reproducibility and reliability of the data collected, it was found that the negatively charged AgNPs are effective in the inhibition of both E.coli and B. megatarium at an M.I.C of 0.05 mM to 0.75 mM. However, the positively charged nanoparticles behave differently in both types of bacteria tested, with a lower M.I.C for the E.coli at around 0.025 to 0.050mM and a higher concentration than the maximum tested for the B. megaterium. We are in the process of further deducing the range with regards to the latter sample.In order to understand the mechanism of inhibition, whether bacteriostatic or bacteriocidal, experiments performed included diluting the last hourly sample tested at the highest concentration of 0.1 mM and placing it within fresh media. It was determined that the mechanism is bacteriostatic, thus using this data, we can now conduct disc diffusion to understand the cooperative property of the AgNPs with antibiotics such as penicillin and chloramphenicol. Since a bigger zone of inhibition does not always indicate the effectiveness of the disks used, knowing the M.I.C will help in analyzing how quick the diffusion rate is and allow us to compare this zone of inhibition with bacteria tested in the future. We expect to eventually determine how chemical variations of the nanoparticles contribute to their antibacterial activity when used to either combat wound infections or reduce biohazards.

U23. Pigmentation in the Photosynthetic Bacterium *Rhodospirillum centenum*: Mutagenesis and Sequencing Strategy for Selected Mutants

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Rhodospirillum centenum is a photosynthetic organism that also has the ability to perform phototaxis. Therefore, photopigment production is critical for this bacterium. *R. centenum* produces pigments including bacteriochlorophyll (Bchl) and a number of carotenoids (Crt). In this study, random mutagenesis with a mini-Tn5 transposon was performed. The transposon was introduced into *R. centenum* by conjugation of a suicide vector. After conjugation, transposition events that represent potential mutants were selected for on media containing spectinomycin. From approximately 3750 *R. centenum* conjugated strains containing the transposon, four mutant strains were observed with various pigment phenotypes and were subjected to spectral analysis. Strain 2-4 was chosen for further study because it produces absorbance-shifted carotenoid pigments. Strain 2-15 was selected because it makes neither Bchl nor Crt pigments and is unable to grow photosynthetically. Sequence data of these mutants will be generated by an inverse PCR method.

U24. Assessing the Emergence of Antibiotic Resistant Bacteria in Corpus Christi: An Undergraduate Crowdsourcing Approach

James T. Gonzalez, John Ramirez, Alexa Raney, and J. Robert. Hatherill

The increasing emergence of antibiotic resistant bacteria (ARB) is effectuating one of the most pressing crises currently being imposed on multiple fields of healthcare worldwide. Both anthropogenic and natural factors contribute to this phenomenon including the inappropriate prescription and overuse of antibiotics as well as their dissemination in the environment via improper disposal, partial metabolism, and ubiquitous use throughout the agricultural industry. These factors provide selective evolutionary pressures which may exacerbate mechanisms by which bacteria acquire antibiotic resistant genes (ARGs) in nature. Natural mechanisms contributing to ARB include random genetic mutations, which select for resistance and horizontal gene transfer (mediated by mobile genetic elements and bacteriophages). As anthropogenic and natural factors producing ARB become increasingly

complimentary, the rate of emerging ARB will continue to escalate. The PARE assessment aims to develop a profile of ARB in the environment surrounding Corpus Christi, TX. During this project, soil samples were collected from various locations potentially harboring ARB throughout the city. These samples were then subjected to serial dilutions prior to being plated on 3 series of plates, each with differing antibiotic concentrations. To attain statistically significant CFU counts, serial dilutions were conducted in triplicates prior to incubation. The CFU counts were compiled for differing experimental conditions and conducting a student's T-test. It is proposed that crowdsourcing the collection of data forming a profile of ARB present in Corpus Christi will be critical in developing an understanding of this emerging threat. Furthermore, we postulate that these data will serve to aid in developing a more detailed understanding of emerging ARB dynamics on a nationwide scale as it is added to the national PARE dataset.

U25. Evaluating Anti-Viral Properties and Cell Toxicity of Silver Nanoparticles

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Sexually transmitted infections (STIs) have become an increasing problem in recent years. Our goal is to discover whether silver nanoparticles (AgNPs) could be used as an effective anti-viral against STIs. The AgNPs we use are from the Omary lab at the University of North Texas, and have varied surface properties. We have been testing negatively and positively charged AgNPs. We use the herpes virus, mouse cytomegalovirus (MCMV), to determine the anti-viral properties of these AgNPs. Although currently working with cells, mouse IC-21 macrophages, this system has the potential to be taken into the natural host. We use these cells because these cells are phagocytic and are more likely to react negatively and die than other cells because these cells take in the toxic debris. We have also tested the negatively charged AgNPs with NIH3T3 fibroblasts that don't appear to react as negatively to the nanoparticles. In toxicity tests, we have found the negatively-charged particles to kill the mouse cells at concentrations as low as 0.01mM and at a concentration of 0.1 mM the cells are dead within 6 hours, but the positively charged have no apparent toxicity as high as 0.25 mM. Therefore, we are testing the positively charged nanoparticles by mixing them with the cells and virus at varying times. For example, we would mix the virus and the nanoparticles first before introducing the mixture to the cells like we would see in condoms or we would mix the cells with the nanoparticles and then add the virus, resembling vaginal creams. With the negatively-charged toxicity to mammalian cells, we are testing it for viral binding, this could be useful for pretreatments of non-cellular bodily fluids. We also plan on looking into macrophage activation.

U26. Microbial Hg Methylation and Methane Synthesis in Caddo Lake Nevada King1, Sara Janssen2, Javid F. McLawrence3, Cara L. Case3, John R. Reinfelder2, Anil Somenahally3, Ri-Qing Yu1

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Caddo Lake in northeastern Texas is one cypress-Spanish moss dominated lake ecosystem. Contamination of mercury (Hg) especially methylmercury (MeHg), which is a neurotoxicant in major fish species and reptiles, has been reported in this lake decades ago. Due to the feature of bioaccumulation and biomagnification, Hg contamination in the lake fishes cause health concerns on the wildlife and local people. However, the source of MeHg in this lake, primarily from microbial Hg methylation mechanisms, has been very little studied. We investigated the lake for the past two years, by taking sediment and plant samples in several lake wetland habitats which showed high MeHg levels in fish from previous studies. Total Hg, MeHg, sulfate and other biogeochemical factors were analyzed. For the first year analyzed Total Hg concentrations in lake sediment (123.2 – 147.7 ng dw g-1) were significantly higher than those in Spanish moss (*Tillandsia usneoides*) tissues (27.1-39.8 ng dw g-1). However, MeHg levels in Spanish mosses (1.2-1.4 ng dw g-1) were obviously higher than those in the sediment. As an epiphyte, Spanish moss generally has little direct connection with its host plant (cypress). Where the MeHg in moss tissues comes from is a mystery due to the fact that MeHg is usually synthesized by anaerobic microbes in lake sediments. Among the samples of interest are several invasive species that also likely contain microbial

Hg methylators. We further extracted genomic DNA from all the sediment and plant samples, and conducted the detection of functioning genes including the Hg methylation genes (*hgcAB*), methyl-coenzyme M reductase genes (*mcrA*) and others. Our results indicated that all lake sediment samples showed microbial Hg methylation genes which could lead to microbial Hg methylation, while one Spanish moss sample showed a likely weak *hgcAB* gene band. Most of the aquatic invasive plant species contained *mcrA* genes which lead to the production of greenhouse gas methane. We hypothesize that Spanish moss-cypress-sediment habitats might play an important role in MeHg synthesis and Hg cycling in this ecosystem.

U27. Examining the Role of Exopolysaccharide in *Myxoccocus xanthus* Social Motility

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Social (S) motility is type IV pili-mediated flagella-independent group movement on a solid surface. It has been best studied in the gram-negative soil bacterium *Myxococcus xanthus*. The cells move due to extension and retraction of the polar-localized pili. A unique characteristic of *M. xanthus* S-motility is cell density-dependent colony expansion. Our previous quantitative analysis and mathematical modelling data support the hypothesis that this expansion is dependent on the accumulation of exopolysaccharide (EPS), a sugar-based polymer material excreted by *M. xanthus* cells onto the agar surface: low-density colonies delay expansion until a threshold concentration of EPS accumulates, whereas, high-density colonies expand upon plating as they quickly produce EPS above a threshold value. To test this hypothesize and determine the threshold at which EPS triggers cell movement, we examined the effects of EPS on colony expansion and single cell motility. First, EPS was isolated from *M. xanthus* wild-type cells grown for 3 days on nutrient agar plates using a cold phenol extraction method to strip EPS from the cells' surface. The EPS was then purified by isopropanol precipitation, dialyzed against water, and stored in solution at 4°C. To observe the effects of EPS on colony expansion, 3-µl drops of the diluted EPS solution (100%, 75%, 50%, and 25%) were spotted and dried onto soft agar plates (0.5% agar) containing 1% casitone with 0.2% yeast extract. Next, 3-µl drops of a diluted cell culture ($1x10^8$ cells/ml) of *M. xanthus* DK1218, a social motile only strain, were carefully spotted directly adjacent to each dried EPS spot. The plates were incubated at 32˚C in a humid chamber for 96 h and each spot was imaged at least once a day with a digital camera attached to a dissecting microscope. To observe the effects of EPS on single cell motility, 3-µl drops of *M. xanthus* DK1218 ($2x10^7$ cells/ml) were spotted directly onto a dried 3-µl spot of the 100% EPS solution on a 0.5% casitone soft agar pad on a microscope slide. The slide was imaged immediately after plating every 15 sec for 30 min by differential interference contrast (DIC) microscopy with a 40X objective using an Olympus 81X inverted microscope. Cells spotted directly onto the agar pad served as the control. Qualitative and quantitative analysis showed that the presence of purified EPS increases the movement of *M. xanthus* colonies and single cells. More quantitative experiments are planned to determine the concentration of carbohydrates in the EPS solutions, the colony expansion and single cell motility rates on different EPS concentrations, and the minimal amount of EPS necessary for stimulation of colony expansion and single cell motility.

