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Abstract Book

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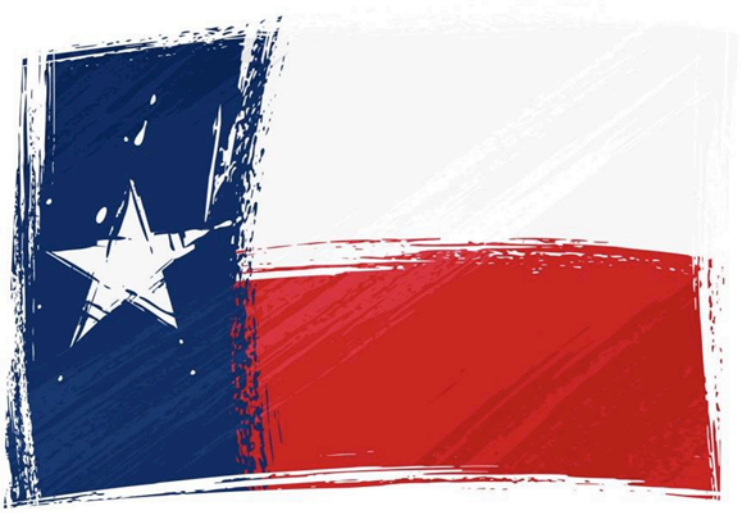

Undergraduate Student Oral Presentations

1. A Multimeric qPCR Assay to Survey Avian Retroviruses

*Faith Cox[1], Tory Perlichek[2], Jason Hardin[2], and Dustin Edwards[1]
[1]Department of Biological Sciences, Tarleton State University, Buffalo, TX
[2]Upland Game Bird Program, Texas Parks and Wildlife Department, Buffalo, TX

Reticuloendotheliosis virus (REV) and lymphoproliferative disease virus (LPDV) are immunosuppressive avian retrovirus affecting Galliformes. Modern testing for REV utilizes a duplex quantitative polymerase chain reaction (qPCR) technique developed by Texas Veterinary Medical Diagnostic Laboratory. Here, we modified their technique to a multiplex qPCR assay adapted to simultaneously test for REV and LPDV. The pan-avian constitutive *GAPDH* gene was designed as an internal control in testing for DNA or cDNA from RNA viral genomes. REV was replicated in chicken fibroplast DF-1 cells and sequence targets were amplified with PCR. LPDV inserts were amplified with PCR from proviral DNA (courtesy of Nicole Nemeth, University of Georgia). PCR products were purified with the "freeze and squeeze" method. Plasmids were constructed by TA cloning in pCR2.1 vectors, and confirmed by Sanger sequencing, to contain the target REV *LTR*, REV *Env*, LPDV *Gag*, LPDV *Env* and *GAPDH* genes. After plasmid DNA purification, DNA molecule copy number was determined and serial diluted to form a standard curve of 1×10^8 to 1×10^1 molecules/μL. TaqMan probes for the REV *LTR*, REV *Env*, and *GAPDH* target genes were redesigned to contain double non-fluorescent quenchers to decrease false positives and Taqman probes for the LPDV *Gag* and LPDV *Env* genes were designed to have single non-fluorescent quencher probes. Reactions were performed in triplicate to establish the standard curve, with slopes of the log concentration measured, allowing for precise determination of the identity and quantity of virus in a sample. A total of 206 dried and 21 whole blood samples collected from January 2018 to February 2019 by Texas Parks and Wildlife Department biologists from La Salle, Comel, Menard, Wilks, Mason, Cottle, and Gillespie counties. DNA was extracted, and viral DNA measured by qPCR to determine viral prevalence rates.

2. Does *Lactobacillus gallinarum* protect *Caenorhabditis elegans* from the effects of infection with *Salmonella enterica* serovar Montevideo*?*

*Blanca A. Garcia, Patricia J. Baynham
Department of Biology, St. Edward's University, Austin, TX

Lactic-acid bacteria (LAB) are continually recognized to be in the microbiota of humans and animals. While the mechanisms around the probiotic effects of these bacteria are not completely understood, it has been noted that some LAB have the ability to extend a model animal's lifespan. *Salmonella enterica* includes various strains of bacteria that are able to cause gastrointestinal disease in various organisms. The Centers for Disease Control and Prevention

(CDC) estimates that *Salmonella* is attributed to 1.2 million illnesses, leads to 23,000 hospitalizations, and is the cause of 450 deaths in the United States annually. Agar overlay assays were performed to determine the inhibition of pathogens against the LAB strains. The pathogen strains *Salmonella enterica* Montevideo gfp-GM, *Salmonella enterica* St. Paul-gfp, *Salmonella enterica* Typhimurium-gfp, and *Listeria monocytogenes* NADC2783-gfp were paired against the LAB strains *L. gasseri* ATCC 33323, *L. gallinarum* ATCC 33199, *L. acidophilus* NCFM*, L. crispatus* JCM 5810. From this assay, *L. gallinarum* showed the greatest inhibition of *S.* Montevideo. *Salmonella* Montevideo is known as the sixth most common serovar. In order to examine whether LAB can protect against *S.* Montevideo infection, *Caenorhabditis elegans* was selected as the model organism. In this study, *Lactobacillus gallinarum* was tested to see if it protects against *S.* Montevideo-gfp infection in free-living nematodes. Adult nematodes that were fed *L. gallinarum* or *E. coli* OP50 were challenged with *S.* Montevideo-gfp. The *L. gallinarum* feeding for 96 hours led to a reduction in fluorescence in the C.*elegan* gut suggesting decreased colonization. Life expectancy increased in nematodes fed with the LAB and then challenged with *S.* Montevideo compared to those fed OP50. Antagonistic activity of LAB against pathogens has been highly documented and attributed to the production of metabolites such as organic acids. This in addition to competitive exclusion, likely prevents the colonization of the intestine by pathogens. Previous studies have shown that *L. gallinarum's* antagonistic activity is due to this. Because *L. gallinarum* showed inhibitory properties against *S.* Montevideo and increased life expectancy of *C. elegans* they could be a potential probiotic candidate for treatment. Ongoing research will focus on performing a CFU assay and *L. gallinarum* should be studied further for properties that could be used as prevention of infections.

3. Comparative Analysis of Lytic and Lysogenic Phage Genomes in Search of Phages With Therapeutic Potential

*Sara Henry, Gregory D Frederick and Frederick N Baliraine
Department of Biology and Kinesiology, LeTourneau University, Longview, TX

Bacteriophages go through either of two life cycles, lytic or lysogenic. Because of the way each life cycle occurs, it can be expected that viruses would require different genes in order to either effectively integrate into a host's DNA or destroy the host cell. To determine which key genes are shared between or unique to lytic and lysogenic phages, a comparative analysis of genomes from lytic phage clusters A, B, C and O and lysogenic phage clusters P, Q, T, X, Y and Z was undertaken using bioinformatics software such as phamerator, as well as the gene lists on phagesdb.org. The lysogenic phages contained genes such as antirepressors and CRO proteins that were absent in the analyzed lytic phages. Surprisingly, however, integrases were found in some lytic as well as lysogenic phages. In the lysogenic state, the presence of a functional integrase can be expected, because this phage-encoded enzyme facilitates the integration of the phage's DNA into the DNA of the bacterial host to become a prophage, thereby rendering the phage dormant and suppressing the usual anti-bacterial properties displayed during the lytic

state. The mutualistic lysogenic phage-bacterial host relationship may increase the host's fitness with a display of superinfection immunity which curtails the effectiveness of phage therapy. In some cases, it even may trigger the bacteria to release virulence factors, including toxins. Although lytic phages would therefore be the ideal candidates for phage therapy, our data indicate that not all lytic phages would be ideal therapy candidates, except if it can be doubtlessly confirmed that such integrases, albeit present, are non-functional. Thus, it is not enough just to know whether a phage is lysogenic or lytic. Rather, knowing which specific genes are present in each phage genome as well as knowing their functions and how they affect a phage's life cycle, is crucial to deciding which phages to use in "phage cocktails" that could be used for phage therapy as a way of mitigating the current threat of antibacterial drug-resistant infections.

4. Survey of Wolbachia in Texas Arthropods

*Tiffany Lee, Cecilia Cabrera, Haley Greenia, Faith Cox, Kenneth Underhill, Russell Pfau, and Dustin Edwards
Department of Biological Sciences, Tarleton State University, Stephenville, TX

Wolbachia is a symbiotic bacterium that infects a wide variety of arthropods. *Wolbachia* is able to manipulate its host reproductive processes to induce cytoplasmic incompatibility, male killing, feminization, and parthenogenesis. In addition, *Wolbachia* harbors temperate bacteriophage WO, which provides opportunity for genetic transfer and exchanges leading to evolutionary modifications which could be involved in the different induced phenotypes. The objective of our study was to determine the prevalence rate and host range of *Wolbachia*-carrying species in Texas. During 2016–2019, arthropods were collected from across the state. Mitochondrial DNA was extracted by homogenizing tissue with a sterile pestle in HotSHOT cell lysis reagent and a portion of the *cytochrome oxidase I* (COI) and *Wolbachia* genes were amplified by polymerase chain reaction (PCR). Presence of *COI* and *Wolbachia* PCR products were verified by agarose gel electrophoresis. The *COI* PCR products were sequenced by Sanger capillary sequencing and species identities determined by BLASTn software. Phylogenetic analyses and genetic distances were determined using MEGA X software. Of the 121 arthropods identified, 11 (9%) were infected with *Wolbachia*, including mosquito species *Aedes albopictus*, *Aedes aegypti*, *Culex pipiens*, and *Culex quinquefasciatus*. This is the first known sample of a natural *Wolbachia* infection in *Aedes aegypti*. Determining prevalence rate and host range of *Wolbachia* could be of importance in controlling populations of insects carrying human pathogens.

5. The spatial distribution and abundance of gut bacteria within honeybees and bumblebees

*Alexia Martin, Jo-anne Holley
Department of Integrative Biology, University of Texas at Austin, Austin, TX

Honeybees and bumblebees have a defined set of bacteria within their mid- and hindguts. This bacterial community positively affects bee health by aiding digestion, assisting insecticide resistance, and providing some pathogen defense. Honeybees and bumblebees are closely related and share some characteristics; they both feed on honey and pollen and live in social groups. Honey and bumble bees also share some five species of bacteria. In honey bees the bacteria sit along the wall of the digestive tract in a clear orientation, *Snodgrassella alvi* along the gut lumen and *Gilliamella apicola* towards the center of the gut. While it is thought *S. alvi* and *G. apicola* share the same metabolic niches in bumble bees, the spatial distribution and abundance of bacteria has not been confirmed. Fluorescent *in situ* hybridization (FISH) microscopy was performed to analyze the spatial distribution of bacteria within the guts. *Snodgrasella alvi* was found within the ileum of *Bombus impatiens,* and research is continuing to confirm the presence and orientation of *G. apicola*.

Graduate Student Oral Presentations

6. Photo-chemically activated silver nanoparticles (AgNPs): analysis of antibacterial activity and possible mechanisms

*Bibas Basnet and Laura Hanson, Ph.D.
Department of Biology, Texas Woman's University, Denton, Texas

Silver nanoparticles (AgNPs) are being discovered as promising antimicrobial agents recently, owing to their extraordinary physical, chemical and biological properties relative to the original bulk material silver compounds. The purpose of this study was to investigate the antibacterial property of two novel, photo chemically synthesized, silver nanoparticles, and to evaluate the potential antibacterial mechanisms involved. We showed that photo-chemically synthesized, negatively charged (-) AgNPs made in poly(acrylic acid) (PAA), were bactericidal. However, photo-chemically synthesized, positively charged (+) AgNPs made in chitosan, have no evidence of any antibacterial activity beyond that of chitosan alone. The (-) AgNPs have antimicrobial activity against Gram-negative bacteria like *Escherichia coli*, and *Pseudomonas aeruginosa* and Gram-positive bacteria like *Staphylococcus epidermidis* and *Bacillus megaterium*. However, the (-) AgNPs showed more efficiency when they were made using high molecular weight (MW) PAA than low MW PAA. The antibacterial activity of (-) AgNPs was inversely correlated to NaCl and presence of 2.5% glucose. These (-) AgNPs showed cooperativity with kanamycin but not with ampicillin. Our findings support a model where multiple mechanisms of activity work in combination to control bacterial growth.

7. Pioneer Species Affect Community Composition and Function During Recovery from Physical Disruption of the Mucosal Microbiome of *Gambusia affinis*.

*Javier A Gomez, Lindsey A Burcham, Madison B Cowdrey, Jeffrey M Belanger, & Todd P Primm, Department of Biological Sciences, Sam Houston State University

Microbiome communities have major effects on the health of humans, and when disrupted, can have negative impacts, including opportunistic infections and inflammatory conditions. When disrupted by antibiotics, microbiome communities in humans and research animals become temporarily dominated by one species, presumably serving as a pioneer during secondary succession. The mechanisms behind and results of this domination are still unclear. We use the fish *Gambusia affinis* to model mucosal microbiomes and have an experimental system to add a selected bacterial strain after disruption to be the dominant organism. The fish skin microbiome was depleted and disrupted by a physical rinse, and then *Escherichia coli* strain K-12 was the introduced pioneer species. K-12 dominated after eight hours of exposure and became a rare species after two and ten days of recovery. The genus *Chryseobacterium* was the dominant taxon after two days in all groups, whether K-12 was present or not. However, the K-12 exposed fish did have a different final community composition and final biochemical profile on day ten. These results suggest that the introduced pioneer, despite being transient, influenced the final community composition and function. Further understanding of pioneer effects may allow us to use probiotics in a predictable fashion to restore disrupted microbiomes.

8. Effects of host mediated microbiome engineering under drought stress in the wheat rhizosphere

*Michael D. Jochum[1], Kelsey McWilliams[1], Elizabeth Pierson[2], Young-Ki Jo[1]
[1]Department of Plant Pathology & Microbiology, Texas A&M University, College Station, TX
[2]Department of Horticulture, Texas A&M University, College Station, TX

By conducting six rounds of artificial selection on a root rhizosphere microbiome using the concept of host-mediated microbiome engineering, we selected a microbial community that was able to delay the onset of drought symptoms by 5 days in wheat seedlings. When compared to the control, the engineered rhizosphere demonstrated a statistically significant increase in physiological measurements of total plant biomass, and root system architecture phenotypes of length, dry weight, and surface area. The engineered microbiome also demonstrated a statistically significant increase in water retention over a 10-day period, and retained efficacy at the 10-2 dilution series, compared to dilution at 10-3 or the control. Result from 16s rRNA next generation amplicon sequencing of the root rhizosphere microbiomes in rounds 0, 3, and 6 revealed taxonomic increases in proteobacteria at the phylum level and betaproteobacteria at the class level. Phylogenetic diversity analysis revealed significant decreases in alpha diversity in round 6, divergence in speciation with beta diversity between round 0 and round 6, and changes in overall community composition. A functional metagenome inference resulted in increases from round 0 to round 6 in KEGG ortholog level 2 gene families associated with cell motility, cell signaling, and metabolism. In summary, artificial selection using host mediated microbiome engineering of the wheat rhizosphere using drought stress as a selective marker was successfully able to increase the delay in the onset of drought stress, alter root system architecture, increase water retention, and change the taxonomic and functional metagenome of the root rhizosphere in wheat seedlings.

9. Exploration of Genome Length, Burst Time, and Burst Size of *Streptomyces griseus* Bacteriophages.

*Jindanuch Maneekul and Lee E. Hughes
Department of Biological Sciences, University of North Texas

The study of bacteriophage growth is one of the crucial tools used to gain insight into this biological system. There are two main parameters involved in phage growth that can be measured: burst time and burst size. Unlike for other phages isolated on different strains of bacteria, publications focusing on the burst time and burst size of *Streptomyces* phages are available. We have found no prior reports studying possible correlations between a *Streptomyces* phage's genome length and its burst time and burst size. Since the phages will use the host resources to replicate themselves after infection, the different sizes of the phage genome should influence the replication rate. We, therefore, hypothesized that the smaller genomes should burst the cell faster than the larger ones. As well, the shorter genomes would have greater burst sizes because they should replicate faster. Here, we obtained 16 phages of various genome length. All phages were isolated on *Streptomyces griseus* and available in our phage bank at the University of North Texas. Because the filamentous nature of the host caused several difficulties during the experiment, we isolated single cells by sonication and centrifugation. After the cell number was determined by viable cell count, the cells were infected with each type of phage using a multiplicity of infection (MOI) of 0.5. The results show that phages' burst times range between 45 and 405 minutes and burst sizes from 12 to 1500 The statistical analyses show that there is no correlation between either genome size and burst time ($R= -0.00718$, $P=0.97894$) or genome size and burst size ($R= -0.32677$, $P=0.21670$). Although this study did not find any correlation between genome size and burst time/burst size, it provides a foundation for further studies to determine what regulates these two traits.

10. The effects of gold mining on microbiome composition in a freshwater ecosystem

*Caroline E. Obkirchnner, Santos Gonzalez, Ibeth Caceres, Carmen Montaña-Schalk, and Madhusudan Choudhary
Department of Biological Sciences, Sam Houston State University, Huntsville, TX

Mercury (Hg) contamination of freshwater ecosystems due to gold mining operations causes major human health issues and environmental problems around the world. Once in the aquatic environment, Hg is methylated by microorganisms to produce methyl mercury (MeHg), which becomes bioaccumulated and biomagnified through different trophic levels. MeHg is a neurotoxin, which creates a health hazard to fish consuming populations. Characterization of the microbiome structure existing in these aquatic systems is necessary to fully comprehend the nature of the microbial methylation reaction in situ. Here we tested whether a) the concentration of Hg and MeHg in fluvial sediments at gold mined sites will be higher than at non-mined sites and b) whether the microbiome composition at mined sites are significantly different than non-

mined sites in a tropical river in South America, the Mazaruni River, Guyana. Results revealed higher concentrations of Hg and MeHg in soil sediments collected from gold-mined sites when compared to non-mined sites. Microbial community structures at mined and non-mined sites were significantly different at levels of phyla, families, and genera. *Proteobacteria***,** which is a phylum in which mercury methylation has been extensively reported, was found significantly higher in abundance at mined sites. Furthermore, some known mercury methylators, such as *Geobacter* and *Desulfosporosinus* were found in higher abundance at mined sites. Future work includes the cloning and sequencing of *hgcA* and *hgcB* gene sequences from the soil samples collected from the mined sites as these genes represent important basis for identifying Hg-methylating microbes. The cumulative impact of these data will contribute to the development of innovative biomonitoring and bioremediation tools for mercury and its bioremediation from freshwater ecosystems.

11. Investigation on mechanism of temperature sensitivity in a mouse cytomegalovirus mutant

*Sushila Pathak[1], Clive Sweet[2] and Laura K. Hanson[1]
Department of Biology, Texas Woman's University, Denton, Texas[1]
School of Biosciences, University of Birmingham, Edgbaston, Birmingham, United Kingdom[2]

Cytomegalovirus can cause complications like hearing loss or motor disability in the new-born and retinitis or hepatitis in immunocompromised patients. Since human cytomegalovirus (HCMV) and mouse cytomegalovirus (MCMV) have similarity in gene sequence and function, we infect mouse cells with MCMV to explore the function of viral pathogenesis factors. The M139, M140, and M141 genes of MCMV belong to the US22 gene family and are respectively homologous to US22, US23, and US24 of HCMV. Many members of this gene family are involved in pathogenesis. The M139, M140 and M141 proteins form a stable complex and deletion of any one of these genes leads to replication impairment of MCMV in macrophages and reduced virulence in mice. The pM140 (M140 protein) is required for stable viral gene encapisdation in macrophages but the function of pM139 (M139 protein) is unknown. Dr. Clive Sweet's lab showed that pM139 also has a role in fibroblasts as truncation of 79 aminoacids towards the C-terminal end of pM139 makes MCMV temperature sensitive in fibroblasts but not in macrophages. We aim to understand the mechanism of temperature sensitivity of this mutant MCMV which may clarify the role of pM139 in infection. Our preliminary results indicated that the temperature sensitive defect in Sweet's mutant is unrelated to alteration in virion stability, complex formation with M140-M141, and occurs after the step of virus DNA replication. Preliminary data from mass spectrophotometry indicated that pM140 may be interacting with viral capsid protein (M86) and heat shock protein 90 (HSP90). Since pM139 can complex with pM140 and the C-terminal truncation of pM139 makes MCMV temperature sensitive, we are investigating whether pM139 interacts with M86 and/or HSP90, a protein involved in cellular stress response. We are also working to determine if exogenous expression of pM139 can rescue temperature sensitivity in the mutant virus.

Undergraduate Student Posters – Basic and Environmental Microbiology

U1. Inducing Programmed Cell Death to Test for Kin Recognition Among *Chlamydomonas reinhardtii*

Armon Do Amini* and Anne R. Gaillard
Department of Biological Sciences, Sam Houston State University, Huntsville, TX

For a unicellular organism to exhibit cooperative behavior and increase the fitness of the species, there must exist a capacity to recognize kin among a mixed population. Kin recognition has been observed in the amoeba *Dictyostelium discoideum* when the organism is placed under environmental stress. *D. discoideum* forms a fruiting body consisting of cells who exhibit altruistic behavior by sacrificing themselves to form the stalk that supports the sorus. The cells that form the sorus on top of the stalk are able to survive and proliferate the species. The model organism *Chlamydomonas reinhardtii* is a unicellular green alga that has been used to study programmed cell death (PCD) in unicellular organisms. In this study, *C. reinhardtii* and the relative species *Chlamydomonas smithii* were induced to undergo PCD with heat stress at 42°C to test the hypothesis that the level of cell death will increase in a population of clones when compared to a population of two genetically diverse species. Kin recognition in *C. reinhardtii has* not been well investigated, thus this study aims to identify if the organism exhibits cooperativity when stressed.

U2. Molecular and Microscopic Characterization of Symbiotic Nitrogen Fixation Mutants in *Medicago truncatula*

Hala Samara, Jeanne Beckwith*, Logan Boling, and Catalina Pislariu
Department of Biology, Texas Woman's University, Denton, TX

Legumes are essential for sustainable agriculture, due to their symbiotic association with soil bacteria, collectively called rhizobia. Through symbiotic nitrogen fixation (SNF), legumes acquire nitrogen with help from rhizobia, which convert the inert molecular nitrogen (N_2) into useable ammonium (NH_4^+). SNF injects around 50 million tons of N_2 per year into the agricultural systems (Vance, 2001). One of the widely used model legumes to investigate SNF is *Medicago truncatula* (barrel medic), a relative to alfalfa. As a result of *M. truncatula* association with its symbiont, *Sinorhizobium meliloti*, specialized new organs (root nodules) develop on its root system, where rhizobia fix N_2. A visible marker of functional SNF is the accumulation of leghemoglobin, which gives nodules a pink color. SNF is a complex process that involves regulation of thousands of host and bacterial genes, with much still needing characterization. Various functional genomic resources are available for *M. truncatula,* facilitating the exploration and characterization of new symbiotic genes. These include genome sequence, transcriptomic databases, and mutant collections (d'Erfurth et al., 2003; Young et al., 2011; Pislariu et al., 2012). Our lab is using the tobacco retrotransposon (*Tnt1*)-insertion mutant population,

developed at Noble Research Institute (Ardmore, OK), to investigate SNF. To microscopically assess the putative *Tnt1*-insertion symbiotic mutants, different rhizobial strains expressing biomarkers were developed. These genetically engineered strains constitutively express either the reporter *hemA::LacZ* that yields a blue precipitate following histochemical staining, or the *J23119::eforRed, which* results in pink color under visible light. The advantage of engineering rhizobia expressing visible chromoproteins such as *eforRed* is that the infection process can be visualized *in situ*, without sample destruction, as in histochemistry. The phenotypes of putative *Tnt1*-insertion symbiotic mutants are thoroughly examined and characterized using bright field, stereo, and confocal microscopy. Representative results will be presented.

References

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U3. Quantifying gut colonization of antibiotic-producing bacteria in Wild-Type *C. elegans*

Cole A. Calderon*, Elizabeth Rodriguez, Kate Weil, Dr. Teresa Bilinski
Depatment of Natural Sciences, St. Edward's University, Austin, TX

Caenorhabditis elegans is a soil nematode that has been cultivated and studied in the lab as a model organism to investigate a multitude of cellular, genetic, molecular, research questions. However, the native gut microbiome of wild *C. elegans* has not been as assiduously researched. Wild-type *C. elegans* are commonly found in the topsoil in decomposing plant matter. Wild-type *C. elegans* can serve as a better model for understanding real-world host-microbe interactions since they have a native microbiome. Previous research in this field has shown that nematodes prefer some bacterial strains over others, including the most commonly fed bacterial strain, *Escherichia coli OP50*. Also, specialized functions of soil microbes, such as antibiotic production may affect the rates of gut colonization of different strains through competitive interactions. However, past research has not established methods to quantify bacterial gut colonization of *C. elegans,* especially with the goal of characterizing differences in gut colonization between bacterial strains. The goal of this study is to investigate how differences in the taxonomy and function of bacterial strains affects the gut colonization of the wild-type *C. elegans* strain PX179 isolated from Hendricks Park in Eugene, OR using cost-effective quantification methods. Notably, the wild-type *C. elegans* strains in this study has been seen to

have behavioral and growth differences compared to the established model N2 strain. This research compared gut colonization between *E. coli* and antibiotic-producing bacteria such as *Pseudomonas fluorescens* and soil bacterial isolates. Bacterial colonization was quantified using methods that were adapted from previous studies and optimized. Preliminary data suggests that this method for quantitation is consistent across multiple nematode generations. In addition, gut colonization varied between bacterial strains. This suggests that the *C. elegans* may display a preference for specific bacterial species, and/or certain bacterial species may be less effective in colonizing the *C. elegans* gut environment. Future work will investigate competitive interactions between the bacterial strains within the *C. elegans* gut microbiome by comparing their gut colonization rates.

U4. Isolation of wild nematodes with the goal of investigating gut microbiota-host interactions

Emily M. Cooper*, Valerie A. Miranda, and Daniel A. Gold
Department of Biological Sciences, Saint Edward's University, Austin, TX

The microbiome, which is composed of bacteria, viruses, protozoa and fungi, has been increasingly recognized to have an important role in organismal health and disease. These microbes are found in high frequencies both in and on an organism, resulting in constant host-microbe interactions. Wild soil nematodes, similar to humans, have a microbiota within their gut that is specific to their diet and environment. Despite the widespread use of the model soil nematode *C. elegans* in laboratories around the world, the natural history of the microbiota--including pathogenic microbes--of wild soil nematodes has yet to be fully explored. Furthermore, the microbial interactions of other nematode species--specifically those that are not lab-adapted--is vastly understudied. Investigation into these wild host-microbial interactions could yield significant advances in the study of human diseases caused by pathogenic microbes. Utilizing nematodes as a model organism to study microbial interactions--specifically in the gut--provides great feasibility, while still serving as an accurate representation of human pathogen infection mechanisms. The aim of our study is to identify and characterize microbes, with an emphasis on understudied eukaryotic microbes that infect wild soil nematodes. Having successfully isolated nematodes of the *Acrobeloides* genus from soil samples in the Central Texas region, we will utilize cellular biology techniques including diagnostic PCR, metagenomic sequencing, and light microscopy to identify any microbial species that may be present. The findings of this study will help to fill in the gaps of knowledge on the natural history of microbial interactions in wild soil nematodes, and could potentially provide a method to study human pathogens.

U5. Assay determination of function vs character of microbiome species on *Gambusia affinis*

Madison B Cowdrey*, Lindsey A Burcham, Jeffrey M Belanger, Javier A Gomez, & Todd P Primm, Department of Biological Sciences, Sam Houston State University, Huntsville, TX.

Some patients whose microbiomes are disrupted by antibiotics have severe side effects while others are fine, yet we cannot predict the outcome because the system is currently poorly understood. With next-generation DNA sequencing technology, determining composition of microbial communities is routine. However, as the field of microbiome research matures, research must move past simple organism numbers to the functional level. In order to look at a vertebrate mucosal microbiome, our lab uses the fish model organism *Gambusia affinis* because of its easily obtainable skin mucosal microbiome. Using strains isolated from the fish, Microgen GN A+B assays and differential media were employed to determine over 24 different possible biochemical activities from each strain. We are now moving to examine enzyme activities directly in the microbiome. Assays are now being developed for neuraminidase (first step in degradation of sugars from host mucus) and nitrate reductase (involved in nitrogen assimilation and energy metabolism). Our model system will be used to examine how different biochemical pathways are regulated during recovery from disruption, hopefully revealing interventions that can benefit patients.

U6. An analysis of diversity and resistance of environmental bacteria from three elementary schools in Houston, TX.

Nia D. Griffith* and M. Bowden
Natural Sciences Department, University of Houston-Downtown, Houston TX 77002

Research has shown that the microbiota of an environment and the microbiota of the living organisms that inhabit the environment contribute to, affect, and change each other. This study sought to assess the differences in the microbes that inhabit selected areas of three elementary schools in three different neighborhoods in Houston, TX. Census data of the neighborhoods has shown significant stratification of average income, ethnic make-up, and lifestyle factors between them. The researchers have hypothesized that the bacterial diversity may be higher in the lower income and more diverse neighborhoods, and that resistance to antimicrobials may be higher in the higher income area. Samples were collected from the three locations in the schools at three time points over a six-month period. The samples had their microbial DNA extracted and this mixed sample was analyzed utilizing next generation sequencing (NGS) techniques of the 16S rRNA. The metagenomic data was used to calculate the diversity of the microbiota. A portion of each sample was cultured and the culturable strains were tested for resistance to common antibiotics and disinfectants. The α-diversity for each sample was calculated, including both the species richness as well as diversity indices. The β-diversity was then calculated comparing the individual locations, the variation over time, and between the different schools. The ξ-diversity was calculated between the schools over time. The results of these calculations and the resistance data was compared, and conclusions were then drawn.