U28. Analysis of mutation effects on viral protein functions in immune macrophages

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Cytomegalovirus (CMV) has a prevalence of 60-100% worldwide. Infection in humans is typically asymptomatic but can cause severe disease in the immune-compromised. In the US, it is the most common infectious cause of birth defects, which can include hearing loss or mental retardation. Murine cytomegalovirus (MCMV) is used as a model due to similarities in the genetic structure of this virus to human CMV and in the diseases caused. Protein m140 of MCMV (homologue of HCMV US23) is required for the virus to grow efficiently in murine macrophages. Growth in macrophages correlates with

disease severity in the host. Deletion of the m140 protein results in an attenuated virus that does not cause disease, even in immune-compromised animals. This protein normally forms a complex with two other proteins, m139 and m141, and m141 is protected from degradation. Earlier work has shown that a specific region in the m140 protein sequence is necessary for m141 to be protected. We are tagging deletion mutants of m140 with GFP (green fluorescent protein) to determine the regions required for complex formation, cellular localization, and how these affect the ability to efficiently grow in macrophages. We are focusing on how this relates to the ability to stabilize m141. As these phenotypes may not all be connected, we are also analyzing constructs with smaller deletions. Identifying the importance of various functions of m140 to the ability of MCMV to efficiently grow will not only enhance our understanding of the virus, but may also lead to new ways to inhibit viral replication by targeting this protein.

U29. MRSA-ful or MRSA-less?

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Staphylococcus aureus is a major cause of both nosocomial infections and community acquired infections. It ranges anywhere from life threatening disease such as, bacteremia to something as manageable as food poisoning. It may be harmless when dormant in the nasal cavity, but may cause damage when an impairment of the skin occurs. Asymptomatic carriers exist without anyone's knowledge, possibly including the carrier themselves. Although asymptomatic, the organisms can still spread to susceptible hosts. CDC reports about a quarter of the population carries *S. aureus* in their nasal passages, and of those, 2% are a carrier of Methicillin Resistant *Staphylococcus aureus* (MRSA).
This is a result of bacterium having developed a resistance to antibiotics, MRSA specifically being resistant to oxacillin. MRSA infections are most often skin and soft tissue infections, but have also been associated with pneumonia, osteomyelitis, and bacteremia. A survey and a collection of cultures were executed from students for two classes: Biology 4401 and Biology 1402 over the course of three semesters. The survey included whether certain factors affected the carrier rate such as team sports, animals, and traveling. Out of the 510 students that participated from all semesters, 121 fermented mannitol. After the samples were tested for fermentation of mannitol, fermenters were then transferred to tryptic soy agar plates for further testing. Testing included coagulase tests, catalase tests, and gram staining. Cultures that were coagulase positive, catalase positive, gram stain positive staphylococci, and mannitol fermenting were presumed *S. aureus*. Of the original 121 fermenters, 84 inferred to be *S. aureus*. *Staphylococcus aureus* organisms were then tested for resistance against seven antibiotics using the Kirby-Bauer disk method: oxacillin, penicillin, erythromycin/azithromycin, trimethoprim sulfa, doxycycline, and ciprofloxacin. Zones of inhibition were measured in millimeters and compared to the standard zone diameter interpretive standards for *S. aureus*. Of the fermenters, three exhibited resistance towards oxacillin, thus making them MRSA-ful.

U30. Membrane Protein Expression of Engineered Escherichia coli Strains After Single / Multiple Plasmid Transformation

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The performance of bioengineered strains of bacteria is integral to the companies that depend on these strains to produce products. These products can range from biofuel to medication. The Joint BioEnergy Institute (JBEI) in cooperation with Lawrence Berkeley National Laboratory has set out to explore and improve the performance of bioengineered bacteria. One key component of the performance metric is Membrane Capacitance. Membrane Capacitance measures how readily a bacterial strain is able to utilize modified metabolic pathways to express additional membrane proteins. JBEI researchers selected bacterial Escherichia coli strains used to produce biofuels and then subjected them to two distinct assays in order to measure their Membrane Capacitance. The two assays consisted of first using a Tecan 200 liquid plate handler and second a Fluorescence Activated Cell Sorter (FACS). The assay performed with the Tecan 200 liquid plate handler measured expression of fluorescent tagged membrane proteins over a twenty four hour period. By contrast, the FACS measured the expression of fluorescent proteins in each individual cell after twenty four hours of incubation. It was found that strains of E. coli that had been

transformed with multiple bioengineered plasmids had marked decreases in fluorescent protein expression when compared to strains transformed with one bioengineered plasmid. This result was still upheld when the bioengineered strains made use of high-copy Origins of Replication. It is hypothesized that successive transformations of bioengineered plasmids impede the ability of a bacterial strain to express membrane proteins.

U31. Antibiotic Sensitivity of Bacteria in Single- and Multi-Species Biofilms

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Bacterial antibiotic resistance is an increasing global concern as treatment of infections largely depends upon the efficacy of antibiotics. Antibiotic resistance is especially notable in biofilm or sessile and attached bacteria, which cause nosocomial infections through colonized implants. Treatment of such infections is much more difficult than other infections as the presence of biofilm, which is more resistant to antibiotics, continually ensures colonization in other tissues, resulting in chronic infections. Some severe infections can be multi-species, further complicating the treatment. The purpose of this study is to assess the antibiotic sensitivities of bacteria in single and multiple species infections to determine antagonistic and synergistic relationships. The two bacterial species under study are the gram-negative *Escherichia coli* and *Serratia marcescens*, both of which are present in the human body. *S. marcescens* colonies attain a red pigmentation, which facilitates identification and distinction from the white *E. coli* colonies. Seven antibiotics were tested on the two bacteria using Kirby-Bauer Disk Diffusion assays. Of these, kanamycin and chloramphenicol were found to be most effective. Further quantification is being conducted using the minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) assays of bacteria existing in single- and multi-species communities exhibiting either planktonic or sessile growth. The results of this study will determine synergism or antagonism between the two bacterial species, which may lead to more effective treatment design against severe bacterial infections.

U32. The Effect of Subinhibitory Concentrations of Tetracycline on Non-Plasmid-Based Erythromycin Resistance in Escherichia coli

Beth Russell and James Masuoka
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Antibiotic resistance is a significant and continually expanding clinical challenge. Bacteria often gain resistance to an antibiotic via horizontal transfer of plasmids, and revert to a susceptible state when cured of those plasmids. However, chromosomally located resistance elements can also be lost. Lacey, et al. concluded that a strain of *Staphylococcus aureus* with chromosomal resistance elements lost that resistance in vivo due to treatment of patients with an antibiotic to which the strain was susceptible. We hypothesized a similar loss of resistance could occur in *Escherichia coli*. Other students in the lab isolated a strain of *E. coli* (QH020816-1.1) with resistance to erythromycin and tetracycline. When cured of its plasmids by acridine orange, this strain became susceptible to tetracycline yet remained resistant to erythromycin. From this, we concluded that while the resistance to tetracycline was located on a plasmid, the erythromycin resistance genes are on the chromosome. The minimum inhibitory concentration (MIC) for tetracycline was determined using an E-test [illegible] cured strain with a subinhibitory concentration (0.5 x MIC) of tetracycline to provide selective pressure. The strain was pressured for a total of 10 days. A sample was taken and subcultured every 24 hours. A linear increase in the zone of inhibition was observed for days 1-5 by Kirby-Bauer using15 mg erythromycin discs. After five days, the diameter of the inhibition zone stayed ~20mm. Identity of each subculture, along with the original strain and acridine orange-treated strain, was confirmed by API 20E strips. We performed plasmid preps on the original strain, the acridine orange-treated strain and the sample subcultured after three days of tetracycline pressure. Plasmid DNA was evident in the original strain, but not in either strain following acridine orange treatment. Current work involves amplification of known chromosomal resistance genes using PCR to identify any alterations in those sequences from wild-type. From this, we hope to gain insight regarding possible mechanism(s) leading to the decrease in resistance so we can better understand how bacteria revert to susceptibility. This information could lead to strategies of

antibiotic use that will increase the lifespan of these drugs.