U7. Exopolysaccharides Drive Density-dependent Colony Expansion of Socially Motile *Myxococcus xanthus* Cells

*Kimberley Kissoon[1], M. Gabriela Bowden[1], Zhaoyang Zhang[2], Oleg Igoshin[2], Heidi B. Kaplan[3]
[1]Department of Natural Sciences, University of Houston-Downtown, Houston, TX; [2]Department of Bioengineering, Rice University, Houston, TX; [3]Department of Microbiology and Molecular Genetics, McGovern Medical School, UT Health, Houston, TX,

Social (S) motility is type IV pili-mediated flagella-independent group movement on a solid surface, which is best studied in the gram-negative soil bacterium *Myxococcus xanthus*. It is related to twitching motility in the gram-negative pathogen *Pseudomonas aeurginosa*. The cells move due to extension, adhesion, 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 supports 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 directly upon plating as they quickly produce EPS above the threshold value. We validated the model's predictions by studying long-term (up to 96 hours) colony expansion of a S-motile only strain (DK1218) of *M. xanthus* at different initial cell densities on 0.5% agar nutrient plates. Our data strongly suggest that EPS is critical for S-motility colony expansion. To test this further, we generated an EPS biosynthesis mutant (*epsZ*-) in wild-type and S-motile only backgrounds. First, we examined colony expansion in the of *epsZ*- mutants and observed that both strains moved much less than their EPS-producing parents. It appeared that the *epsZ*- S-motility only strain expanded only due to cell growth. Then, we examined the effects of purified EPS on colony expansion of the *epsZ*- S- motility only strain and its parent by spotting cells of these strains (3 x 105 cells/3 µl) adjacent to or overlapping increasing concentrations of purified EPS on 0.5% nutrient agar plates at 32°C for 48 h. Our analysis showed that the presence of purified EPS increased *M. xanthus* colony expansion of all strains tested with increasing EPS concentration. These results support the conclusion that EPS is absolutely required for *M. xanthus* S-motility colony expansion. We are currently examining the effects of EPS on single cell motility.

U8. Characterization of *E. coli* Population Diversity in Locally Resident Canada Geese and Migratory Seagulls

Tarrah A. Miller*, Shanice D. D. Toussaint, James Masuoka
Department of Biology, Midwestern State University, Wichita Falls, TX

The emergence and dissemination of antibiotic resistant pathogens continues to plague healthcare by increasing healthcare costs and mortality rates. Migratory birds are able to transfer antibiotic resistant strains to local organisms. We hypothesized that the seasonal seagulls that

arrive each winter serve as a source of new strains of bacteria and that are then introduced into the local environment and then picked up by our resident waterfowl. The aim of this project is to compare *Escherichia coli* strains isolated from both resident Canada geese and migratory seagulls. Fecal samples were collected from two separate Canada goose individuals. Ten presumptive *E. coli* colonies (5 from each individual) were selected for further testing. Of the ten, nine were identified as *E. coli* and one was *Enterobacter cloacae*. Of the *E. coli* isolates, variation in sugar utilization and patterns of antibiotic resistance suggested the presence of different strains. Current efforts are focused on carrying out the same methodology on specimens obtained from seagulls that migrate into the area each winter. By comparing the metabolic and antibiotic resistance patterns, we hope to determine common strains to both species as well as those that are unique to one or the other. By extending these comparisons over time, we will better understand how antibiotic resistant bacteria move between populations.

U9. Examining horizontal gene transfer between two auxotrophic strains of c*hlamydomonas reinhardtii* through programmed cell death

Tyler Musgrove* and Anne R. Gaillard
Department of Biological Sciences, Sam Houston State University, Huntsville, TX

Horizontal gene transfer (HGT) is a form of transferring genetic material between organisms other than by vertical transfer from parent to offspring. HGT has been commonly observed in prokaryotic organisms; however, the mechanism of HGT in eukaryotic cells is not well characterized. *Chlamydomonas reinhardtii* is a single-celled green alga that is commonly used as a model organism. *C. reinhardtii* has been observed to undergo programmed cell death (PCD) when exposed to environmental stressors, such as moderate heat stress. PCD includes chromatin condensing and DNA laddering, as well as cell membrane permeability and detachment from the cell wall. This study tested the hypothesis that *C. reinhardtii* participates in HGT through PCD. Once the cell has started the PCD process, DNA laddering occurs and the membrane becomes permeable, which may allow genetic material to leave one cell and move into another cell. The outcome of a successful HGT event could be beneficial to a surviving cell by acquiring a new gene. To test this hypothesis, two auxotrophic strains of *C. reinhardtii* were induced to undergo PCD together to test for the presence of HGT between the strains. So far, no evidence has been obtained to support the hypothesis of HGT during PCD; however, more experiments are underway.

U10. Microbial analysis in plant-soil interaction between Sideoats grama and Bermuda grass

Marissa Narvaez*, Brenda G. Rushing, and J. Rodolfo Valdez Barillas
Biology Program, Texas A&M University-San Antonio, San Antonio, TX

Urban grassland ecosystems dominated by non-native invasives are a restoration challenge due to the inability of native perennial grass seeds to germinate and become established. The goal of

this study is to understand how changes in the soil microbial community can affect the establishment of native grass **Sideoats grama (*Boutelua curtipendula*)** in areas where non-native and invasive **Bermuda grass (*Cynodon dactyloides)*** occurs. We designed a series of indoor, controlled experiments where Sideoats grama and Bermuda grass were treated with soil samples collected from areas where natives or invasive species are naturally established. We assessed the response of Sideoats grama and Bermuda grass, when growing alone and in competition, to native and invasive soil treatment by estimating changes in shoot and root biomass. To assess the corresponding changes in the soil microbial community, we identified the soil microbiome composition using 16s rRNA gene sequencing and the soil microbial metabolic profiles using a Biolog MicroStation. The results showed that Sideoats soil has a more diverse microbial population than Bermuda. The results from the plant data showed that Sideoats and Bermuda grew to greater biomass with their own natural soils. Bermuda showed poor growth when inoculated with Sideoats soil. Interestingly, Sideoats grew better with Bermuda soil compared to its own soil inoculum. Since the native habitat of Sideoats is widely distributed in various regions, it may be compatible with a broad range of microbes. Our experiment suggests that Sideoats is a good candidate for reestablishing the native grass population where Bermuda grass dominates.

U11. The Regulation of Nutritional Metal Versus Toxic Metals by the *Saccharomyces cerevisiae* Nonsense-mediated mRNA Decay Pathway.

Cheryl Pai*, Annika Gunderson, Bessie Kebaara
Department of Microbiology, Baylor University, Waco, TX

Nonsense-mediated mRNA decay (NMD) is a eukaryotic pathway specializing in targeting and degrading mRNAs that prematurely terminate translation. The NMD pathway is responsible for regulating the expression of mRNA and can be found in multiple organisms such as humans and yeasts. A large array of mRNAs are degraded by NMD including those involved in pathways attributed to metal transport and homeostasis. This metal include nutritional metals such as copper and iron, and environmental toxins, such as cadmium. Regulation of mRNAs involved in metal hemostasis has been seen in the model yeast *Saccharomyces cerevisiae.* The concentrations of these metals create different conditions for the cells resulting in different regulatory behaviors regarding NMD functioning. In this study, we examining the growth of yeast cells with a functional and non-functional NMD pathway under copper and Iron deplete and excess copper conditions. In addition, we examined the growth of yeast cells with a functional and non-functional NMD pathway in the presence of cadmium. We found that in toxic copper concentrations NMD mutants were more resistant compared to wild-type cells, with less distinguished resistance in copper deplete conditions. In trace iron conditions, we found that the mutant presented decreased resistance when compared to the wild-type strain. Finally, we generated a wild-type and NMD mutant strain carrying a functional gene for cadmium resistance, to study the effects of growth on the cells.

U12. Construction of *Myxococcus xanthus* Type IV pili- and EPS-deficient strains for motility analyses

Katelyn Payne* and M. Gabriela Bowden
Natural Sciences Department, University of Houston-Downtown, Houston TX 77002

Myxococcus xanthus is a predatory soil bacterium that moves by gliding onto solid surfaces. This Gram-negative bacterium uses a multitude of extracellular signals to stalk its prey in swarms. *M. xanthus* uses two clear methods of movement, adventurous (A) motility and social (S) motility. Our research project focuses on S-motility, which requires exopolysaccharide (EPS) and Type IV pili production. To study the role of EPS in social motility, our laboratory has constructed in-frame, markerless *epsZ* mutants in the DK1622 (wild type) and DK1217 (A- S+) background strains. To understand the role of Type IV pili in S motility, a *pilA* deletion construct was transduced into the *epsZ* mutants using myxophage Mx4 transduction. The resulting *epsZ pilA* mutant strain is predicted to be defective in the production of both EPS and Type IV pili. The motility of these strains will be analyzed by microscopy. In addition, to monitor the behavior of mutant strains cells while moving within a swarm of mixed cultures, myxophage Mx4 transduction was performed to introduce a GFP marker into the described mutant strains, of. The motility of the fluorescently labeled strains mixed with unlabeled wild-type, *epsZ* or *pilA* mutant cells will be quantitated using time-lapse video microscopy.

U13. Characterization of Metacaspases during Programmed Cell Death in *Chlamydomonas reinhardtii*

William Perez*, Celso S. G. Catumbela, and Anne R. Gaillard
Department of Biological Sciences, Sam Houston State University, Huntsville TX

Programmed cell death (PCD) in the unicellular green alga, *Chlamydomonas reinhardtii*, exhibits similar characteristics to apoptosis, a well-known form of PCD in animals. Canonically, apoptosis is regulated by cysteine proteases known as caspases; however, C. *reinhardtii* instead possesses two related proteases known as metacaspases, which can be categorized as types I and II in accordance with their structure (MCA1 and MCA2, respectively). Here, we investigate the potential effect of MCA2 disruption during heat stress-induced PCD in C. *reinhardtii.* Our experimental results do not support that MCA2 is the main protease responsible for the initiation of PCD. This suggests that in the absence of MCA2, MCA1 may substitute for its PCD roles. However, previous studies on MCA1 suggest that MCA1 may act as a negative regulator of PCD. More trials need to be run to further understand the potential role of metacaspases in *Chlamydomonas reinhardtii.* Development of an MCA1 and MCA2 double knockout strain is also underway to better understand metacaspase regulation of PCD phenotypes.

U14. Using qPCR to differentiate between fungicide resistant and sensitive isolates of c*ercospora beticola*

Joshua F. Rios*, Jonathan Neubauer, Melvin Bolton
St. Edward's University - USDA – ARS, Northern Crop Science Laboratory, Fargo, ND, USA

Cercospora leaf spot (CLS) is caused by the fungus *Cercospora beticola,* which is the most serious foliar disease of sugar beet (*Beta vulgaris* L.). It causes significant reductions to crop yield worldwide. CLS disease control is achieved by timely fungicide treatment, but control has become an issue as resistance to TOPSIN (Thiophanate-methyl) fungicide has become highly

prevalent. Identification of sensitive and resistant isolates to fungicide application is crucial for treatment and monitoring of disease progression. The purpose of this research was to streamline differentiation between sensitive and resistant isolates of *C. beticola* using qPCR methods.

U15. Investigating colonization between different bacterial isolates in the wild-type nematode microbiome

Elizabeth Rodriguez*, Cole Calderon, Kate Weil, and Teresa Bilinski
Department of Natural Sciences, St. Edward's University, Austin, TX

Recent research has demonstrated the importance of host-microbe interactions to understanding many aspects of host physiology and health yet, little is known regarding these interactions in wild species. *Caenorhabditis elegans* is a model organism commonly raised in the lab however; the original isolate was a free-living bacterivorous soil nematode. A small number of previous studies have characterized the gut microbiome of wild *C. elegans* however; there has been less research on processes leading to the bacterial colonization of their gut, and these effects on host-microbe interactions. Previous work suggests that *C. elegans* prefer soil bacterial species found in places similar to their natural habitats over common lab strains of bacteria, such as *Escherichia coli*. The goal of this study was to test the hypothesis that the gut of wild *C. elegans* would be colonized to a greater degree by bacterial strains for which they display a higher preference. In order to test this, wild *C. elegans* were fed different bacteria and the bacterial colonization was measured via culture-based methods. To ensure adequate recovery of bacteria within the gut microbiome of the nematode, sonication was incorporated to effectively break the tough cuticle of *C. elegans* and allow for quantification of bacteria in the microbiome. This method was observed to be more accurate than previous methods used, and this allowed for the determination of the concentration of *E. coli* and a bacterial isolate from Wild Basin Nature Preserve within the gut microbiome of a wild *C. elegans* strain. Both bacterial species were shown to have colonized the nematode microbiome via this new method. Additionally, the wild *C. elegans* strain took longer to proliferate when fed the soil bacterial isolate, indicating the different bacterial food source had an effect on the growth and/or reproduction of the *C. elegans.* The development of this culture-based procedure will allow for quantitative measurements and comparison of the relative abundance of bacteria colonizing wild nematode gut microbiomes. Future research will continue to investigate this along with bacterial preference of wild *C. elegans* and their immunological responses to the different species.

U16. Identification and Evaluation of Quorum Sensing Inhibitors

John Rowland*, Ian Patterson, and Jennifer A. Hennigan
Department of Biology, Hardin-Simmons University, Abilene, TX

Many bacteria use quorum sensing to control the coordinated expression of virulence factors, toxins, or pigments, as in *Serratia marcescens*, *Chromobacterium violaceum*, or *Pseudomonas aeruginosa*. Quorum sensing pathways provide cellular targets that differ from established antibiotics and their inhibition could eliminate or reduce the release of virulence factors, causing bacteria to become less pathogenic. As with traditional antibiotics, competing species of bacteria may be a good source of novel quorum sensing inhibitors (QSIs). In this study, soil samples were collected from Rolling N Ranches, LP in Crews, Texas. Bacteria were isolated from the soil and screened for the production of QSIs using the QS sensor strain *Chromobacterium violaceum*. A

bacterial isolate was found that shows quorum-sensing inhibition of *Chromobacterium violaceum*. Additionally, FDA-approved drugs could be another untapped source of QSIs. For example, the diabetes medication metformin was shown to act as a QSI previously in some bacteria. We tested metformin against *Serratia marcescens* to evaluate its ability to inhibit the production of prodigiosin. Our analysis of QSIs could lead to the identification of an effective QSI that reduces the pathogenicity of specific bacterial species.

U17. RNA-seq for eukaryotic microbes: benchmarking splice-aware alignment and differential gene expression analysis tools

Utkarsh Singh[1] *, Tandav Argula[1], David Burks[2], Rajeev K. Azad[2, 3]
[1]Texas Academy of Mathematics and Science, University of North Texas, Denton, Texas 76203, United States of America, [2]Department of Biological Sciences, University of North Texas, Denton, TX, [3]Department of Mathematics, University of North Texas, Denton, TX

The pipeline chosen for RNA-Seq analysis heavily influences the downstream results. Variance in alignment strategies lead to remarkably different sets of differentially expressed genes. On the other hand, differential expression analysis tools generate non-converging differentially expressed gene sets using the same read alignment data. Therefore, it is important to assess the performance of RNA-Seq analysis tools in order to identify the best performing tools, understand the strengths and weaknesses of different tools and strategize integration of methods of complementary strengths. In this study, we selected five of the most popular tools for splice-aware sequence alignment, as well as three tools for quantifying differential gene expression, to benchmark their relative performance. Using publicly available RNA-Seq datasets derived from the arbuscules of *Manihot esculenta* infected with *Rhizophagus irregularis*, as well as simulated RNA-Seq datasets using the transcriptomes of these organisms, we have identified a pipeline that yields optimal performance with respect to alignment accuracy, expression estimation, and reproducibility.
We follow this benchmarking with a survey of the variance across pipelines and study of the effects of variance on standard downstream analyses, including enrichment and functional characterization. Our study suggests the need for a fresh look at the conclusions of past studies that used RNA-Seq datasets subjected to potentially inaccurate pipelines.

U18. Assessment of the Threshold of Similarity for Defining Species of Rhizobacteria by Analysis of Spectra Generated by MALDI-TOF

Phi Tran. University of Houston - Clear Lake, Houston, TX

To avert widespread famine, growers must double agricultural productivity this century. Plant growth promoting rhizobacteria (PGPR) can increase the yield and sustainability of row agriculture and help humankind avert this coming food crisis. Development of effective microbial products will require screening of millions of isolates. Typically, microbial discovery programs use 16S rRNA gene sequencing to identify and sort isolates and bioassays to evaluate prospects. Matrix-assisted laser desorption ionization – time of flight (MALDI-TOF) systems can accelerate this bioprospecting. MALDI-TOF systems can identify bacteria for pennies an isolate, at a throughput of hundreds an hour, by matching spectra to reference databases. Sorting isolates based on these spectra requires setting a threshold of similarity to define a species. To determine this threshold for PGPR, we compared the cosine similarity (CS) of protein spectra to

the percent identity of rRNA gene sequences for two libraries of rhizobacteria. Parameters, such as signal to noise ratio, used to calculate CS were optimized with a custom script written in R. CS values were used to define clades and two isolates from nine clades were tested for traits associated with PGPR *in vitro*. Similarity of ribosomal sequences showed a hyperbolic relationship with CS and a CS of > 0.88 corresponded to 99.7% identity of rRNA genes. High CS values did not consistently correspond to traits associated with plant growth promotion. Of the nine pairs, defined by cluster analysis of spectra generated by MALDI-TOF, only five showed similar phenotypes, as assessed by bioassays. This suggests that MALDI-TOF can accelerate initial identification of rhizobacteria but it cannot replace bioassays for screening for PGPR.

U19. The Breakdown: A Comparison of Plastic Degradation by *Rhodococcus rhodochrous* and *Pencillium chrysogenum*

Allison White* and Teresa Bilinski
Department of Biology, St. Edward's University, Austin, TX

Plastic accumulation in the environment poses a significant threat to the health of our ecosystems. Plastic is relatively recalcitrant to degradation in the environment, and thus persists as a pollution problem and causes landfills to overflow with waste. Recent research has demonstrated the potential for soil microorganisms to degrade plastic. The goal of this experiment was to use soil incubations to compare the ability of *Rhodococcus rhodochrous, Penicillium chrysogenum,* and the native soil microbial community to degrade two types of commonly used plastic: antimicrobial HDPE and a generic plastic used in trash bags. It was hypothesized that the generic trash bag would be degraded more quickly than the antimicrobial HDPE. Additionally, it was hypothesized that all three microbial treatments would result in degradation; however the rate of degradation would be faster for plastic in incubations that were inoculated with either *R. rhodochrous* or *P. chrysogenum*. These hypotheses were tested using a full-factorial soil incubation experiment. Three microbial treatments were added to the two types of plastic and plastic degradation was characterized after 10 weeks. In addition, the growth rates of the three microbial treatments were measured and pre- and post-incubation soil conditions were characterized. The weight of the plastic did not significantly change during the incubation; however differences in surface area and texture were observed, which is evidence of microbial degradation. Future work will include further quantification of plastic degradation using measures of plastic integrity, such as percent elongation, as well as image analysis. In addition, PCR screening will be conducted for presence of known plastic-degrading microorganisms on the plastic surface and in the soil from the incubations. Finally, DNA extraction and sequencing will be performed to characterize the microbial communities on the plastic and within the soil from the incubations. This research informs ways in which plastic degradation can be promoted in the environment using soil microorganisms, and investigates the ability of the native soil environment to assist in plastic degradation.

U20. The effect of copper on growth and mRNA expression in *S. cerevisiae* cells with and without a functional nonsense-mediated mRNA decay pathway

Angelo Wong* and Bessie Kebaara
Department of Biology Baylor University, Waco, TX

The nonsense-mediated mRNA decay pathway (NMD) is a physiological mechanism in most eukaryotic organisms that recognizes and degrades mRNAs containing premature termination codons and various natural mRNAs. Of particular interest is NMD's effect on natural mRNAs involved in copper homeostasis or regulation of copper intake. Copper, being an essential micronutrient, plays a cofactor role in cellular processes, but can be toxic to the cell at high concentrations. The pathway has been found to regulate numerous mRNAs involved with copper homeostasis, including *COX19*, *MAC1*, *FRE2*, *CTR2*, *FRE2*, *CRS5*, and *PCA1*. These mRNAs are sensitive to different environmental conditions such as high levels of copper. The degree and rate of regulation remains largely unknown, but the NMD pathway has been shown to play an important biological role as *S. cerevisiae* cells with a mutant NMD pathway exhibits a higher level of tolerance to copper. This observation was found through analysis of growth curves for cultures with and without an active NMD pathway. The next question was at what rate does the expression of mRNA change when grown in varying copper conditions with and without a functional NMD pathway. To test this question, RNA steady-state levels were evaluated at differing time points after switching from regular growth media to high copper conditions. RNA blot hybridization was conducted to assess *S. cerevisiae's* changes in gene expression and produce northern blots. Through examination of gene expression level, we can gather support for NMD's rate of regulation and more information regarding *S. cerevisiae's* tolerance to copper.

Undergraduate Student Posters – Pathogenic Microbiology

U21. Developing a Transcriptomic Atlas for the Gregarine Parasite *Blabericola migrator*
Nicholas Barrett*, Matthew Fencl, Mauricio Padilla, Kaitlyn Stark, Joseph Mathis, and Dr. Daniel A. Gold
Department of Biological Sciences, St. Edward's University, Austin, TX

Organisms in the genus *Cryptosporidium* are important waterborne protozoan pathogens that infect humans and other vertebrates around the world, causing a debilitating and sometimes fatal diarrheal disease called cryptosporidiosis. The work needed to combat cryptosporidiosis necessitates the use of a model organism as a stand-in for human infections. Mice are commonly used as such a model, but this research requires extensive resources and infrastructure. Recent molecular phylogenetic studies have shown that Gregarines, a diverse, widespread, invertebrate-infecting group of protozoan pathogens, are closely related to *Cryptosporidium* spp., and share many unique features, such as their epicellular lifecycle in the intestinal epithelia. This leads to the hypothesis that, Gregarines might be capable of modelling the molecular and cell biological features of cryptosporidiosis. Since gregarines infect invertebrates, creating a model system using these pathogens reduces costs and risks that are associated with all *Cryptosporidium* spp. Using the Gregarine model system, our goal is to conduct transcriptomic analyses on the various life cycle stages of these parasites, which will allow us to identify key parasite regulatory factors involved in infection. Such findings could then be applied to *Cryptosporidium* spp., and aid in the discovery of various drug targets against these pathogens. Currently, we have been optimizing RNA isolation procedures from the varied lifecycle stages of the Gregarine *Blabericola migrator* (host: Madagascar Hissing Cockroach).

U22. Discovery of a novel iron-acquisition gene in *Bacillus anthracis*
R. Samuel Baugh*, Jacob A. Malmquist
Texas Christian University, Fort Worth, TX

As the threat of antimicrobial-resistant infections continues to rise, the need for novel antibiotics grows. Targeting virulence factors in bacterial pathogens is one potential strategy for antibiotic development because inhibiting virulence would decrease the ability of the pathogen to evade the host immune response. This strategy may decrease the development of resistance since the treatment is not directly bactericidal and there is less selective pressure put on the bacteria population. Our goal is to discover new virulence genes in *Bacillus anthracis* that could potentially be a therapeutic target. Specifically, we are interested in finding genes that allow *B. anthracis* to acquire iron from the host. For bacterial pathogens, iron is critical for growth and often a limiting nutrient in the host. It has been linked with proper functioning of electron transfer proteins and superoxide dismutase enzymes. In *B. anthracis* infection, iron is acquired from host hemoglobin through a hemolytic pathway, but the complete mechanism of this is unknown. Approximately 1000 transposon mutants of *B. anthracis* were screened for the inability to acquire iron from hemoglobin, and five were deficient in acquiring iron from hemoglobin in *in vitro* assay*s*. Of those five mutant strains, only one (9F12) also exhibited an *in vivo* phenotype using the wax worm model of infection. The gene disrupted in the 9F12 transposon mutant is the dUTPase/aminopeptidase gene. Our aim in this study is to confirm that the disruption of the dUTPase gene leads to the inability to acquire iron from hemoglobin in *B. anthracis.* Using targeted mutagenesis, we created an insertional mutant strain to disrupt the dUTPase gene and we are currently testing it, along with WT and 9F12, for the ability to grow in iron-limited conditions with or without hemoglobin. Confirmation of this phenotype will demonstrate that the dUTPASE/aminopeptidase gene is important for iron acquisition from hemoglobin and will support further studies to understand the role of this gene in the virulence of *B. anthracis*.

U23. Determining the Mechanism of *Pseudomonas* Elastase Induced Activation of EGFR

Shandis Fancher*, Dustin DeLong, Dustin Patterson, and Ali Azghani
Department of Biology and Biochemistry, University of Texas at Tyler, Tyler, TX

Pseudomonas aeruginosa is a Gram-negative bacterium, which functions in an opportunistic manner in immunocompromised patients. Several exoenzymes and proteases play a significant role in the pathogenesis of *P. aeruginosa* in Cystic Fibrosis associated chronic lung inflammation. We have reported that elastase isolated from *P. aeruginosa* (PE) is an activator of inflammatory pathways *in vitro*. This study examines the mechanisms of PE-induced inflammatory responses of lung epithelial cells in an effort to determine a more effective treatment modality. The working hypothesis is that PE activates Epithelial Growth Factor Receptors (EGFR) indirectly through a secondary soluble ligand. Indirect interactions between PE and EGFR expressed on human pulmonary epithelial cell line A549 were analyzed using Virus Like Particle (VLP) encapsulated elastase. Cells were maintained in DMEM/F-12 with 10% FBS and 1% antibiotic-antimycotic solution. For experiments, cells were grown to confluence in 6-well plates, semi-starved in 2% FBS overnight, and starved in HBSS for 2 hours before treatment. Target treatments included commercial PE, recombinant PE (rPE), and rPE encapsulated in Virus Like Particles (VLP/rPE) constructed in the lab. Cells were treated with HBSS and empty VLP as negative controls and EGFR as a positive control. Cells were lysed post-treatment for protein extraction and concentration determination, and they underwent analysis via SDS-PAGE. Western Blots were performed using primary antibodies targeting phosphorylated EGFR and mitogen activated protein kinase ERK. We found that EGF, free PE,

and rPE activate EGFR and downstream ERK1/2 while encapsulated PE failed to do so. In conclusion, our preliminary data indicate that PE activates EGFR by direct ligand/receptor interactions, the nature of which will be explored in successive studies. This project was supported in part by the UT-Tyler interdisciplinary research fund to Drs. Patterson and Azghani.

U24. Determining transmission of the parasite b*labericola migrator* in a laboratory colony of g*romphadorhina portentosa*

Matthew Fencl*, Nicholas Barrett, Joseph Mathis, Kaitlyn Stark, Mauricio Padilla, and Daniel A. Gold. Department of Biological Sciences, St. Edward's University, Austin, TX

Protozoan parasites in the genus *Cryptosporidium*, which cause the serious diarrheal disease cryptosporidiosis, have a massive impact on global human and veterinary health. Following ingestion, the parasites attach to host intestinal epithelial cells, causing diarrhea and gut dysbiosis. Due to the nature of how *Cryptosporidium* is spread (primarily in water), it can affect large numbers of people, with particularly devastating effects on children, killing approximately 525,000 every year. Relatively little is known regarding the host-pathogen interactions between *Cryptosporidium* and gut epithelia, in part due to the technical difficulty, cost, and safety considerations of studying these parasites. Thus, we elected to utilize a closely related group of parasites, Gregarines, which are also members of the phylum Apicomplexa and parasitize the intestinal epithelia of invertebrates in a similar manner to *Cryptosporidium*. We propose that Gregarines may be a tractable model of many aspects of *Cryptosporidium* biology, particularly their physical and molecular interaction with the host gut epithelia. The specific gregarine-host interaction we are developing is between *Blabericola migrator* and the Madagascar Hissing Cockroach *Gromphadorhina portentosa*. A focus of this research is enhancing the infectivity of the cockroach colonies to allow for genetic manipulation of *B. migrator*. To do this, we will compare the efficacy and impact of directly infecting cockroaches uninfected by *B. migrator* to infected cockroaches. This will also indicate how many *B. migrator* parasites can be harvested from a single, infected cockroach. We can further determine if infected cockroaches that are not currently demonstrating signs of parasitism can be reinfected.

U25. Bacterial fluorescence imaging detects planktonic bacteria and biofilm *in vitro*

*Andre Gomez[1], William Little[1], Andrea J. Lopez[1], Klara C. Keim[1], Monique Y. Rennie[2], Liis Teene[2], Allie Clinton Smith[1] Department of Honors Studies, Texas Tech University, Lubbock, TX, USA[1] MolecuLight, Toronto, Canada[2]

Chronic wounds commonly harbor polymicrobial biofilms, and certain combinations of microbes may result in more aggressive infections. Culture-based diagnostics identify dominant microorganisms as well as their antimicrobial susceptibility, however the lag time to obtain those results (3 days – 4 weeks) significantly impacts wound care and treatment. Bacterial fluorescence imaging with the MolecuLight *i:X* imaging device uses safe violet light to detect fluorescent properties of bacteria. Many species of bacteria utilize aminolevulinic acid (ALA) to produce porphyrins, which fluoresce red under specific wavelengths of light. The MolecuLight *i:X* imaging device allows physicians to detect bacterial bioburden in a wound in real time, and can direct specimen sampling to the area with the heaviest bioburden, improving diagnostic capabilities. Prior work demonstrated its detection of bacteria from *in vivo* and *in vitro* monomicrobial planktonic cultures. We have investigated its capability to detect biofilm

using our polymicrobial *in vitro* biofilm model, consisting of Bolton's broth and bovine plasma, which is representative of the chronic wound environment. *Staphylococcus aureus*, *Escherichia coli*, and *Enterobacter cloacae* were selected as representative wound pathogens. When grown in the *in vitro* wound-like model for seven days, followed by the induction of porphyrin production by the addition of ALA for 24 hours, we demonstrated that the device can readily detect bacterial fluorescence from both monomicrobial and polymicrobial biofilms. These data demonstrate that bacterial fluorescence imaging detects porphyrin-positive species of bacteria growing both planktonically and as a biofilm, as well as monomicrobial and polymicrobial communities, which further validates the clinical capability and relevance of the device for use in wound care.