U33. Characterization of novel zinc oxide morphologies and their antimicrobial properties

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Increased resistance to antimicrobials is recognized by the World Health Organization (WHO) and Centers for Disease Control and Prevention (CDC) as a global public health threat. As the occurrence of drug resistant strains of microorganisms continues to increase, many first-line treatments are being rendered ineffective. As new mechanisms for antimicrobial resistance emerge and spread, common illnesses become more difficult to treat, resulting in increased morbidity and mortality rates among otherwise healthy individuals. New and novel drug treatments are needed to contest this growing threat. Heavy metals, such as zinc, have long been demonstrated to serve as antimicrobials. Current applications of zinc oxide use a non-distinct, or mixed, morphology.
In this study, novel synthesis techniques allowed for the creation of zinc oxide nanostructures featuring increased polar termination, or distinctly flat surfaces, referred to as “plates” and structures featuring increased non-polar terminations, or long hexagonal surfaces, referred to as “rods.” The antimicrobial effects of the two different morphologies were studied with *Escherichia coli, Pseudomonas aeruginosa,* and *Staphylococcus aureus.* Preliminary time courses performed by determining CFUs/mL on time 0hr and time 4hr showed marked bactericidal activity with *S. aureus* and demonstrated some growth inhibition with *E. coli* and *P. aeruginosa*. The completion of a 10-hour time course (CFUs counted every hour) with *S. aureus* clearly demonstrated a significant effect of both plate and rod morphology, with the culture containing 10g/L ZnO plates being completely killed by hour 5. Overall, the plate morphology was shown to be a more effective antimicrobial. This suggests oxygen radical production off the polar faces of the ZnO as a possible mechanism of action.

U34. Antibiotic Side Effects: Looking at Intestinal Motility

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Antibiotics are powerful medicines used to treat bacterial infections. While antibiotics are effective tools, they can also be responsible for disrupting the ecology of the body’s normal microbiota. Gambusia affinis is a vertebrate model used for human mucosal microbiomes. Previous work demonstrates that exposure of fish to the broad-spectrum antibiotic rifampicin causes community composition changes in the gut microbiome and several negative effects on the host fish, including increased susceptibility to a bacterial pathogen and lack of weight gain (failure to thrive). Transit time for food in the gut was examined as a potential factor related to lack of weight gain. Experimental groups of fish were exposed to antibiotic for three days while control groups were vehicle exposed. After this, fish consumed fluorescently-labeled 70kDa dextran beads embedded in food. Water samples were taken over a thirty-six-hour period and fluorescence intensity measured. Results showed no significant difference in the gut motility rate with fish exposed to rifampicin compared to control. This suggests that failure to thrive may be linked to other factors, such as gut inflammation or altered metabolic activities of the gut microbiota.

U35. BeardedLady: Isolation, Characterization, and Analysis

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The purpose of this research lab was to isolate and analyze bacteriophage (or phage) that infect the bacterium *Streptomyces xanthochromogenes*. To do this, we collected soil samples and proceeded to isolate, purify, and characterize the phage found within the samples. My phage, BeardedLady, was taken from a soil sample collected in Denton, TX that was then enriched and purified by performing multiple spot tests and phage titer assays according to the *Streptomyces Resource Manual*, with minor variations. In addition to experimentation in our lab, BeardedLady was also selected to undergo genomic sequencing

at the University of Pittsburg. My lab partners and I are currently annotating this genome, making decisions concerning the 76 putative genes presented in the sequence. The genome size of BeardedLady is 49,941 base pairs, with a GC content of 66.2%. BeardedLady was also determined to belong to the BD1 subcluster. This information closely resembles my findings obtained in the lab and suggests that our phage is fairly stable. Our results, along with other material collected by my classmates, will contribute to the limited knowledge available concerning phages able to infect *S. xanthochromogenes* bacteria. Further analysis of BeardedLady, and similar bacteriophage, will also increase the awareness of opportunities that these underrated biological organisms provide for future research.

U36. Annotation of Bacteriophage 'Wyatt2' and Investigation of the Gene 28
Doug Burns, Joshua Martinez, J. Robert Hatherill, and Daisy Zhang
Del Mar College

According to the World Health Organization (WHO), we are headed for a post-antibiotic era where infections and minor injurers can kill once again. At least 2 million people infected with antibiotic resistant bacteria in the US and more than 23,000 deaths reported annually. Phage therapy can be used as an alternative to antibiotics. Research shows that bacteriophage is host specific. Scientists have developed applications for treating infections by introducing bacteriophage into bacterial infacted wounds by injection. The bacteriophage, named 'Wyatt2', was discovered from costal bend area and its genome was sequenced and annotated using bioinformatics software 'DNAmaster'. The genome of 'Wyatt2' was compared to a few other published L1 bacteriophages using a genomic comparison software 'Phamerator'. It was discovered that the bacteriophage Wyatt2's genome contains an important Holin-producing gene at ORF 28. Holins are a diverse group of small proteins produced by dsDNA bacteriophages in order to trigger and control the degradation of the host's cell wall at the end of the lytic cycle. Holin forms three helical transmembrane domain proteins when completely folded. These helical structures are parts of the Transmembrane2 (TM2) site, also called pores in the bacteria cell membrane. More research is needed on how the pores are formed, but there is much research suggests that holing proteins might behave like biological detergent by forming endolysins on the pores to set up for lysins to cleave peptidoglycan in the bacteria cell wall. This is also a critical part of the bacteriophage lytic cycle. Culturing and extracting bacteriophage for holin protein production could have medical uses such as permeating pores to increase the effectiveness of current antibiotics against antibiotic resistant bacteria.

U37. Isolation and characterization of *Streptomyces griseus* bacteriophage Paradiddles
Canyon Calovich-Benne, Baylee Green, Jocelyn Gonzales, Alessandra Martinez, Nikita Suri, Subhayu Nayek, Swapan Bhuiyan, and Lee E. Hughes
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Bacteriophages are vast and dynamic. They are the most abundant organism with 1031 organisms. Phages are being researched heavily in the fields of therapy and infections. Paradiddles was isolated and purified at the University of North Texas on the host bacterium *Streptomyces griseus*. After various analyses were carried out on Paradiddles; we have concluded through restriction digest, gel electrophoresis, and sequencing at Pittsburgh Bacteriophage Institute that Paradiddles is part of the BE cluster and BE1 sub-cluster. Paradiddles is currently being annotated. Paradiddles has 230 genes, 47 tRNA's, and 1 tmRNA. This is important because *Streptomyces* phages are usually small in genome size. With this large size, it shows that Paradiddles is unique and needs to be studied further as to why it is so large.

U38. Isolation and Lysogen Study of Novel Bacteriophage 'Osoo'
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It is estimated there are 10^34 bacteriophage on earth, and they are virus that attack bacteria. The name is derived from the Greek word "Phaigin" meaning 'to devour'. Bacteria can harm the human body in multiple ways. They can produce toxins that harm the human tissue, and also damage the hosts by fast multiple replication that overwhelms the human body's immune system. Antibiotics are what we usually use to treat bacterial infections. Phage therapy is being used as an alternative in medical treatment when

bacteria is showing increasing antibiotic resistant. Other than destroy their bacteria hosts, Phage can also incorporates its genome into the bacteria genome and turn their hosts into lysogen. In this study, a novel bacteriophage named 'Ooso' was isolated from Oso Creek, South Texas. The total genomic DNA is isolated from 'Osoo', followed by restriction enzymes digest. The restriction pattern suggest that 'Osoo' is a Cluster A phage. 'Osoo' was fixed using a 1% uranyl acetate on copper grid and a transmission electron microscope was used for imaging. The database of 'Osoo' is generated on phagesDB.org. A lysogen of 'Osoo' is isolated and will be used for future study.

U39. Terminators of Cluster BE and Reannotation of Peebs

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Bacteriophages are viruses which infect bacteria. The isolation of bacteriophage (phage) and its subsequent study is imperative for the furthering of genomic studies and using phage as tools. Through the SEA-PHAGES program at the University of North Texas, the *Streptomyces* cluster four BE phage were analyzed. The BE cluster phage in this study are Jay2Jay, Karimac, Peebs, and Samisti12. A DNA Master annotation analysis on Peebs' genome showed 36 out of 240 gene call starts that required changing. The previous method into calling a gene showed biases towards certain aspects of the genome that could result in a poor call. After the genome analysis on Peebs, terminators were determined for each of the four cluster BE phage. Using the FindTerm program, the top four terminators for Jay2Jay, Karimac, Peebs, and Samisti12 were chosen. In the future I would like to observe the effects point variants and deletions have on the efficiency of the selected terminators.

U40. Isolation and Bioinformatic Characterization of the *Streptomyces* Bacteriophage Austintatious

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Our world exists in a continuous cycle of evolution, and one crucial area in which researchers must stay ahead of the curve is the development of antimicrobial agents. The leading source of antibacterial development originates from the genus *Streptomyces*, which is a gram-positive bacterium utilized in bacteriophage genomics. For this project, phages are first isolated from soil samples followed by purification and further analysis using a wide array of bioinformatics tools. These tools include Phage Evidence Collection And Annotation Network (PECAAN), DNA Master, Starterator, and Phamerator. The tools utilize systems such as GLIMMER and GeneMark to find genes in microbial DNA. In the present study, the bacteriophage Austintatious was isolated from soil in Denton, Texas, using host bacterium *Streptomyces venezuelae* ATCC 10712. Austintatious is classified in the BC3 subcluster and contains 36,213 base pairs with 56 genes and a high GC content of 72.6%. This and other bioinformatics studies will lead to a further understanding of the great diversity of bacteriophages present in our environment.

U41. Isolation and Annotation of the Bacteriophage Ozzie

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Bacteriophages are viruses that infect the bacteria. The data presented in this research was derived from the isolation and characterization of a phage. Streptomyces phage Ozzie was isolated using the host bacterium *Streptomyces xanthochromogenes*. Ozzie was found by following the standard procedures in the *Streptomyces Phage Laboratory Manual*. The soil sample used for isolation was found on August 27th, 2016 in Gunter, TX. Ozzie is in the process of being annotated by a team of three SEA PHAGES students. Ozzie has a genomic size of 49,961 base pairs and a GC% of 66.2, and is one of the twenty-seven members of the BD1 cluster. This specific cluster has an average genome size of 50,633 base pairs, an average GC% of 65.9, and on average has 74.3 genes. It is important to research bacteriophages because they are the most numerous cellular organism on Earth. Bacteriophages have a wide impact on the world, they are important in terms of the evolution and ecology of bacteria, they have

a large impact on the global carbon cycle, and lastly they could even be the solution to the antibiotic crisis. The research and annotation that is being done on Ozzie, might just provide other insights to the importance of bacteriophage research.

U42. Isolation, Purification, and Annotation of Diane a *Streptomyces xanthochromogenes* Bacteriophage

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The objective was to find a unique bacteriophage from a collected soil sample by isolating, purifying, and characterizing that phage. The phage Diane was isolated from an enriched soil sample that was then purified through a spot test and phage titer to characterize plaque morphology. The plaques have an average diameter of 1.0mm and are generally clear and circular. The phage was characterized by isolating and purifying DNA and then performing a gel electrophoresis. The structure of the phage was characterized by electron microscopy. The electron micrograph suggests a morphotype of Siphoviridae. Host Range results were negative for other *Streptomyces* bacteria that were tested. The genome was sequenced, revealing a genomic length of 50,483 base pairs, a GC content of 66.7%, and BD2 sub-cluster classification. It likely includes 83 genes, 40 of which have suggested functions. The genome was annotated using several analysis programs and online databases. These include PECAAN, DNA Master, HHPred, Phagesdb BLAST, NCBI BLAST, Phamerator, Glimmer, and GeneMark. The results suggest Diane is a lytic, genetically unique, Siphoviridae phage that only infects *Streptomyces xanthochromogenes*. This information helps further the knowledge of bacteriophages that infect *Streptomyces xanthochromogenes* because not many phages from this bacterial host have been discovered.

U43. Isolation and characterization of *Streptoyces* BD2 Phage Daudau

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Through the SEA-PHAGES program at UNT, the bacteriophage Daudau was identified and isolated from an enriched soil sample from Arlington, Texas. Daudau was replicated and isolated using the bacterial host Streptomyces xanthochromogenes, using the standard procedures from the *Streptomyces* Phage laboratory manual. The purpose of this research is to isolate and annotate a phage so that future research can be conducted. After the isolation process was completed we obtained a gel electrophoresis picture and an electron microcopy picture and found it to be a lytic phage. Daudau belongs to the BD2 subcluster, of which only 15 total phages have been identified and only 2 submitted to Genbank's database. Daudau is similar to the phages R4 and ELB20. There is a total of 87 genes coded, 50,602 base pairs for this phage's genome and a total GC content of 67.1%. The annotation of the phage's genome required tools such as: PECAAN, Phamerator, Genemark, DNA Master, and BLAST tools from NCBI and Phagesdb.

U44. Elucidation of Evolutionary Alterations in the Mycobateriophages.

Nicholas Leonard, Conner DeJager, Frederick Baliraine, Gregory D. Frederick
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Bacteriophages are small and diverse viruses that use bacterial organisms as hosts to reproduce and diversify. The diversity of their genomic characteristics can be attributed to many factors and stimuli. However, the overall "malleability" of their genomic DNA can contribute to successful evolutionary adaptations. The acquisition of both intergenic and protein producing DNA sequences into their genome is a very unique and important subject in phage genomics. By accepting integrating pieces of DNA, a bacteriophage's genome has the ability to reconfigure its original protein-producing sequence into one that can also incorporate, and benefit from, new informational DNA. This not only creates new protein products for the phage, but also transforms existing genes into new ones. Without this function, phages would surely acquire and integrate useless and contextual pieces of intergenic information into their genomes. This inherent ability of bacteriophages is integral to the proliferation and evolution of these

viruses, and contributes significantly to their microbiological impact. This study describes the investigation of the evolutionary background of several mycobacteriophages.

U45. Post Isolation Differentiation of Mixed *Streptomyces* Phages Thestral A and B
Claudia McCown, Megan Webb, Swapan Bhuiyan, and Lee E. Hughes
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Advancements in bacteriophage manipulation show positive progression towards the uses of bacteriophage in the medical field and industry. Recently at the University of North Texas, a student isolated a novel *Streptomyces* Phage on the bacteria *Steptomyces xanthochromogenes.* This phage was given the name Thestral and was sent for routine sequencing. However, the phage was not a single isolate, and two phage sequences were obtained from the single sample. They were given the names, Thestral A and Thestral B. Both sequences are in the BD2 subcluster. Because of requirements of phage databases, a physical sample of each individual phage must be acquired in order to document these samples as new and unique. Techniques of bacteriophage isolation vary among samples. In order to separate the two phages, plaques made by the phage lysate were taken and tested on the DNA of each phage with two unique primers to differentiate between the two phages. Once identified using DNA, the phage samples were amplified and currently in the process collection. To analyze the PCR product, electrophoresis gels of the product were run. Bands of about a thousand base pairs in designated lanes confirmed the presence of Thestral A or Thestral B. After several trials, positive results of both phages were confirmed. Once two pure phage samples are collected, the sequences of Thestral A and B can be released. Their release will open doors for more study of the Thestral phages as well as all *Streptomyces* phages.

U46. Annotation of Streptomyces Phage SqueakyClean Paola Medina, Peter Anghel, Sierra Shepard, Subhayu Nayek, Swapan Bhuiyan, Dr. Lee Hughes
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The Phage Hunters Advancing Genomics and Evolutionary Science (PHAGES), is a national research and education that focuses around a research course in bacteriophages, sponsored by the Howard Hughes Medical Institute's Science Education Alliance. BIOL 1750 is a laboratory course at the University of North Texas in which undergraduates perform research activities on bacteriophages genomics. In this course students contributed to research by trying to find a unique bacteriophage that infects the bacterium Streptomyces. Streptomyces are characterized as gram-positive aerobic bacteria of complex form; members of this genus are prolific producers of antibiotics. Students isolated phages with Streptomyces xanthochromogenes from soil samples, purified the phage, and analyzed the genome. The goal was to obtain DNA and create a restriction digest. As the course advanced each student discovered a phage that was added into the phages database and some accomplished to be sequenced. Sequenced phages are currently being annotated with the new goal of studying their genomes. SqueakyClean, found by Peter Anghel, is the bacteriophage that is currently being annotated. Starts and stops for each gene have been chosen and a few genes have been deleted. Genes show different coding potentials, functions and characteristics that are considered for annotation.

U47. Isolation and Characterization of *Streptomyces* phage NootNoot
Sandra Meridew, Ryann Morgan, Anna Moussa, Syeda Shahid, Swapan Bhuiyan, Subhayu Nayek, Nikita Suri, and Lee E. Hughes
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This research was performed in order to obtain, identify, purify and characterize a new and unique phage. Soil samples were collected from various locations, and each sample was isolated using the host bacterium *Streptomyces griseus.* Along with the bacterium, a detailed *Streptomyces* resource guide, provided by SEA-PHAGE program, was utilized which presented instructions on executing an isolation

and analysis on bacteriophage. NootNoot, the phage which was successfully isolated during this research, is a *Streptomyces griseus* bacteriophage, and was found in Keller, TX. The average diameter of the plaques found were around .5mm-1mm. A DNA sample of NootNoot was sent out to be sequenced at a local lab and has been placed into the BE1 cluster. There is a total of 60 reverse genes, 168 forward genes, 46 forward tRNA's, 1 reverse tRNA, and 1 tmRNA. As of now, these genes are still being annotated allowing for more genomic information to be found for the categorization of NootNoot.

U48. Streptomyces griseus – Analysis of BryanRecycles Bacteriophage

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Bacteriophages are the most abundant life forms on Earth. It is important to study bacteriophages to better understand infection and coevolution. Bacteriophages can be used as a therapy to treat bacterial infections. In our attempt to discover more potentially useful phages, our research group isolated phages from soil samples collected from a variety of locations. The bacterial strain *Streptomyces griseus* was used as a host to isolate the bacteriophage from a soil sample collected in Denton, Texas. The bacteriophage was named BryanRecycles. The phage has been sequenced and is being annotated. BryanRecycles is in the subcluster BD1. BryanRecycles is smaller than an average phage, containing 50,066 base pairs. BryanRecycles is a promising phage that needs to be further analyzed to discover more intricate features.