U26. Mortality during *Pseudomonas aeruginosa* chronic infection of *Drosophila melanogaster* dependent on mating experience of host

Virginia E. Green*, Eli Wallace, Deanna M. Soper, and William L. Cody
Department of Biology, University of Dallas, Irving, TX

Evolutionary trade-offs are extremely significant in understanding why certain phenotypes evolve and persist in different species. Such a trade-off may exist between reproduction and bacterial resistance in *Drosophila melanogaster*; recent studies suggest that reproduction may negatively affect immunity, which in turn influences the coevolution of virulence in pathogens such as *Pseudomonas aeruginosa*. Characterization of this trade-off will further illuminate the immunological cost of reproduction in fruit flies, leading to a more complete understanding of the coevolutionary dynamics of bacterial virulence and host resistance. While decreased immune function following mating has been noted previously, acute models of infection using thoracic puncture have traditionally been used rather than the chronic oral route. Given the influence of route of infection on host immune response, a thorough analysis of the effects of sexual experience must examine whether induced immunodeficiency is also observed against chronic infection. In this study, we orally infected groups of virgin and experienced flies with *P. aeruginosa* and compared mortality rates to determine whether either group displayed increased resistance to infection. Experienced female flies showed increased mortality after chronic infection compared to virgin females. These observations suggest that the act of mating reduces female flies' ability to resist oral bacterial infection. These findings may shed light on the coevolutionary past of host- pathogen pairs and suggest that coevolution infection models may need to be expanded to account for such factors as host experience.

U27. The influence of cell type on the expression of cytomegalovirus pathogenesis factors

Cassandra Grizer and Laura Hanson, Ph.D.
Department of Biology, Texas Woman's University, Denton, Texas

Sexually transmitted infections (STIs) have become a huge problem in recent years. We use mouse cytomegalovirus (MCMV) as a model herpesvirus to investigate. HCMV is also the leading infectious cause of birth defects and CMV is a huge problem in transplant patients- it is estimated that 15 cents out of every dollar we spend on transplantation is dealing with this virus. The m140 promoter is a DNA sequence that transcription factors bind to in order to regulate transcription and thus expression of that gene. The m140 gene product is an important pathogenesis factor that is required for efficient replication of the virus in macrophages. We are studying the promoter to have a better understanding of how the expression of this viral protein

is regulated in different cell types. There are three major classes of genes for transcription in CMV like viruses: immediate early, which does not require any viral transcription factors to be active, early, which requires at least one viral factor to be activated, and late which are normally active after viral DNA replication. The majority of gene regulation studies have been done in fibroblasts, but a few studies done with other cell types have found that variations in host transcription factor expression may allow expression of some early viral genes without viral transcription factors. We used various cells types, 3T3 fibroblasts, B35-P7 neuroblastomas, SGC1 salivary glands, and IC-21 macrophages to investigate. We will be using plasmids with known transcription factors, ieIII, ie1, and ie3. We are interested in the B35-P7 neuroblastomas because another viral early promoter had altered activity in neuronal cells, so we aim to see if this is a more general characteristic of early promoters in these cells. We are interested in the SGC-1 cells as they are a salivary gland tumor line, and cytomegalovirus is shed for a very long time from the salivary gland. We are interested in the IC-21s as the m140 gene product is required for efficient virus replication in macrophages. To investigate we are using a plasmid, which has the m140 gene fused to the GFP gene sequence under the control of the normal m140 promoter. The GFP only fluoresces in the cell's nuclei with successful induction of the m140 promoter using known transcription factors. We did experiments with the co-transfection of the various cell types to see which transcription factors could induce the m140 promoter. Although viral transcription factors are required for expression in the fibroblasts, we will also be evaluating for expression in the other cell types in the absence of viral transcription factors.

U28. The Clinical Significance of *Staphylococcus aureus* Small Colony Variants

Klara C. Keim* and Allie Clinton Smith
Department of Honors Studies, Texas Tech University, Lubbock, TX, USA

A novel phenotype of *Staphylococcus aureus* (SA) called *Staphylococcus aureus* Small Colony Variants (SA-SCV) have been identified principally associated with chronic and recurrent infections. This phenotype is induced spontaneously as a result of a combination of environmental stressors, including harsh conditions and polymicrobial interactions. SA-SCVs exhibit altered phenotypes as a result of metabolic dormancy caused by electron transport deficiency, leading to increased biofilm production and alterations to antimicrobial susceptibility. SA-SCVs typically exhibit altered colony morphology and biochemical reactions compared to wild-type *S.aureus*, making them difficult to detect via routine diagnostic procedures, and there is evidence that SA-SCVs can be unidentified or misdiagnosed in a clinical setting. The major clinical implication of SA-SCVs are inherent alterations in antimicrobial susceptibility and their contribution to chronic or recurrent infections, such as skin and soft-tissue infections, foreign-body associated infection, cystic fibrosis, sepsis. There is evidence that SA-SCVs contribute to patient morbidity and mortality as a result of diagnostic difficulties and limited treatment options. New detection methods may need to be developed that can be incorporated into routinediagnostics, which would allow for better assessment of specimens and introduce new considerations for treatment.

U29. An investigation of *Pseudomonas aeruginosa* cyan fluorescence with the MolecuLight *i:X* bacterial fluorescence imaging device

*William Little[1], Andrea J. Lopez[1], Andre Gomez[1], Klara C. Keim[1], Monique Y. Rennie[2], Allie Clinton Smith[1] Department of Honors Studies, Texas Tech University, Lubbock, TX, USA[1]
MolecuLight, Toronto, Canada[2]

Chronic wounds are a current area of major clinical concern, resulting in immense morbidity and mortality of a large patient population annually. These wounds do not typically respond to normal courses of antimicrobial treatment and often require drastic therapies, including amputation of the affected limb. Many different bacterial species are known to cause infections in chronic wounds, with *Pseudomonas aeruginosa* often playing a major role in these wounds' virulence and persistence. MolecuLight has developed a bacterial fluorescence-imaging device to detect the fluorescent properties of many chronic wound pathogens to aid in real-time visualization and direct specimen sampling. Bacterial species that produce the exoproduct porphyrin will fluoresce red under the MolecuLight *i:X* device. While *P. aeruginosa* is a known porphyrin producer, this organism typically fluoresces blue-green cyan under the device both *in vitro* and *in vivo*. It is thought this is due to the production of additional exoproducts with fluorescent properties, such as pyocyanin. We have partnered with MolecuLight to elucidate the mechanisms of cyan fluorescence production of *P. aeruginosa* in order to optimize the detection and utilization of the device with *P. aeruginosa*-infected chronic wounds.

U30. The Clinical Significance of Polymicrobial Interactions on Antimicrobial Susceptibility in the Diagnostic Laboratory

*Andrea J. Lopez[1], Mohamed Fokar[2,3], Andre Gomez[1], William Little[1], Allie Clinton Smith[1]
Department of Honors Studies, Texas Tech University[1]
Center for Biotechnology and Genomics, Texas Tech University[2]
Institute of Agricultural Biosciences, Oklahoma State University[3]

Chronic wounds are a frequently encountered condition in healthcare, characterized as slow-healing, difficult to treat, and often harboring a polymicrobial, biofilm-associated bacterial infection. One well understood consequence of biofilm infection is that bacteria are protected by a self-produced extracellular matrix, significantly altering the efficacy of antimicrobial drugs. Another less well-studied condition present in biofilm-associated infections is their polymicrobial nature, which research suggests also significantly alters antimicrobial susceptibly. The current methodologies for antibiotic susceptibility testing in a clinical laboratory setting are performed by using a single species planktonic bacterial suspension and conducting a minimum inhibitory concentration (MIC) assay. Given that this protocol requires a single species suspension, but chronic wounds typically harbor a consortium of bacteria that can synergize their activities regarding antimicrobial susceptibility, we hypothesize that monomicrobial and polymicrobial MIC results may be significantly different. To study this hypothesis, we have chosen four common wound pathogens, *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Enterococcus faecalis*, and *Acinetobacter baumanii*, which often synergize to cause polymicrobial infections, and determined their antimicrobial susceptibility individually and in combination, imitating the wound environment. Our studies suggest that polymicrobial interactions do significantly affect antimicrobial susceptibility, which has important implications for clinical microbiology diagnostics.

U31. The role of ClpX and ClpP in antibiotic resistance in *Bacillus anthracis*

Quinn Losefsky*, Shauna McGillivray
Texas Christian University, Fort Worth, TX

Bacillus anthracis is a Gram-positive bacterium that causes anthrax in humans. It is a significant microorganism in that many proteins important to virulence or pathogenesis are highly conserved in many other pathogenic bacteria. Our lab has previously identified the protein ClpX in *Bacillus anthracis* as metabolically significant in antibiotic resistance. Specifically, *B.* anthracis lacking the *clpX* gene (ΔClpX) are significantly more susceptible to antibiotics that target the bacterial cell wall such as penicillin than the wild type. ClpX has multiple functions; primarily it interacts with ClpP to form a proteolytic complex that degrades dysfunctional or obsolete proteins. ClpX also has an independent chaperone function, moving proteins around the cell. This project has focused on determining if the pathway of decreased antibiotic resistance in mutant *B. anthracis* is dependent on ClpX interactions with ClpP, or if ClpX can function independently. To test this, a point mutation (I265E) was made in the *ClpX* gene at the site that has been previously identified as the site of interaction between ClpX and ClpP in *Staphylococcus aureus*. The ClpX genes in *B. anthracis* and *S. aureus* exhibit a high degree of conservation particularly in this region, and it is expected that this site will also be critical for ClpX and ClpP interaction in *B. anthracis*. The mutated *ClpX* gene (I265E) has been confirmed with sequencing and has been transformed as an inducible expression plasmid into the ΔClpX *B. anthracis* strain. Preliminary assays to determine the antibiotic resistance of the mutant strain have shown marked decrease in resistance to penicillin as compared to the wild-type or the complemented strain.

U32. Exploration of pyomelanin-mediated cooperation in chronic infections

Jiwasmika Baishya, J. Fowler, M. Moncrief*, O. Shabaneh, C. A. Wakeman
Department of Biological Sciences, Texas Tech University, Lubbock, TX

Biofilms are often associated with chronic infections and some are known to harbor multiple species of microorganisms. Pathogenic microorganisms evolving at the host-pathogen interface often possess adaptations for increased survival via evasion of host immune system responses. Interestingly, these adaptations can sometimes be associated with decreased production of molecules traditionally viewed as virulence factors. We have observed this specific trend in *Pseudomonas aeruginosa* isolates in the lungs of cystic fibrosis patients. These isolates have shown phenotypes that indicate metabolic interdependencies, which hinder the microorganism's fitness in the absence of resources it is acquiring either from the host or from other organisms within the polymicrobial community. Our lab focuses on polymicrobial interactions between *Pseudomonas aeruginosa* and *Staphylococcus aureus*. We aim to look at the hypothesized cooperation of these two species in order to have a better understanding of what would be best to treat the infection. Our lab aims to understand the cooperation between microbes mediated by pyomelanin, an antioxidant pigment byproduct of a mutated tyrosine degradation pathway. We hypothesize that pyomelanin can be used as a community resource to protect against oxidative stress by the microbes that cause the lung infections in cystic fibrosis patients. We have observed that the presence of *Staphylococcus aureus* leads to greater pyomelanin production in both laboratory strains and cystic fibrosis isolates. We are attempting to determine whether or not the co-culture of *Pseudomonas aeruginosa* and *Staphylococcus aureus* can be mutually beneficial to both organisms due to increased oxidative protection mediated by this increase in pyomelanin production.

U33. Is the madagascar hissing cockroach-infecting gregarine, b*labericola migrator*, susceptible to the antimicrobial drug paromomycin?
Mauricio Padilla*, Nicholas Barrett, Matthew Fencl, Kaitlyn Stark, Joseph Mathis, and
Daniel A. Gold. Department of Biological Sciences, St. Edward's University, Austin, TX

Worldwide, infectious diarrheal diseases account for a significant portion of infant morbidity and mortality. Cryptosporidiosis, caused by the protozoan pathogen *Cryptosporidium spp.*, ranks second as a causal agent for these infant deaths, and is also implicated in the deaths of many immunocompromised individuals. The technical challenges and costs involved in developing a working laboratory system in which to conduct transgenic experimental studies of *Cryptosporidium* have slowed the research into treating cryptosporidiosis. In light of this difficulty, alternative organismal models for studying the basic cell and molecular mechanisms of host-pathogen interactions of *Cryptosporidium* should be sought. Fortunately, recent molecular phylogenetic re-classifications of protozoa have revealed that *Cryptosporidium*, in the phylum Apicomplexa, is closely related to a sub-class of invertebrate-infecting, apicomplexan parasites called Gregarines. With the knowledge of this re-classification, we intend to mirror previous work that studied the effects of the aminoglycoside antibiotic paromomycin on *Cryptosporidium* (Vinayak et al, 2015) to Gregarines to determine their capability as an alternative model for studying *Cryptosporidium*. Developing Gregarines as a tractable laboratory model system, specifically *Blabericola migrator* (host: Madagascar Hissing Cockroach), we intend to gain key insights into the evolutionarily conserved molecular and cellular mechanisms involved in *Cryptosporidium* parasitism. To do this, we must first determine if Gregarine parasites are as susceptible to paromomycin as *Cryptosporidium* parasites are. We will accomplish this by administering the drug to the food of one infected cockroach population and withholding it from a second infected population as a control and assessing both the numbers and viability of parasites isolated from frass. We expect that ascertaining the susceptibility of *B. migrator* to paromomycin is the first step to determining the use of Gregarines as a tractable laboratory model for *Cryptosporidium* so that the drug and its resistance gene product, NeoR, can be used as a selectable marker system for genetic manipulation of the parasite.

U34. Effect of Growth Conditions on the Cell Surface Hydrophobicity of *Candida albicans*
Melissa Rose* and James Masuoka
Department of Biology, Midwestern State University, Wichita Falls, TX

Candida albicans is an important commensal fungus that inhabits the gastrointestinal and genitourinary tracts of healthy humans. *C. albicans* can also opportunistically cause diseases such as vaginal and oral candidiasis ("yeast infections" and thrush, respectively). Individuals immunocompromised by chronic infections, or by drugs given for transplantation or cancer treatment, are at particular risk. Adherence is the first step in colonizing the host, and cell surface hydrophobicity (CSH) is an important factor in this process. Hydrophobic cells were demonstrated to adhere better to mouse tissues than hydrophilic cells and hydrophobic cells are more resistant to phagocytic killing. Both of these characteristics contribute to the ability of *C. albicans* to cause disease. The human host provides a range of growth conditions that may influence the ability of microbes to effectively colonize the host. For example, growth temperature affects cell surface hydrophobicity. Cells grown to stationary phase at 23 °C are almost entirely hydrophobic, while cells grown at 37 °C are almost entirely hydrophilic. We

hypothesized that other growth conditions would influence CSH. To test this hypothesis, we determined *C. albicans* CSH grown in media of differing nutrient composition. Of particular interest was the amount of yeast extract, as previous studies suggested this affected length of lag phase. We also adjusted the growth medium pH to reflect that of the blood or the vaginal mucosa. Finally, we determined CSH at growth temperatures between 23 and 37 °C to see if the transition was linear. Our results demonstrated that this transition was not linear, but more sigmoidal. pH had little effect on CSH. Nutrient composition had some effect, but appeared to show strain differences. By understanding how cell surface hydrophobicity changes with these parameters, we will gain a better understanding of how *Candida albicans* successfully colonizes multiple niches within its host.