U49. Isolation, Characterization, and Annotation of *Streptomyces griseus* bacteriophage Blueeyedbeauty

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Bacteriophages are considered to be viruses that infect targeted bacteria cells. This research aims to describe the unique bacteriophage, Blueeyedbeauty, which was obtained, isolated, purified, and characterized at the University of North Texas. The host bacterium used throughout the process was *Streptomyces griseus*. Many procedures, such as spot testing, enrichment, lysate titers, and dilutions were used to come to the final conclusions. In the end, Blueeyedbeauty was isolated and documented as a newly discovered bacteriophage. As of right now, Blueeyedbeauty is considered to be a Singleton and could possibly be included in the newly found cluster with several other unclassified phages. Blueeyedbeauty was sent off to be sequenced and has been returned to allow us to annotate the bacteriophage's genes. Out of the 264 genes, over fifty genes have functional properties. By studying these genes further, we will be able to continue gathering information about Blueeyedbeauty's characteristics.

U50. Discovery and Annotation of Bacteriophage Oliynyk using Bacterium *Streptomyces xanthochromogenes*

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Bacteriophages are viruses that infect bacteria. They are composed of a capsid or protein head, which holds nucleic acid, and a hollow protein tail. The bacterium *Streptomyces xanthochromogenes* served as the host for this experiment. Using standard procedures found in *Streptomyces Phage Laboratory manual*, Streptomyces phage Oliynyk was isolated from a soil sample located on the University of North Texas campus on 12 September, 2016. Oliynyk has been sequenced at the University of Pittsburgh and found to be a member of cluster BD1. Its genes are currently being annotated. Multiple genes have been found to match phage Izzy, and few others to match Caliburn and Aaronocolus. These phages are found in sub cluster BD1, which makes sense as to why Oliynyk shares similar genes. Although, Oliynyk's

genes may seem common, many of them are quite unique, because they cannot be found to match any other genes when searching through various databases. Also, Oliynyk is estimated to have 79 genes, which is 5 genes more than its most genetically similar phage, Izzy. Oliynyk also has a GC content of 65.9% and a length of 49976 base pairs. The phage Oliynyk has been isolated from bacterium *Streptomyces xanthochromogenes*, and is unique, because it contains unrecorded genes not found anywhere else, and if all current genes remain after annotation, will be the phage with the most genes in sub cluster BD1.

U51. Adaptation and Replication of Bacteriophage Recombineering with Electroporated DNA Point Mutation in Streptomyces Bacteriophages

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Bacteriophages are one of the most abundant life forms on the planet living resiliently in all corners of the earth. With relatively simple and small genomes they make for ideal organisms for genomic studies such as gene expression or phylogeny. Previous studies conducted by the University of Pittsburgh have shown that strains of bacteriophages can be consistently created that contain just a single point mutation difference from the wild type genome. This protocol, dubbed the BRED protocol, is made possible by the induction of two phage derived genes (Che9c genes 60 and 61) in plasmid pJV53 that when expressed, substantially increase recombination frequencies between phage genomes and mutant oligos. This process has the potential to silence, or knockout, genes allowing researchers to predict the function of the gene. This protocol was designed for use in mycobacterium phages, the goal of this experiment is to first replicate the results of the BRED protocol in Mycobacterium phage EagleEye and then adapt this protocol for use in *Streptomyces* phages, successfully creating a mutant of a *Streptomyces* bacteriophage using the BRED protocol.

U52. Latency Period Evaluation of *Streptomyces griseus* Infected with Bacteriophage BryanRecycles

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Bacteriophages are a group of diverse viruses that infect and replicate in a bacterial host. Phages display a profound level of uniqueness, and express a distinct set of characteristics that sets them apart from other organisms. Phages are used to study genetics, and some can be utilized as a genetic transfer vector. A latency period for a phage is the time the phage remains within the cell before lysis. To identify the latency period for a *Streptomyces* phage, cells of *Streptomyces griseus* were infected with phage BryanRecycles at a 10:1 multiplicity of infection (MOI). A viral titer assessment was conducted at 0, 30, 60, 90, 120, 150, 180, 210, and 240 minutes post-infection to evaluate progression of phage lysed out of bacterial cells. This data could be used for future endeavors that will analyze gene expression at these time intervals, and used as an additional comparative tool amongst phages within the same cluster.

U53. Using The DnaB Helicase Gene In The Mycobacteriophage ‘Wyatt2’ To Further Understand *M. tuberculosis*

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In 2015, it was reported about 1.8 million people died from tuberculosis. *Mycobacterium tuberculosis* causes infectious tuberculosis. Mycobacteriophages are viruses that infect mycobacterial hosts, such as *Mycobacterium tuberculosis (TB)* and *Mycobacterium smegmatis (M. smeg)*. In this project we purified, sequenced, and annotated a novel Mycobacteriophage named ‘Wyatt2’. ‘Wyatt2’ is classified as a cluster L, sub cluster L1 with 122 putative genes and 9 putative tRNAs. *M. smeg* is the host bacteria used to isolate Wyatt2 because it’s closely related to *TB*. Based on GeneMark coding maps, we found that ‘Wyatt2’ had higher coding potential to *TB* then *M. smeg*, which may suggest a closer lineage. We counted 122 genes in total and compared each gene between the *M. smeg* and *TB* GeneMark coding

maps. In fact, about 96.7% of genes from ‘Wyatt2’s genome had coding potential with *M. tuberculosis* while about 73.8% of genes with *M. smeg*. During annotation of Wyatt2’s genome, gene number 68 in the sequence had similar characteristics to DnaB helicase. DnaB functions as a helicase by unwinding DNA. This process starts when DnaA loads a DnaB-DnaC complex onto the DNA. Once the DnaB reaches the replication fork DnaC is released, DnaB then begins to unwind the DNA. The DnaB helicase in Wyatt2’s genome has the potential to give further insight of the function of DnaB in *TB*. Future studies include the mechanism for DnaB, which could potentially create a drug target to stop or hinder the replication of pathogenic multi-drug resistant *TB*.

Graduate Student Posters

G1. Comparative Analysis of Four Cluster BI1 Streptomyces Phages.
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The vast diversity of bacteriophages presents an opportunity to discover novel isolates and study their genetic composition. Our research focuses on isolating bacteriophages from the diverse and industrially-important genus *Streptomyces*. As part of this project, we isolate, sequence, and annotate phages from soil using several *Streptomyces* species as hosts. In this report, four novel *Streptomyces* phages, DrGrey, OlympicHelado, Rima, and Spectropatronm, belonging to the BI1 cluster were isolated, annotated, and compared to one another. The phages in this study have a GC% content from 59.4 to 59.6 and range from 55,707 kbp to 56,189 kbp in length. Three of the phages, DrGrey, OlympicHelado, and Spectropatronm, were isolated using *Streptomyces griseus* ATCC 10137 as the host, while the fourth, Rima, was isolated on *Streptomyces azureus* NRRL B-2655. Interestingly, OlympicHelado and Rima, novel members of the cluster found on two different host species, were isolated from the same New York soil sample. The other phage isolates were found from different soil samples collected in Texas. Based on our annotation results, Phamerator data, and dot plot analysis, the phages are very similar. They only differ by a few genes, most prominently towards the right end of the genome. OlympicHelado contains 88 putative genes and Rima contains 87 putative genes. Of the putative genes, 83 are homologous. OlympicHelado and Rima share 99% average nucleotide identity with each other. DrGrey contains 80 putative genes and share 89% identity with OlympicHelado, Rima, and Spectropatronm. Spectropatronm contains 84 putative genes and share 98% identity with OlympicHelado and Rima. They all follow typical synteny with structural genes in the left end of the genome and functional genes in the right end. These results show that phages isolated using same host, contain similar characteristics, and phages isolated from same soil, contain similar characteristics despite their host being different.

G2. Preliminary Analysis of Select Programmed Cell Death Genes in Chlamydomonas reinhardtii
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Programmed cell death (PCD) can be defined as any form of cell death which is coded for by the cell's own genome. PCD is an essential process to mammals during embryonic development, as it allows for the development of complex structures, such as differentiated digits. PCD is also necessary in adult mammals, as aged, malfunctioning, and damaged cells also undergo PCD to prevent the proliferation of these abnormal cells. Much of the PCD machinery appears to be conserved throughout the tree of life, suggesting that PCD may have arisen very early in evolutionary history. Thus, the study of more evolutionarily "simple" organisms may lead to insights regarding the evolution of PCD. The unicellular green alga *Chlamydomonas reinhardtii* serves as a representative intermediate in the early evolution of higher plants, and has provided a number of insights into general and plant specific-processes. Previous studies show that, when faced with an environmental stressor, *C. reinhardtii* undergoes PCD that is similar to PCD observed in other organisms. However, many of the essential PCD genes in other organisms appear to be absent in *Chlamydomonas*. The goal of the current study is to identify conserved aspects of the PCD machinery in *C. reinhardtii*. In order to achieve this, we first extracted the names and amino acid sequences of proteins from all eukaryotes with the keywords "programmed cell death" and/or "apoptosis" from the NCBI Proteins database, which we compiled into a local database. The predicted *C. reinhardtii* proteome was then used to query this local database using the BLASTp algorithm. This produced *C. reinhardtii* proteins which are sequentially similar to proteins involved in the PCD process. From this list of *C. reinhardtii* proteins, several were selected for further study for their involvement in *C. reinhardtii* PCD. Currently, a genetic approach is being used to determine the involvement of these proteins in *C. reinhardtii* PCD.

G3. Recovery Analysis of a Mucosal Microbiome after Antibiotic Exposure using a Fish Model

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It is well-established that antibiotics can cause a major disruption to the homeostasis of the human microbiome that can inflict consequential side-effects hazardous to our health. The early recolonization events following disruption and how they link to the eventual stable community structure are unclear. The focus of this research is to observe potential factors involved in driving the bacterial community structure during recovery from antibiotic exposure. Factors include environmental conditions, host mucosal components, and/or bacterial antagonism. Previous work in our lab has shown that antibiotic can alter the community composition of the fish microbiome, and this can result in negative host effects, including susceptibility to infection and lack of weight gain. The mucosal skin microbiome of a small vertebrate fish, *G. affinis*, exposed to a broad-spectrum antibiotic will be monitored in two different recovery conditions, sterile versus aquarium water. Change in the bacterial community's composition will be detected by 16S rRNA sequencing/profile analysis along with estimating the bacterial load via a semi-quantitative method using PCR amplification of the V2 region of the 16S rRNA gene. An indirect assessment of the community diversity inter/intra fish skin microbiotas will be analyzed by the community fingerprinting method Enterobacterial Repetitive Intergenic Consensus (ERIC)-PCR. In conjunction with bacterial load, the amount of skin mucus proteins on the fish will be quantitated by using the Alcian Blue method. Bacteria in the water column and water quality will also be inspected throughout the experiment. Findings from this study will observe changes within the fish skin microbiota to better understand microbial restructuring dynamics during recovery from a disruptive event and the relative importance of mucus and the environment.

G4. Modification of intracellular calcium signaling by Community Acquired Respiratory Distress Syndrome Toxin of *Mycoplasma pneumoniae*

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Mycoplasma pneumoniae (*Mp*) is the leading cause of community acquired pneumonia in children and demonstrates an ability to exacerbate chronic conditions such as asthma. The ADP-ribosylating and vacuolating toxin, Community Acquired Respiratory Distress Syndrome (CARDS) toxin, is the major virulence determinant of *Mp*. Deciphering the mechanisms through which CARDS toxin performs its pathogenic functions is key to understand *Mp*-mediated pathogenicity. Intracellular calcium signaling has been shown to play an important role in a variety of mechanisms during bacterial toxin-mediated pathogenesis. Among these mechanisms, the induction of pro-inflammatory cytokine expression and the initiation of cytoskeletal rearrangements hold some of the most significant implications for host cell damage. Here, we examine the role of intracellular calcium signaling in CARDS toxin-mediated pathogenesis and its impact on specific downstream events observed during *Mp* infection.

G5. Characterization of *Mycoplasma pneumoniae* CARDS Toxin-Mediated Cytoskeletal Reorganization

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Mycoplasma pneumoniae (Mp) is a respiratory bacterial pathogen and the primary cause of community acquired pneumonia. Mp is also responsible for a broad spectrum of other respiratory tract infections, including tracheobronchitis, bronchiolitis, pharyngitis, and asthma. Community-acquired respiratory distress syndrome (CARDS) toxin, which is the major virulence determinant of Mp, is able to recapitulate Mp infection-associated pathologies in murine and primate models. The amino terminal region of CARDS toxin exhibits ADP-ribosylating activity, and the carboxy terminus induces vacuole formation. In addition, CARDS toxin triggers cytoskeletal reorganization in epithelial cells and tracheal organ cultures. Exploitation of the host-cell cytoskeleton by microbial toxins facilitates pathogen entry into target cells, dissemination within and between infected tissues, avoidance of phagocytic cells, and intimate

attachment to host cell surfaces. Here, we describe how CARDS toxin exploits the host-cell cytoskeleton and induces cytoskeletal rearrangement. Further, we reveal the role of ADP-ribosylating and vacuolating activities of CARDS toxin in mediating morphological changes and identify and characterize the targeted cytoskeletal proteins. Understanding the mechanisms of CARDS toxin action provides a basis to generate vaccines and therapies to combat Mp infection and develop new strategies to use toxin derivatives for the treatment of human disease.

G6. Antimicrobial susceptibility assessment of clinical & environmental *Clostridium difficile* isolates in relation to CRISPR-Cas

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Clostridium difficile is the most common causes of healthcare-associated infection in the US & worldwide that is posing a significant challenge to the healthcare industry. Resistance to the commonly used antimicrobials is a hallmark of C. difficile infection. Mutant selection and acquisition of resistance gene are the common mechanisms of antibiotic resistance. In addition to various factors affecting this resistance mechanism, exposure to antimicrobials as well as CRISPR-Cas associated adaptive immune system may also play a vital role. This study aims to assess the differences in antimicrobial susceptibility patterns based on presence/absence of the CRISPR-Cas system stratified by sources of isolation (clinical vs. environmental). A total of 47 clinical C. difficile isolates and 15 environmental isolates were used in this study. Previously ribotyped C. difficile isolates were CRISPR typed based on the presence of Cas1 gene which is a suitable marker for the presence of potentially active CRISPR-Cas system. 48-hour MIC was determined from the overnight culture of each of the C. difficile strains using microdilution method for 4 different antibiotics (Vancomycin, Metronidazole, Fidaxomicin, and Ridinilazole). MIC50, MIC90, and geomean of the MICs were calculated for each of the antibiotics. 48-hour MIC for a standard strain (R20291) was used to compare the antibiotic sensitivity as well as resistance pattern of the 47 clinical strains (39 CRISPR-positive and 8 CRISPR-negative) and 15 environmental strains (12 CRISPR-positive and 3 CRISPR-negative). Ribotypes looked at included O27 (29), O14-20 (6), O78-126 (18) and others (9). The range of MIC for clinical strains for various antibiotics were as follows- Vancomycin (0.25 to 16 g/ml), Metronidazole (0.24 to 7.66 g/ml), Fidaxomicin (0.03 to 1.92 g/ml) and Ridinilazole (0.03 to 0.24 g/ml). For environmental strains the MICs were Vancomycin (0.5 to 4 g/ml), Metronidazole (0.24 to 1.92 g/ml), Fidaxomicin (0.06 to 0.48 g/ml) & Ridinilazole (0.06 to 0.48 g/ml). There seemed to be a subtle difference in the MIC based on the source of the strains especially those with higher MICs however the MIC50 was comparable between them. The geomean of MIC (vancomycin, Metronidazole, Fidaxomicin, Ridinilazole) based on presence of CRISPR gene is higher among the CRISPR negative (0.85, 1.87, 0.24, 0.15) compared to CRISPR-positive strains (0.90, 0.48, 0.06, 0.08). There was no significant difference in the MIC by ribotypes. The presence of CRISPR in C. difficile might have the potential to protect against the development of antibiotic resistance against commonly used antibiotic.

G7. The Physical and Genomic Characteristics of Actinobacteriophage Sebastisaurus

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Bacteriophages are abundant and diverse microorganisms present in a variety of environments, including but not limited to water, plants, animals, and soil. They play a vital role in the ecology and evolution of bacteria. Sebastisaurus was isolated from soil collected in Easley, South Carolina in July of 2016. It was the first phage isolated using *Streptomyces xanthochromogenes* as the host. It was isolated through enrichment and then purified. Physical properties such as plaque size and host range were observed. DNA was then extracted and sequenced using MiSeq, an Illumina sequencer. This was followed by bioinformatical analysis to determine the genome length, GC content, and cluster information. This phage has a genome length of 41609 bp and a GC content of 62.1%. It is part of the BB cluster, while most of the *S. xanthochromogenes* phages isolated and sequenced recently, during a research class, were identified as belonging to the BD cluster of Actinobacteriophages. Besides Sebastisaurus, the BB

cluster includes the well-known and widely used phiC31 phage, as well as three other phages. Sebastisaurus is the first and currently only phage to make up the BB2 subcluster, since the other four phages in the cluster share more similarities and make up the BB1 subcluster. Phages help in the understanding of genetics and bacteria, and may aid in combating antibiotic resistance.

G8. TGF-β signaling is required for a variety of immune challenges by the model organism *C. elegans*
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The innate immune response coordinates several molecular activities, including a cell-cell signaling pathway called TGF-β (Transforming Growth Factor-β), conserved in species from simple animals to humans. In *C. elegans*, a TGF-β signaling pathway is required for an innate immune response to fight fungal and bacterial infection. While the response to fungus is mediated by a non-canonical TGF-β pathway, the specific pathway that responds to bacterial infection is unknown. To determine this, we have challenged normal and TGF-β mutant nematodes with Gram positive and negative bacteria. We have quantitated and compared nematode survival, avoidance behavior, and intestinal pathogen colonization. We will look for cross-talk between TGF-β and other pathways upon exposure to pathogens. This will establish the specific TGF-β pathway nematodes use upon immune challenges. This work may aid in sensitizing parasitic nematodes, which are becoming resistant to current anthelmintics, causing major crop losses.