U35. *Staphylococcus aureus p*revalence in an asymptomatic population in South Central Texas
Loan Yen Vu* and Dr. Robert Jonas
Department of Microbiology, Texas Lutheran University, Seguin, TX

Staphylococcus aureus is a common skin bacterium that is found as a commensal species in many humans. For this research, we looked at the carrier rate in a population of mainly young women, ages 15-18. *Staphylococcus aureus* was targeted to find a specific strain called MRSA (*Methicillin Resistant Staphylococcus aureus*) in students' noses that participated during the Texas Lutheran University STEM woman's day, Nursing Boot Camp and the New Braunfels Science Fest in July and October 2018. Four in twenty-seven (14.8%) TLU participants carried *S. aureus* in their noses identified by the growth on Mannitol Salt agar plates. Only one (3%) of those turned out to have the MRSA gene, which confers the ability to resist many common antibiotics.

U36. Testing for the Development of Bacterial Resistance to Glycoside Hydrolases
Rachel L. Washburn*, Whitni K. Redman, Derek Fleming, Kendra P. Rumbaugh
Department of Surgery, Texas Tech University Health Sciences Center, Lubbock, TX

Chronic wounds are an enduring personal, medical, and economic issue due to their tendency to support persistent infections. Bacteria in chronic wound infections frequently live in biofilms where they are surrounded by a self-produced matrix of extracellular polymeric substances (EPS). The EPS inhibits the action of many antibiotics, acting as a protective shield surrounding bacteria. We have previously shown that certain glycoside hydrolases (GHs) can disperse biofilm-associated bacteria *in vitro* and *in vivo* by hydrolyzing α-1, 4 and β-1, 4 linkages of EPS polysaccharides. These dispersed bacteria are then more easily killed by antibiotics. We postulate that GHs represent a promising new class of adjunctive agents that would increase antibiotic efficacy. Since GH's are not bactericidal themselves, they should not put pressure on bacteria to develop resistance. However, this theory has not been experimentally tested. As the utilization of GHs in clinical settings requires repeated topical application to wound beds, the aim of this study was to test whether bacteria can evolve resistance to GHs. To accomplish this, *Pseudomonas aeruginosa* and *Staphylococcus aureus* were used to create polymicrobial *in vitro* biofilms with four technical repeats. The biofilms were treated with 5% GH for two hours. Efficacy was determined by calculating the number of bacteria that dispersed from the biofilm. Bacteria treated with GHs were used to initiate new cultures and biofilms, which were treated with GHs

in a reiterative process. Upon ANOVA comparison between GH groups and vehicle control groups, there was one significant difference that showed increase in dispersion both in the *P. aeruginosa* enzyme and vehicle groups. While we saw an overall 20% reduction in dispersal from the first round of treatment to the last, the high variability between passages indicates that further biological repetitions are required to determine if this observation is consistent and significant.

U37. Determining the Efficacy of Multi-Enzyme Cocktails to Degrade Biofilms

Garrett S. Welch*, Whitni K. Redman, Derek Fleming, Kendra P. Rumbaugh
Department of Chemistry and Biochemistry, Texas Tech University, Lubbock, TX

Chronic wounds are the result of a disruption in at least one of the four physiological wound healing phases, leading a wound to persist longer than 6-8 weeks. Chronic wounds affect over 6 million people in the United States alone, with experts expecting that number to increase. A common factor that can lead to the persistence of a wound is a biofilm-associated infection. This infection often leads to the disruption of the second physiological wound-healing phase: inflammation. Bacteria in biofilms are more tolerant to antibiotics, antimicrobials, and host immune system defenses when compared to their free-floating, planktonic, counterparts. This increased tolerance has been attributed to the self-synthesized extracellular polymeric substance (EPS), made up primarily of exopolysaccharides and extracellular DNA (eDNA), as well as the physiological changes that occur in biofilm-associated bacteria. Due to this increased tolerance, treating biofilm-associated infections has proven difficult with traditional methods. Since experts estimate that 80% of all human bacterial infections are biofilm-associated, many researchers have begun to develop treatments that target the biofilm directly, reducing its integrity and dispersing the associated bacteria into their more susceptible planktonic form. This method of treatment focuses on using catabolic enzymes to target various parts of the EPS. Glycoside hydrolases (GH) target linkages in the exopolysaccharides while DNase targets linkages in the eDNA. We have previously shown that two GHs, α-amylase and cellulase, reduce the integrity of the EPS and lead to bacterial dispersion. However, these enzymes are not universally effective on biofilms made by complex communities of bacteria, suggesting that multi-enzyme cocktails may be required. In order to determine efficacy, we subjected biofilms, grown *in vitro* either in multi-well plastic plates or in a clinically relevant wound model, to different enzyme cocktails containing cellulase, α-amylase, xylanase, alginate lyase and/or DNase. We determined that the efficacy of enzyme cocktails depended upon the specific bacteria present, rather than the sheer number of species present. We next hope to test our cocktail in a murine wound model. By attacking different components of the EPS with multiple enzymes, we hope to create a cocktail that degrades the complex biofilms found in various human infections. We believe that a cost-efficient enzyme cocktail coupled with current antibiotics could combat biofilm-associated infections more effectively.

U38. Elucidating the temperature-dependent physiology of *Pseudomonas aeruginosa* biofilms

Kiddist Yihunie*, Peyton Webster, Karishma Bisht, Jeanette Rimbey, Austin G. Brown, Jake Sellers, Andrew Salazar, Jennifer Herrera, Alex Luecke, Jessica L. Moore, Richard M. Caprioli, Eric P. Skaar, and Catherine A. Wakeman
Department of Biological Sciences, Texas Tech University, Lubbock TX;
Department of Chemistry, Vanderbilt University, Nashville TN;
Department of Pathology, Microbiology, and Immunology, Nashville TN

Biofilms are surface-associated groups of microorganisms that adhere and interact with each other through the use of an extracellular polymeric substance. Biofilm growth can aid in microbial survival in a constantly changing environment. *Pseudomonas aeruginosa* is a common environmental isolate and nosocomial pathogen that is known to form biofilms in all the various niches it occupies. The ability to form robust biofilms contributes to the antibiotic resistance and immune evasion mechanisms of *P. aeruginosa*. We hypothesize that the fluctuations in temperature in the different niches that *P. aeruginosa* occupies drive the formation of biofilms specifically adapted to survival within that niche. Studies have shown that exposure to different environmental factors, such as temperature fluctuations, can result in the expression of specific morphologies and behaviors within biofilm communities of other microorganisms. Using MALDI IMS, we have demonstrated that *P. aeruginosa* biofilms grown under these different temperature conditions exhibits dramatically different protein expression profiles, which supports the contention that these biofilms are uniquely adapted to different niches. The objective of this project is to identify the genes present in *P. aeruginosa* required for biofilm adaptation to a range of temperatures (room temperature, 30°C, 37°C, and 40°C) using a commercially available library containing over 5,000 unique mutants.

Undergraduate Student Posters – Phage and Antimicrobial Resistance

U39. Discovery of novel bacteriophages located in Galveston and South Padre Island

Adey Awah*, Jolene Ramsey, Ryland Young
Department of Biochemistry & Biophysics, Texas A&M University, College Station, TX

Bacteriophages, or phages, are viruses that infect a specific bacterium, replicate inside the host, and eventually kill, or lyse the cell. Using these bacteriophages to eliminate not only pathogenic bacteria but harmful industrial strains such as bacteria that stunt and decrease the yield of crops is a long-term goal. In this study, the objective was to locate phage for bacteria that possess a potential for further research. The host bacteria chosen, *Vibrio natriegens,* is a model organism in development because of its robust genetic system and molecular tools available for its manipulation in the lab. While phages infecting V. natriegens have been previously isolated, they have not been sequenced or characterized at the molecular level. To locate phage specific to *V. natriegens*, environmental samples were retrieved from salty marsh areas. After screening various samples, four phage positive for infection of *V. natriegens* were found. Two of these were effective at lysing the bacteria in laboratory conditions, and therefore amplified for characterization. Amplification of phage allows extraction of genomic DNA and enables viewing the morphology using transmission electron microscopy. The two phage isolated, called Galveston and Phriendly, were found in samples from Galveston and South Padre Island, respectively. Through TEM, the morphology of Phriendly was identified as a podophage, and through genome sequencing Phriendly and Galveston have a genome size of 50.2 and 53 kb, respectively. In ongoing investigations, these phages will be compared to others known to infect *Vibrios* at the genomic level.

U40. Isolation of Bacteriophages Rowley and Gustopher

Kayla Bahr*, Leah Dowell, Kenneth Underhill, Michaela Aguirre, Shey Andrews, Abigail Ballard, Matthew Bristerpostma, Faith Cox, David Kiker, Tiffany Lee, Stacy Luke, Tiffany Lujan, Haze Murphy, Abbigail Ramirez, Rheaven Sandoval, and Dustin Edwards
Department of Biological Sciences, Tarleton State University, Stephenville, TX

Rowley and Gustopher are bacteriophages, which were isolated from a *Microbacterium foliorum* bacterial host at 29° C in PYCa media. Transmission electron microscopy from purified samples identified both Rowley and Gustopher to have *Siphoviridae* morphology. Rowley is a Cluster EE bacteriophage that was direct isolated from a vegetable garden soil sample in Stephenville, Texas, and it has a relatively small genome that is 17,542 bp in length with 68.7% G+C content and a 9 bp overhang of 5'-CCCGCCCCA-3'. Rowley has 26 predicted protein-encoding genes, including a lysin A. Rowley was determined to be 100% identical to the bacteriophage Scamander, which had been previously isolated from a different vegetable garden over 1,600 km away. Gustopher was isolated from a water sample from the Bosque River in Stephenville, Texas using enriched isolation method. Gustopher was unique among our bacteriophage isolates in that plaques appeared within six hours as compared to 48–72 hours for other isolates. Rowley and Gustopher both could be valuable in understanding therapeutic use against antibiotic resistance and genetic diversity in bacteriophages.

U41. Parameter Estimation and Simulation of Bacteriophage Infection Model

Abigail Ballard*[1], Shey Andrews[1], Michaela Aguirre[1], David Kiker[1], Matthew Bristerpostma[1], Dustin Edwards[1], and Keith Emmert[2]
[1]Department of Biological Sciences, Tarleton State University, Stephenville, TX
[2]Department of Mathematics, Tarleton State University, Stephenville, TX

Bacteriophages are an emerging focus in research due to their potential use in treating antibiotic resistant bacterial infections. Tarleton State University, as part of the international HHMI SEA-PHAGES bacteriophage discovery program, is working on an interdepartmental project between the biology and mathematics departments to create a mathematical model for the complexity of bacteriophage infection of host cells. We constructed a Compartmental Diagram using $3n+1$ differential equations to model interactions between bacterial cells and bacteriophage, in which n different types of phages are capable of infecting a cell. Susceptible cells are represented as Class S. Class C_j represents those bacteria, which have become infected with the j^{th} phage where the bacteriophages are circularizing. Bacteria in Class C_j will then move to either a lytic or lysogenic cycle. Class I_j represents the cells infected by the j^{th} bacteriophage and are currently following a lytic cycle. Class R_j represents the cells infected by the j^{th} bacteriophage, which are currently following a lysogenic cycle and exhibiting bacteriophage homoimmunity. We assume it possible to move from Class R_j to Class I_j when suitable environmental stress occurs. The Classes C_j, I_j, and R_j are considered immune to infection by a different virus. Finally, Class V_j represents j^{th} virus in the environment produced from lysis of infected cells. A simple mass-action interaction term, $\alpha_j S V_j$, controls the creation of newly infected susceptible bacteria and removes spent virus. A single, constant carrying capacity is used to control the growth of all classes of bacteria. Local asymptotic stability criteria for selected equilibria are then derived. Currently, we are designing experiments to evaluate and verify the model that has been created. *Microbacterium foliorum* will be used as a host for bacteriophages to establish parameters, which that can be used to verify the model.

U42. Characterization of Streptomyces phage TomSawyer and the use of Bioinformatics in Genomic Analysis in Order to Annotate the Genome

Brenda Banda*, Morgan Marshall, Maxine Nguyen, Kevin Mariena, Adam Tesso, Michael Tran, Subhayu Nayek, and Lee E. Hughes
Department of Biological Sciences, University of North Texas, Denton, TX

In this laboratory, we isolate and analyze bacteriophages that infect *Streptomyces griseus*. For this study, Streptomyces phage TomSawyer was isolated by enrichment in Harrisonburg, VA in 2017 by Sawyer Kearns. This phage was found to be in the BE cluster and BE2 subcluster. TomSawyer was sent to the Pittsburgh Bacteriophage Institute, where it underwent DNA sequencing. It has a GC content of 49.3%. This phage also seems to be lytic. The genome contains 133961 bp with terminal repeats of 12182. We adopted the phage for genome annotation in our laboratory. We used bioinformatic software including Glimmer and GeneMark, which predicted 262 genes. Each gene then had to be individually examined for the correct start and probable functions that it could have. In total, we had two rounds of annotations – one primary annotation and a secondary review by another peer. The software we used was primarily PECAAN because it was a collection of most of the bioinformatic software we needed.

U43. P1 Phages: So many, so similar, yet so unique

Garret Beene*, Korrin Taylor, Frederick N Baliraine and Gregory D Frederick
LeTourneau University, Department of Biology and Kinesiology

Bacteriophages are viruses capable of infection and replication of their genomes within bacterial cells. Phage genomes encode specific proteins, which allow lytic reproduction or lysogeny of the virus within the host. Characterization of phage genomes contribute significantly to the functional understanding of the genetic systems and the evolution of viruses and their hosts. In the fall 2018 semester, LeTourneau University undergraduate students, participating in the Howard Hughes Medical Institute SEA PHAGES program, isolated bacteriophages capable of infecting *Mycobacterium smegmatis* mc^2155. Of the nine isolated phages whose genomes were sequenced seven, including KilKor, Phalm, CactusJack, Meggido, TChalla, BelcherPhantom and StressBall, are all members of the P1 subcluster. Currently, there are 33 isolated members of the P1 subcluster with sequenced genomes. Members of the P1 subcluster have an average genome size of 47,962 bp. The average GC content is 67.1%. P1 phage genomes encode an average of 79.3 genes. Subcluster P1 phages infect bacterial hosts in the genus, *Mycobacterium*. They are generally temperate phages. The analysis and comparison of distinct and common genomic features of the subcluster P1 Mycobacteriophages will be described.