G9. The Regulation of PCA1 by the Nonsense-Mediated mRNA Decay (NMD) Pathway
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The requirement of copper as a micronutrient remains throughout organisms ranging from prokaryotes to humans (Pena, Lee & Thiele 1999). Enzymes which require copper to function are involved in several physiological processes. Malfunction of these enzymes are the cause of diseases such as Menkes and Wilson's disease, which are both associated with dysfunctional copper transport. However, despite its necessity, accumulation of excess copper can be fatal to the cell. Homeostatic balance of metal ions is achieved through a number of interrelated proteins responsible for binding, transport and sequestration dependent on environmental conditions. One of these proteins is a copper-transporting ATPase called PCA1 (Adle et al. 2007). It has been previously shown (Peccarelli et al. 2016, Guan et al. 2006) in *Saccharomyces cerevisiae* that *PCA1* is regulated indirectly by the Nonsense Mediated mRNA Decay (NMD) pathway. The NMD pathway triggers rapid degradation of mRNAs that prematurely terminate translation. This includes mRNAs containing PTCs (Premature termination codons) and natural mRNAs lacking PTCs in their coding region. These natural mRNAs contain NMD-targeting features. We have shown that a subset of mRNAs involved in copper homeostasis are regulated by NMD. Here, we investigate the regulation of PCA1 mRNA by NMD.

G10. Functional analysis of a temperature sensitive mutation in a cytomegalovirus virulence protein
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Human cytomegalovirus (HCMV) is a common cause of illness in immuno-compromised patients and a major infectious cause of birth defects. Because cytomegalovirus (CMV) is species specific, mouse CMV (MCMV) is used as a model to study disease mechanisms of CMV. An important gene family in cytomegalovirus is the US22 gene family which includes 12 members in both HCMV and MCMV. The three members of this gene family, M139, M140 and M141 in MCMV are homologous to US22, US23 and US24 respectively in HCMV. A previous work has shown that M139, M140 and M141 proteins form a complex and this complex is required for the pathogenesis of MCMV in macrophages. The deletion of any one of these genes will lead to the replication impairment in macrophages and this defect is associated with the reduced stability of structural proteins of virus such as major capsid protein (MCP). The virus with

truncated M139 protein was shown to be temperature sensitive by Dr. Clive Sweet's lab. This mutant virus has normal replication at 37°C but the replication is impaired at 40°C, like during fever. Our preliminary analysis showed that the level of mutant M139 protein is reduced compared to wild type M139 even at 37°C suggesting that the truncated M139 protein has reduced stability. Also, the analysis of replication of deletion mutants at 40°C suggested that M139 may also have a function outside the complex which was not previously identified. Understanding the reason behind inefficient replication at an elevated temperature will clarify the role of M139 in infection and may lead to the development of new ways to control CMV.

G11. Regulation of Denitrification in *Sinorhizobium meliloti* Under Aerobic Conditions

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Sinorhizobium meliloti is a soil dwelling bacteria capable of forming a symbiotic relationship with the legume alfalfa. The establishment of a successful symbiotic relationship relies on the presence of a functional quorum sensing system (QS). A complete quorum sensing system in gram negative bacteria consists of a LuxR-like response regulator and an autoinducer synthase which produces diffusible signal molecules. Two complete QS systems have been found in *S. meliloti*, as well as several "orphan" LuxR-like regulators which lack an associated autoinducer synthase. *SMc00658* is one such regulator. To determine the potential role of *SMc00658*, microarray analysis was used to compare expression differences between wild type *S. meliloti* and the *SMc00658* mutant. Results suggest that *SMc00658* is involved in the regulation of denitrification in *S. meliloti* under normal growth conditions. The ability to convert nitrate and nitrite to more reduced compounds enhances the survival of these soil organisms and helps *S. meliloti* establish successful symbiosis with its plant host. The genes responsible for producing the enzymes necessary to reduce nitrogen are present on one of the symbiotic plasmids (pSymA) of the *S. meliloti* genome and are under the control of the FixLJ regulatory system, also present on pSymA. Previous studies have extensively explored the regulatory role of the FixLJ system in denitrification under low oxygen conditions. However, the presence of *SMc00658* has not been detected under these conditions, despite the significant overlap of genes whose expression is influenced by both FixLJ and *SMc00658*. This introduces several interesting questions regarding *SMc00658*'s role in denitrification: (1) It is known that FixLJ responds to low oxygen as a signal to start the denitrification process. What signal does *SMc00658* respond to? Is this signal external (such as oxygen) or produced by the cell? (2) Where does *SMc00658* fit in the denitrification regulatory cascade? (3) Under what conditions is *SMc00658* active? Elucidating the function of this LuxR-like regulator will provide better insight into the signals LuxR-like regulators are able to detect as well as the roles that these regulators can fulfill outside of quorum sensing systems.

G12. Protein-protein Interactions May Impact a Virulence Factor in Group B Streptococcus

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Streptococcus agalactiae, group B Streptococcus (GBS), is an important human pathogen, causing life-threatening sepsis and meningitis in newborns. The virulence of GBS in part can be attributed to its ability to produce a polysaccharide capsule that helps with immune cell evasion. CpsA, a member of the LytR-CpsA-Psr protein family, has been recognized as an integral part of capsule synthesis. Previous work has shown that mutations and deletions of CpsA lead to a decrease in capsule production, change in cellular shape and arrangement, and a decrease in virulence. Protein-protein interactions play a role in many virulence mechanisms and may be required for CpsA function. Using a Bacterial Adenylate Cyclase Two-Hybrid (BACTH) system we have demonstrated CpsA interactions using two different methods. By using screening techniques on LB/X-gal and MacConkey/maltose media we have demonstrated CpsA interactions with predicted binding partners CpsC protein and weak interactions with the CpsY protein. We have also quantified these interactions by using β-galactosidase assays. Using the BACTH system has also allowed us to investigate whether these protein interactions are occurring at the N terminus or the C terminus of the proteins. Furthermore, with i*n vivo* MBP-Nanotrap immunoprecipitation assays

(ChromoTek) we are identifying and isolating additional proteins that have direct interactions with the CpsA protein that may have not been a predicted partner in *S. agalactiae*. We expect to see the CpsC and CpsY proteins being pulled down as a positive control since we have already shown interactions with CpsA using the BACTH system. Any proteins pulled down that are of interest can be cloned into the BACTH vectors to confirm these interactions and determine whether the involved interactions occur with the same regions as CpsC and CpsY. Understanding protein-protein interactions of CpsA will help in new drug development as we will know which part(s) of the protein are the most important for interactions and can specifically target these.

G13. Isolation and Characterization of Three Phages Infecting *Streptomyces azureus*

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As bacteria evolve to become immune to antibiotics, it is important to study bacteria and bacteriophages to develop effective antibiotics. One bacterium that may help us in this regard is *Streptomyces azureus*, which produces antibiotic thiostrepton. Isolating novel phages using *S. azureus* as a host, sequencing and annotating the genomes may help us to find new tools that could be used to develop antibiotics. Phages Alsaber, Rowa, and Andalus were isolated using *S. azureus*. They were isolated from enriched soil and sequenced by Illumina Sequencing method. Alsaber was isolated from Denton, TX, and it's siphoviridae phage that creates small clear plaques with a diameter of approximately 0.5 mm. The length of its genome is 48,803 base pair with GC content of 65.9 %. The sequencing shows that it's circular genome with 3' sticky overhang. After completing initial annotation, we found Alsaber belongs to the BD3 sub-cluster. Also, it yields 76 putative genes with no tRNA. Rowa was isolated from New Braunfels, TX. Its plaques are about 1 mm in diameter. The genome draft shows it has 61 genes and 7 tRNAs. The genome length is 42,890 with GC content of 65.9 %. Andalus was also isolated from Denton, TX and from the same soil sample as Phage Alsaber. The restriction enzymes experiment displays similarity between phage Andalus and phages under subcluster BD2. Upon completion of this project, we'll acquire more understanding of *S. azureus* phages and Actinobacteriophage in general, which will expand the research of bacteriophages.

G14. Metagenomic investigation of antibiotic resistance in three urbanized Texas bays

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The purpose of this research is to investigate the prevalence and diversity of antibiotic resistance genes (ARGs) in three urbanized Texas bay systems. Coastal bays adjacent to developed and urbanized land are sinks for runoff and municipal wastewater. These inflows carry residual antibiotic compounds and antibiotic resistant bacteria (ARB) that pose a threat to human and environmental health. This study seeks to investigate Galveston Bay, Copano Bay, and Corpus Christi Bay, Texas. Each bay receives a different type of major wastewater inflow dependent on the type of surrounding developed land. Galveston Bay, Copano Bay, and Corpus Christi Bay primarily receive urban sewage pollution, agricultural runoff, and industrial runoff respectively. Each type of inflow bears a unique suspension of chemical inducers of ARG development. This study proposes that oysters, which accumulate contaminants from the surrounding water via suspension-feeding, are an ideal sentinel organism for the investigation of antibiotic resistance in the coastal environment. Bacterial DNA metagenomes of oyster gut contents collected from Galveston Bay, Copano Bay, and Corpus Christi Bay were sequenced using Illumina HiSeq technology. The metagenomes are being examined to assess the prevalence of ARGs in these bays. Data describing the prevalence of ARGs – a promising proxy for coastal sewage pollution – are needed to aid officials in managing healthy coastal ecosystems and preserving human health.