U44. Phylogenetic and Evolutionary Relationships of Small Genome Bacteriophages

Matthew Brister-Postma*, Abigail Ballard, Shey Andrews, Michaela Aguirre, Tiffany Lee, David Kiker, Kayla Bahr, Hunter Underhill, Leah Dowell, Abbigal Ramirez, Stacy Luka, and Dustin Edwards
Department of Biological Sciences, Tarleton State University, Stephenville, TX

Bacteriophages, viruses that can infect bacteria, are a unique and diverse category of viruses that show wide variability, from host range to genome length, even within their respective clusters. The goal of this study was to characterize bacteriophages that contain genomes of less than 20,000 base pairs, which include Clusters AN, BO, CW, EE, FE, DM, AX, and FD. MEGA X phylogenetic software was used to construct an initial phylogenetic tree to group 83 small genome bacteriophages, using the neighbor-joining method with a Tamura-Nei distance model. Genes were analyzed using Phamerator, HHPred, NCBI Basic Local Alignment Tool-protein (BLASTp), and NCBI Basic Local Alignment Tool-nucleotide (BLASTn) to investigate possible gene functions and evolutionary relationships. Candidate genes were input into Venny software,

which determined that two cluster BO bacteriophages share a LysM-like endonuclease, which is also found in the cluster, BE bacteriophages, which infect plant pathogenic bacteria. This result suggests a common ancestor for these two clusters of bacteriophages, and we plan to further categorize this result with further phylogenetic analysis. Likewise, both cluster AN and FE bacteriophages, which primarily follow lytic cycles, have a unique MerR-like Helix-turn-Helix binding domain, similar to the more temperate cluster A and cluster F bacteriophages. Geospatial analysis is currently being conducted to determine the effect of geographic distribution on genetic variability by using an interactive map with data from the Actinobacteriophage Database. This study could provide insights in bacteriophage evolution and their environmental roles.

U45. The Diversity of Prophage Induction Proteins

Katelyn Gutierrez*, Taylor Zimmermann, Dr. Gregory D. Fredrick, Dr. Fredrick Baliraine
Department of Biology, LeTourneau University, Longview, TX

As increased antibiotic resistance closes the curtain on the antibiotic age, bacteriophages are being pulled out from backstage to become the new stars for treating bacterial infections. The lytic phage is significantly more prepared for the task as they result in the destruction of the infected bacteria cell. The lysogenic phage is less than optimal, because it integrates its DNA into the host chromosome, generally only entering the lytic cycle if effected by stress or damage to DNA. But despite these disadvantages, could lysogenic phages be encouraged into the lytic cycle without having to endure the triggering DNA damage?
In this presentation, we will discuss the diversity of the CrO protein that is involved in prophage induction. Great diversity between phages that have different possible hosts would suggest that it could be possible to force specific prophages into induction without destroying other bacteria. This would allow for the many temperate phages being isolated to be weaponized for therapeutic uses. This could also have implications in halting the continuation of super immune bacterial strands and in understanding the bacteriophage and host relationship through an evolutionary perspective.

U46. Annotating Streptomyces phage Araceli

Joelyn Hanson*, Cameron Theiss, Subhayu Nayek, and Lee E. Hughes
Department of Biological Sciences, University of North Texas, Denton, TX

Phages are a very large population with an estimated 1031 phages in the biosphere. However, only a small portion are known to the scientific world. One of the largest collections, the actinobacteriophages found through the SEA-PHAGES program, are still fewer than 3,000 isolates. What is so interesting about bacteriophages is they are viruses that infect a bacterial host because of their inability to replicate on their own, making them extremely successful in destroying bacteria. These bacteriophages could eventually have the reach to be the counter to antibiotic-resistant bacteria, possibly making bacteriophages the future of medicine. The specific host that I used during my research was Streptomyces griseus. The project will focus on Streptomyces phage Araceli with 84 genes. While annotating Araceli, my partner and I found that it had very few identifiable functions with 13. We were able to determine these functions by looking for a proper E-value with a low number, preferably that of 0 and the query to and from numbers which show the protein alignment. Some functions that were found range to terminase, portal protein, helicase, and DNA polymerase. Araceli compared to the other phages in the same

cluster, lined up with other phages and typically had few moments when it strayed from the similarities. Overall, this process has brought a new light about the smaller world beneath our feet and we feel our hard work with these phages will someday account for something very important.

U47. Annotation and Analysis of *Streptomyces* phage *Tribute*

Rebecca Lamb*, Heather Bullock, Samantha Julsaint, Brandy Mason, Jonah Pfeiffer, Subhayu Nayek and Lee E. Hughes
Department of Biological Sciences, University of North Texas, Denton, TX

Streptomyces phage *Tribute* was discovered in Frisco, TX in 2016 by UNT PHAGES student Meera Patel. This phage was isolated on the host bacterium *Streptomyces griseus* using the HHMI Phage Discovery Guide protocols. *Tribute* is a member of cluster BE, subcluster BE1. This phage appears to be lytic, has a 50% GC content, and was sequenced at the Pittsburgh Bacteriophage Institute. Our team adopted *Tribute* for bioinformatics analysis in our lab and performed annotations by utilizing comparative tools through PECAAN, following the SEA-PHAGES Bioinformatics Guide. Our annotations show the function of most genes in *Tribute* is currently unknown. The functions we have been able to assign include HNH-endonuclease, tail assembly chaperones, minor tail proteins, tape measure proteins, rIIA-like protein, rIIB-like protein, and exonuclease. Annotating and analyzing this phage will further the understanding of basic phage biology. Understanding phage biology could also further developments in using phages as molecular biology tools or in phage therapy for humans with bacterial infections.

U48. Possible Cas4 Gene Encoded by Subcluster A1 Bacteriophages

Tiffany Lujan*, Faith Cox, Camille Trautman, Trey Carter, Nikki Cody, Jackson Roye, Harold Rathburn, and Dustin Edwards
Department of Biological Sciences, Tarleton State University, Stephenville, TX

Bacteriophages are viruses that are able to infect and lyse bacteria. Bacteria have evolved anti-viral defense mechanisms, such as clustered regularly interspaced short palindromic repeats (CRISPR) to combat bacteriophage invasion. We hypothesize phages may have acquired a counter-invasion mechanism by uptaking a CRISPR-Cas-associated gene. Subcluster A1 mycobacteriophage Arlo was direct isolated from host *Mycobacterium smegmatis* mc^2155, and its genome (Genbank MH576971) was predicted to have 96 protein coding genes with gene 67 being annotated as a Rec-B like exonuclease. However, through further characterization using bioinformatics software HHpred and databases Pfam, SCOPe, PDB, BLASTp and NCBI, it showed a 99% identity to a CRISPR-Cas4-associated protein. CRISPR-Cas4 proteins are characterized as containing a Rec-B like exonuclease domain and are relatively uncharacterized as stand-alone genes. Using the software MEGA X to create a phylogenetic tree based on bootstrap statistical method provided high evidence of gene 67 being more closely related to other Cas4 type I-A than Rec-B proteins. MEGA X MUSCLE analysis showed a QXXXY amino acid motif that is found in all CRISPR-Cas4-associated proteins was present in gene 67, but not in RecB proteins. A secondary MUSCLE analysis showed the motif DYK to be highly-conserved in RecB protein sequences at the C terminus, but not present in gene 67 or other cas4 proteins. All proteins were found to contain highly-conserved cysteine residues at the same area on the C-terminus that are thought to be responsible for enzymatic activity. An *in silico* model

of gene 67 using PHYRE^2 software was used for comparative analysis to Cas4 which resulted in 99.8% identity. Current directions include doing a comparative analysis against all tertiary structures analyzed in the phylogenetic tree and characterization of the evolutionary origin of gene 67.

U49. Isolation of Cluster EA2 Bacteriophage Finny

Stacy Luka*, Tiffany Lee, Michaela Aguirre, Shey Andrews, Kayla Bahr, Abigail Ballard, Matthew Bristerpostma, Faith Cox, Leah Dowell, David Kiker, Tiffany Lujan, Haze Murphy, Abbigail Ramirez, Rheaven Sandoval, Kenneth Underhill, and Dustin Edwards
Department of Biological Sciences, Tarleton State University, Stephenville, TX

Bacteriophage Finny was purified from *Microbacterium foliorum* at 29° C in PYCa media by direct isolation method from a soil sample collected from a chicken coop in New Braunfels, Texas. Following initial isolation, two rounds of serial dilutions and plaque assays were performed for bacteriophage isolation and purification. Bacteriophage Finny plaque morphology consists of small-to-medium-sized lytic plaques with turbid halo rings. High titer lysate was stained with uranyl acetate to visualize bacteriophage Finny by transmission electron microscopy, which showed the virus has *Siphoviridae* morphology with an icosahedral capsid. DNA was extracted by a zinc chloride method and the whole genome sequenced at the Pittsburgh Bacteriophage Institute. Whole genome sequence comparison determined that bacteriophage Finny is a Cluster EA2 cluster with a circularly permuted genome 40,313 bp in length with 62.1% G+C content. Bacteriophage Finny genome contains 63 predicted protein-coding genes, including lysin A, holin, RecA-like DNA recombinase, AAA-ATPase, MazG-like nucleotide pyrophosphohydrolase, thymidylate kinase, and ThyX thymidylate synthase.

U50. Complex beauty isolated from the garden: Untangling phage omorphoKaypo variants A & B

Annabelle E. Malinowski*, Kathryn B. Baker, Frederick N. Baliraine, and Gregory D. Frederick
LeTourneau University, Department of Biology and Kinesiology, Longview TX 75602

Mycobacteriophage OmorphoKaypo (OK) was isolated at LeTourneau University in August 2017. Its name is Greek for “Beautiful Garden”, commemorating the campus garden where it was discovered. The soil sample underwent the typical isolation, purification, and amplification processes prescribed by the Phage Discovery Guide developed by the Howard-Hughes Medical Institute SEA-PHAGES program. OmorphoKaypo was found to be a temperate phage, a member of subcluster L1 with an isometric head, siphoviridae morphology, and a non-contractile tail measuring ~266nm long. Despite performing isolation streak-plate dilutions a total of six times and the production a high-quality DNA sample (as indicated by the restriction digest analysis), genomic DNA sequence analysis indicated the original DNA was isolated from a mixed population. Based upon DNA sequence analysis, the two distinct phage in the mixed population were both members of the L1 subcluster. They were found to be identical except for a middle region of approximately 3kb dissimilarity (henceforth called “pt-2”). The remainder of the two genomes, a long initial portion (pt-1) on the left-hand side of the genome and shorter terminal portion (pt-3) are nearly identical in each phage. PCR primers specific to each variant’s pt-2 region were designed. PCR conditions were established which allowed multiplex PCR analysis of isolated plaques to determine whether they were generated by the “A” or “B” variant. PCR

analysis confirmed two distinct phage were present. A third phage type for which neither PCR primer set produced a predicted produce was also identified. This presentation will explore further efforts to untangle the beautiful phage population originally isolated from the garden.

U51. Bacteriophage Distribution: Impact of Geographic Constraints on Diversity
Logan Parish*, Riley Howe, Frederick N Baliraine and Gregory D Frederick
Department of Biology and Kinesiology, LeTourneau University, Longview, TX

Bacteriophages, like all viruses, are remarkable entities, which are incapable of independent reproduction and therefore require compatible hosts for replication. Phages exploit the cellular machinery and assets of their bacterial host cell for viral reproduction. The SEA-PHAGES program explores viral diversity and the evolutionary mechanisms, which play key roles in the diversity of phages. The establishment of this global program allows for the isolation of soil-associated phages and data collection from around the world. The phage isolation process was established by the HHMI SEA-PHAGES program and requires GPS coordinate assignment for each collected phage based on isolation origin. Typically, universities have high diversity in phage isolates, which represent many unique composite bacteriophages. However, in the fall of 2018, students from LeTourneau University isolated six bacteriophage, which were members of the same subcluster (P1). In the previous year, two additional P1 phages were isolated. In total, LeTourneau University has characterized seven P1 isolates that are similar and an eighth phage, which is identical to one of those. Normally, this distribution is highly unusual. The observation lead to subsequent investigation of this seemingly unique situation. The question arose as to whether environmental and geographical constraints might influence bacteriophage distribution within the environment. To investigate this, data from HHMI SEA-PHAGES database was used to investigate potential geographic restriction by phage-cluster or type. GIS mapping was employed to map isolates for ten unique sub-clusters. The results and implications of observations made will be discussed in this presentation.

U52. Exploring the Transfection of Streptomyces Using BryanRecycles Bacteriophage
Karen Lin*, Melissa Mejico, Willow Rock, Yara Zraiqat, Sonya Layton, and Lee Hughes
Department of Biology, University of North Texas, Denton, TX

The computational analysis of genomic data has enabled labs to bypass molecular biology techniques in the elucidation of gene function. However, when bioinformatics cannot supply the function of a gene, wet lab experiments may be designed to solve for the unknown. Analysis of bacteriophage BryanRecycles' DNA sequence has led to a putative location and function of 23 genes, however 54 remain unidentified. In order to observe the effects of individual phage genes on the host bacterium *Streptomyces griseus*, electroporation was optimized to produce high quantities of transfectants. In a number of trials, the variables of the procedure were manipulated, including cell pretreatment, buffer composition, amount of phage DNA, time constant, and electric pulse strength. Should electroporation find success, candidate unknown genes will be chosen to investigate within the *Streptomyces* host.

U53. Bacteriophage Educational Laboratory Exercise Utilizing Raspberry Pi
Rheaven Sandoval*[1], Faith Cox[1], Tiffany Lujan[1], Dustin Edwards[1], and Keith Emmert[2]
[1]Department of Biological Sciences, Tarleton State University, Stephenville, TX
[2]Department of Mathematics, Tarleton State University, Stephenville, TX

Incorporating meaningful real-world experiences into classrooms is a persistent challenge. Bacteriophages are an ideal tool for creating a hands-on collaborative learning environment for high school and undergraduate biology students. As bacteriophages lyse their host, they begin to form cleared areas of host cells called plaques. Bacteriophage plaque assays are a common technique used in virology to visualize and quantitate replication, as well as measure lytic virulence. We have designed a laboratory exercise for high school and lower-level undergraduate courses that combines inexpensive computing devices and open source programming to visualize and quantitate actual or virtual bacteriophage plaque assays. Students assemble Raspberry Pi 3 Model B+ computing device, Raspberry Pi 7 inch Touch Display, and camera module V2 components and program with Python language to acquire time lapse images over a time course of 72 hours. Bacteriophage plaque assays are incubated above a backlit LED light board panel allowing plaque formation to be imaged in a field-of-view camera. A virtual plaque assay can also be performed in classes without laboratory access by using a variable size hole punch on paper to simulate plaque formation. Students are then able analyze the time lapse images of the plaque assay to quantitate plaque forming units and to determine bacteriophage virulence.