G15. Repurposing Auranofin and Ebselen as Antifungals: New Uses for Old Drugs

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Candida albicans is a commensal organism that resides naturally on and in the human body. This fungus has the ability to cause opportunistic infections in immune- and medically-compromised individuals. Candidiasis now represents the third most frequent nosocomial infection in US hospitals, carrying high mortality rates of 30 to 60%. One of the main reasons for this is the very limited arsenal of antifungal drugs available to treat these infections. Despite the dire need for new medications, the antifungal drug pipeline has been mostly dry for more than a decade, and developing new antifungals could take up to 20 years. Drug repurposing, *i.e.* finding new uses for old drugs, represents a potentially faster and more economical alternative for the identification and accelerated development of drugs with antifungal activity. We have used high content screening techniques of a repurposing library (Prestwick Chemical Library) containing 1,200 mostly FDA-approved off-patent drugs, in search for compounds displaying antifungal activity. Selected promising hits from these screenings were further examined for their *in vitro* efficacy against *C. albicans*, including collection strains and clinical isolates, both under planktonic and biofilm growing conditions using the 96 well-plate method developed by our lab. The activity of two leading compounds against *C. albicans* was also tested in combination with conventional antifungals (amphotericin B, fluconazole, and caspofungin) using the checkerboard methodology. Moreover, we also evaluated the activity of two of the leading compounds against different *Candida* spp., since an increasing number of candidiasis are caused by species other than *C. albicans*. We also investigated their activity against *Cryptococcus neoformans*. Results from initial screenings identified the antifungal activity of two organometallic drugs, Auranofin and Ebselen. Both drugs were found to display potent antifungal activity against not only *C. albicans* but also against several other *Candida* spp., including fluconazole and echinocandin resistant strains; as well as against *C. neoformans*. Indifference was mostly observed when these two leading repurposing candidates were used together with clinically-used antifungal drugs, indicating their potential for combination therapy. Overall results indicate that both drugs represent promising candidates to be repurposed as antifungals for the treatment of candidiasis, cryptococcosis, and potentially other fungal infections.

G16. Culturing the unculturable using single cell sorting

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It has been estimated that less than 1% of the world's microbes can be successfully cultured. The limited success of culturing and isolating individual microbes is a consequence of the difficulties that would be involved in analyzing them within their natural environment, which is complex and contains many unknown variables. This method provides a new way to skip the traditional enrichment step in which a majority of the rare biosphere is lost due to not providing the needed nutrients at the onset.
Identification of microbial communities based on 16S rRNA gene target analysis relies on public databases such as National Center for Biotechnology Information (NCBI), which contains a high percentage of uncultured representatives. Increasing the number of cultured representatives within these databases not only strengthens the identification of unknown organisms, but also aids in our understanding of their functionality. This project will utilizes single cell sorting to culture cells from coastal sediment in Oso Bay, Corpus Christi, Texas. Approximately 5 g of sediment was suspended in a 1X phosphate-buffered saline (PBS) solution, gently vortexed to separate cells from sediment matrix, then centrifuged on a low speed. The supernatant containing suspended cells was sorted into 96-well plates at 1, 5, and 10 cells per well using a BD JazzFACS. Each well was filled with either marine broth (e.g., peptone, yeast extract, sodium chloride, and nutrients common to seawater) or Luria-Bertani (LB) broth (e.g., peptides and casein peptones, vitamins). Growth was monitored daily via optical density on a plate reader for 10 days and absorbance was recorded. Visible changes within the wells such as clarity and coloration were also observed. No template negative controls were carried throughout to verify lack of contamination within the media. Cells sorted into LB broth, on average, had greater growth rates whereas the cells grown in marine broth showed lower overall growth. Future studies will include identifying the isolates using 16S rRNA gene targets and manipulating the isolates to simulate environmental perturbations such as changes in salinity, temperature, and nutrient availability. In the future, this method will be applied to deep subsurface biosphere sediments, aiming to cultivate and characterize this microbial community.

G17. Houseflies and Cockroaches as Potential Vectors of Toxigenic *Clostridium difficile*

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Housefly and cockroaches are highly prevalent in the environs and are known transmission vectors for various human and animal pathogens. Spores of *Clostridium difficile* are ubiquitous in the environment and associated with fecal contamination, a known habitat for housefly and cockroaches. The objectives of this study were to investigate the vector potential of these insects by isolation and characterization of *C. difficile* from cockroach and houseflies. We purchased houseflies and cockroaches from a commercial scientific store and exposed them to spores of a toxigenic *C. difficile* (MT2210, Ribotype R027) for a short period (10-100 min) in confined containers. Thereafter insects were maintained in batch or individually and processed to isolate *C. difficile* following standard anaerobic culture method using selective culture media from insect body surfaces and guts over the subsequent 30 days. Live cockroaches were also collected from various community environments to isolate and characterize *C. difficile* using culture and molecular methods. *C. difficile* was universally isolated from the *C. difficile* exposed insects from our laboratory microcosms from insect gut and body surfaces for up to 30 day time period. Interestingly, another batch of 24 houseflies were tested after 134 days (dead flies) from the 2 hours of spore exposure and 15/24 (62.5%) tested positive from direct plating on CCFA and the 9 negatives from direct plating were 100% positive when enriched and plated on CCFA. Of the 120 samples collected as part of our field study, 5 samples (4.2%) were positive for toxigenic (*tcdA* and *tcdB*) *C. diffficile*. Our preliminary data demonstrated the vector potential of both the insects (houseflies and cockroaches) for toxigenic *C. difficile*. Field collections of some cockroaches were harboring toxigenic *C. difficile*. The results will need to be confirmed in various source houseflies and cockroaches with a larger number of samples.

G18. Optimization of a Genomic DNA Extraction Protocol for *Clostridium difficile* and Human Fecal Microbiome Using an Automated Simple Throughput System

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Successful molecular work and sequencing of the bacterial genome requires high-quality genomic DNA (gDNA). However gDNA extraction from *Clostridium difficile* using manual methods is difficult, laborious, time consuming, and expensive. The objectives of this study were to develop an optimized high-throughput protocol for an efficient, cost effective and automated genomic DNA extraction from *Clostridium difficile* and human fecal microbiome. A total of 40 clinical *C. difficile* isolates (5ml taken after at least 18 hours of anaerobic incubation) and 40 human fecal samples (300mg each) were used in this study. Genomic DNA were extracted by using AnaPrep automated platform (Biochain Institute, Inc., Newark, CA) and compared with existing standard protocols for *C. difficile* DNA extraction (QIAamp DNA Mini Kit), and fecal microbiome samples (MoBio, PowerSoil DNA Isolation Kit). The optimized protocol for automated DNA extraction obtained high quantity gDNA in quantities that ranged from 200ng-87µg (mean 56ug). The manual kit obtained high quality DNA but at lower amounts (900ng-17µg DNA; mean 3.25ug). For human fecal samples the quantity and quality of microbial gDNA were comparable with the automated and manual methods with typical yield that ranged from 100ng-38µg. Downstream laboratory investigations using the gDNA for whole genome sequencing of *C. difficile* and microbiome analysis revealed positive results. Our results demonstrated that an automated genomic DNA extraction platform can be used to isolate high quantity and high quality genomic DNA from *C. difficile* and stool microbiome for downstream PCR and genomic works.

G19. Microscopic and Molecular Epidemiology of Asymptomatic Falciparum and Vivax Malaria in South-Central Oromia, Ethiopia

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As per World Health Organization estimates, 3.2 billion people – nearly 50% the world's population are at risk of malaria, a mosquito-borne disease. In 2015 alone, 214 million people got malaria worldwide, 438,000 of whom died of the disease. Whereas global efforts are currently in high gear to eliminate the disease, asymptomatic malaria carriers seriously undermine these efforts, since such individuals won't seek treatment. As such, they serve as a silent, undisturbed gametocyte reservoir of the infection. To bridge the data gap regarding the prevalence of asymptomatic malaria in Ethiopia, finger-prick blood samples of 1094 apparently healthy individuals aged two years and above in south-central Oromia region were screened for malaria parasites using microscopy, the rapid diagnostic test (RDT), and polymerase chain reaction (PCR). PCR was done only on microscopy-positive and RDT-positive samples. By microscopy, overall asymptomatic plasmodium parasite prevalence was 5.0%, while it was 8.2% by RDT. 89.4 % and 77.2 % of the microscopy-positive and RDT-positive samples were PCR-positive, respectively. Age and parasite densities were inversely related. These results indicate that asymptomatic malaria exists in the Oromia region of Ethiopia. Moreover, only the use of the more sensitive molecular diagnostic techniques besides microscopy can ensure getting the true picture of asymptomatic malaria prevalence.