U54. Implications of Phage-encoded DNA Methylase Gene Homology to Relevant Soil-Dwelling Bacterial Species

Christina E Spencer*, Frederick N Baliraine, and Gregory D Frederick
Department of Biology and Kinesiology, LeTourneau University, Longview, TX

Genome annotation of Phage Phalm (P1) revealed two genes with homology to methylase/methyltransferase (MTases) genes in other bacteriophage and soil-dwelling bacteria. MTase function in bacteria is known to be important to cell survival and other aspects of nucleic acid stability. MTases are enzymes, which methylate specific bases within nucleic acid sequences of the host organism. MTases function in protection and epigenetic regulatory processes. MTases also function in restriction-modification systems. For a cell to function properly, nucleic acid modification by MTases assists in directing other cellular enzymes to monitor the nucleic acids. However, the exact functions of MTases within bacteriophage genomes remain unelucidated.

In Phage Phalm and other members of the P1 subcluster, such as Brusacoram, Shipwreck, StevieRay, and Fishburne, MTases genes are located next to proteins of unknown function. However, a gene encoding an endonuclease or a helix-turn-helix DNA binding domain protein always exist within two or three genes on either side of the MTase genes. The location of the MTase genes suggests a hypothesis for the function. MTase genes in temperate bacteriophage are most likely related to protection of the phage genome inside of susceptible hosts. In a phage, MTases most likely protect the phage genome from restriction by host enzymes. Previous investigations indicate that methyltransferase is necessary for stable lysogeny. Further, characterization of phage-encoded MTase could have relevance in host-range determination. NCBI BlastP analysis of genes 53 and 55 in Phage Phalm both align with multiple soil-dwelling organisms. These include *Mycobacterium sp. UM_RHS, Mycobacteroides abscessus, Mycobacteroides salmoniphilum, Rhodococcus, Mycobacteroides chelonae,* and *Mycolicibacterium fortuitum*. The most prevalent alignment for both genes 53 and 55 is to MTase genes in *Mycobacteroides abscessus*. The presence of two MTases that are homologous to slightly different bacterial proteins implies that Phage Phalm frequently inhabits bacterial species possessing more than one restriction system. Thus, acquired MTase genes are conserved

within its genome, protecting future generations. This study describes the application of multiple bioinformatics tools, such as Phamerator, NCBI BlastP, and others, to elucidate plausible significance of MTase genes within bacteriophage genomes.

U55. Testing Synthetic Compounds for Antibacterial Properties

Darwin Argueta*, Idris Akinlusi, and Rachna Sadana Ph.D.
[1]Department of Natural Sciences, University of Houston-Downtown, Houston, TX

Antibiotics are used in-vitro and in-vivo to inhibit the growth of bacteria and to treat bacterial infections and diseases. This could be difficult because bacteria can exchange genetic material through conjugation, transformation and transduction. The effect of these processes and the emergence of mutations have made bacteria resistant to antibiotics. This increase of bacterial resistance calls for the introduction of new antibiotics or a concoction of multiple antibiotics. Possible solutions include finding novel, naturally produced antibiotics or synthetic compounds. Three series of synthetic compounds (SKP, DK-CH, and TTD series) were assayed on gram-negative *P. fluorescence* and gram-positive *B. subtilis* for their antibacterial properties. The Alamar Blue assay was used as a qualitative marker to detect the presence of bacteria based on the percent reduction of the Alamar blue reagent. In general, SKP 638 and 639, DK-CH 26 and 28, TTD 8 and 16 inhibited growth of *P. fluorescence*. SKP 638 and 636, DK-CH 19, 25, 29 and 31, TTD 9 and 15 inhibited growth of *B. subtilis*. These compounds will further be investigated to measure their minimum inhibitory concentration (MIC).

U56. Analyzing Plant Extracts for Bacterial Inhibition of *Staphylococcus aureus*

Selina Lusero Hernandez Gonzalez and Patricia J. Baynham, PhD
Department of Biology, St. Edward's University, Austin, TX

The need for antimicrobial agents has increased, as antibiotic resistance has become one of the major threats to global health. Antibacterial agents have become less effective with time and repeated use. This is a major concern for hospitals, sites with higher risk of antimicrobial resistance, since patients represent an especially vulnerable population due to their preexisting conditions. The World Health Organization (WHO) estimates that by 2050 there will be an estimated 317,000 deaths per year in the United States from antimicrobial resistant pathogens, which is about 13 times the current toll. For many years, traditional healers have used plants to treat infectious diseases and approximately forty percent of current medicines are based on plants compounds. The purpose of this study is to determine which plant extracts are good candidates as alternatives or additions to antimicrobial agents. We tested plants for antimicrobial qualities using the gram-positive bacterium *Staphylococcus aureus* because it is the leading cause of skin and soft tissue infections and is frequently antibiotic resistant. To identify plants with antimicrobial activity we used the Kirby-Bauer Disk Diffusion Method to test a variety of plant extracts resuspended in ethanol. Out of 188 extracts tested, 2 inhibited *S. aureus*. Both extracts were from Madagascar, including *Alberta minor, which* exhibited 10mm of inhibition and *Syzigium emirnense, which* exhibited 14mm of inhibition. *Syzigium emirnense* has previously been used in traditional medicine to treat diabetes, while there have not been any reports regarding the medicinal use of *Alberta minor*. Further testing with these extracts includes performing a minimum inhibitory concentration assay to more precisely determine the concentration needed for therapeutic effectiveness. Additionally, *C. elegans* can be used as a

whole animal screening platform, which will also allow a preliminary assessment of drug toxicity. Adult nematodes would be exposed to *S. aureus* and then distributed into separate wells containing plant extracts. After incubation, staining would reveal nematode survival showing which extracts protected the nematode from *S. aureus* without causing toxicity. Lastly, it would be determined if the plant extracts are nontoxic to implement in treatments.

U57. Investigating soil microbes for anti-microbial properties
Eudes Mvoula*, Angelinda Maldonado, and Sanghamitra Saha
Department of Natural Sciences, University of Houston Downtown, Houston, TX

Antibiotics are critical in clinical applications for treatment of infections caused by pathogenic microbial species. However, in clinical settings, a prevalence of multi-drug resistant bacteria coupled with fewer new drug alternatives has directed research towards discovering new antibiotics. Our objective is to investigate soil bacteria for their potential to produce new antibiotics and characterize the isolates for clinical applications targeted against multi-drug resistant bacteria. Soil samples collected from different areas in Houston, TX were used to isolate soil bacteria. Soil bacteria showing an ability to create a zone of clearance on different tester strains were further characterized using biochemical and microbiological assays. To identify soil bacteria, amplification of bacterial 16S rRNA sequence was done using polymerase chain reaction (PCR). Purified DNA was sequenced and sequence analysis identified them to be belonging to the Bacillus sp. We plan to purify and characterize the compound(s) responsible for exhibiting the anti-microbial properties and test it against tester strains.

U58. Characterizing Soil Isolates Exhibiting Antibiotic Properties
Valeria Vasquez, Stefani Delgado and Sanghamitra Saha
Department of Natural Sciences, University of Houston Downtown, Houston, TX

Drug resistant bacteria are on the rise and the research in new antibiotic discovery has been limited. To combat deadly diseases caused by bacteria, there is an urgent need for new antibiotics. The objective of our research was to screen soil bacteria for their potential as antibiotic producers. Isolated bacteria were screened using tests such as checking for the presence of zones of inhibition. The DNA from selected bacterial samples was isolated and the 16S rRNA sequence was amplified by the Polymerase Chain Reaction (PCR). The amplified DNA was run on agarose gel electrophoresis. The DNA samples were purified and sequenced. Using BLAST, DNA samples were putatively identified to be those belonging to *Bacillus sp.* Further investigations will be conducted to identify the bacterial isolates and characterize the compound(s) exhibiting the antimicrobial activity.

Graduate Student Posters

G1. Characterization of the bacterial and archaeal communities associated with an *Aureoumbra lagunensis* bloom in Baffin Bay, Texas
Paxton T. Bachand[1]*, Kenneth C. Hayes[2], Sarah Tominack[2], Michael S. Wetz[2], Jeffrey W. Turner[1]
[1]Department of Life Sciences, Texas A&M University-Corpus Christi, Corpus Christi, TX
[2]Harte Research Institute, Texas A&M University-Corpus Christi, Corpus Christi, TX

Aureoumbra lagunensis blooms (known colloquially as brown tides) pose a serious threat to environmental health through the disruption of ecosystem processes. In particular, blooms adversely impact seagrass and fisheries habitat through the attenuation of sunlight and the depletion of dissolved oxygen. In the Laguna Madre, where brown tides occur on a near annual basis, previous studies have characterized how variations in physical parameters (e.g., sunlight, temperature, and salinity) and nutrients (e.g., nitrogen and phosphorous species) affect bloom dynamics; however, the microbiological factors affecting bloom dynamics are largely unknown. To investigate the microbiological factors, this study utilized 16S rRNA gene sequencing to characterize the bacterial and archaeal communities associated with a brown tide bloom in Baffin Bay, Texas. This study addresses critical knowledge gaps through the completion of three objectives: 1) identifying and comparing the bacterial and archaeal community diversity between bloom and non-bloom study sites, 2) determining which bacterial and archaeal taxa are significantly enriched by or uniquely associated with bloom and non-bloom study sites, and 3) characterizing how changes in salinity relate to variations in the bloom-associated microbial community. The results of this study are predicted to show that the microbial community plays an important role in the modulation of bloom dynamics across a wide salinity gradient.

G2. Sequence Conservation, Evolutionary Relationships, and Structure-Function Constraint Analysis of RecA

Alex N. Brown*, Hyuk Cho, Anne Gaillard, and Madhusudan Choudhary
Department of Biology, Sam Houston State University, Huntsville, TX

The RecA protein activates the SOS (Save Our Ship) function, and it also plays a major role in homologous recombination. The RecA enzyme is a tetramer, and each subunit consists of three domains, the N-terminus, the C-terminus, and the major central domain. The major central domain is comprised of eight functional subdomains, including ATP binding, DNA binding, LexA interaction, and RecA monomer-monomer interaction domains. C-termini are more diverged among different species, and therefore they possibly play species-specific roles. The N-terminus has been seen to be important in the assembly of the RecA tetramer. This leads to the hypothesis that the central domain is functionally constrained and strongly conserved, while the N- and C- termini evolve rapidly. With explosion of microbial genome sequencing projects, a large number of *recA* genes and their corresponding protein sequences are publicly available on the NCBI database. 61 gene and protein sequences across eight different phyla, representing both pathogenic and non-pathogenic species, were downloaded from the NCBI database. The protein similarities, evolutionary relationships, and structure-function constraints were analyzed using BLASTp, maximum likelihood phylogenetic method, and dN/dS calculator, respectively. Percent identity between protein sequences ranged from 43% to 91%. A comparison of a species tree and a RecA protein tree indicated similar evolutionary relationships at the phyla levels, but the relationships of the species within the phyla differed between the two trees, suggestive of horizontal transfer of *recA* genes between closely related genera. The dN/dS ratios over the different regions or domains further demonstrated that most of the subdomains in the core region, except the ATP binding subdomain, had a dN/dS value less than 0.3, suggesting they were maintained under strong purifying selection. The dN/dS ratios of the N-terminus and C-terminus regions had values greater than 0.3, which suggests that while these regions are under negative selection, they are not as strongly conserved as the core region of the protein. Future work includes the construction of an in-vitro *recA* deletion strain of *Rhodobacter sphaeroides* and further analysis of the role of RecA in the UV damage DNA repair system.

G3. The role of Muc2 sulfation in shaping the adherence and colonization of adherent-invasive *E. coli* in the gut

Erika Flores*, Diana Pereira Vaz and Anne-Marie Krachler, PhD
Microbiology and Infectious Diseases, University of Texas MD Anderson Cancer Center UTHealth Graduate School of Biomedical Sciences, Houston, TX

The intestinal mucosa forms a protective homeostatic barrier between invading bacteria and the underlying epithelium. Alterations in the mucosal composition have been observed in both animal models and patients suffering from inflammatory bowel disease (IBD). The integrity of the layer is attributed to the sulfated mucin, Muc2. Though the physiological importance of the mucus layer is well established, the role of mucin sulfation in protecting against enteric pathogens is unclear. We have shown that adherent-invasive *E. coli*, found in diseased mucosa of IBD patients, harbor sulfo-glycan binding adhesins that belong to the family of multivalent adhesion molecules (MAMs). Mucin-epithelial competition binding assays demonstrate the presence or absence of MAMs critically affects bacterial attachment to epithelial cells, and binding to epithelial cells is decreased in the presence of sulfomucin. Co-incubation of mucin with a sulfatase-producing gut commensal, *Bacteroides thetaiotaomicron*, decreases binding of *E. coli* to mucin and increases attachment of bacteria to the underlying epithelial surface.
Our data show that interactions between MAM adhesins and sulfated Muc2 inhibit translocation of bacteria to the epithelium, and sulfatases secreted by mucin-foraging bacteria such as *B. thetaiotaomicron* inhabiting the same niche may affect the capacity of the mucus barrier to retain AIEC. How these results translate to a disease setting is subject to our current studies.

G4. Comparison and Genetic Analysis of Host Specificity in Cluster BD1 Bacteriophages infecting *Streptomyces*

Hannah Klug*, Nicolas Mercado, Sangita Vasikaran, and Lee E. Hughes
Department of Biological Sciences, University of North Texas, Denton, Texas

Bacteriophages are viruses that infect bacteria. Phages reproduce by attaching to the surface of bacteria, injecting their genome, and hijacking the bacterial cellular machinery to produce phage proteins. The ability for a phage to infect multiple bacterial species is known as host range. In Siphoviridae bacteriophages, it is primarily thought that proteins at the tip of phage tail fibers determine host range. These proteins act as anti-receptors to specific receptors on the surface of bacteria. In Siphoviridae Gram-positive infecting phages, the genes that code for these proteins are typically located between the tape measure protein gene and the endolysin gene. It is hypothesized that phages that have similar anti-receptor proteins will have similar host range. In this study, the host range 12 Cluster BD1 bacteriophages were tested on 9 different *Streptomyces* species, and the genes between the tape measure protein gene and endolysin gene were compared. The 12 phages had high levels of variability in these genes. Five genes in this region had unknown functions and were called Gene A, B, C, D, and E. Genes A-E were BLASTed on NCBI and Phages-DB (blastp) and their results were recorded. The functions of gene A, C, and E remain unknown. The function of Gene D was found to be a minor tail protein. Gene B had significant BLAST hits for a collagen-like protein and a putative tail fiber protein. Gene B was inspected further, and it was found that it contained Gly-X-Y repeats in its amino acid sequence with conservation in its N-terminal amino acid sequence, specifically where the Gly-X-Y repeats were located. Gene B also had strong conservation in the C-terminal end of its amino acid

sequence. Glycine repeats and conservation in the N- and C-terminal of the gene are common factors in known host specificity-related genes. However, there appeared to be no correlation in conservation of Genes A-E and host range. More research needs to be done to identify genes involved in host range specificity in this group of bacteriophages.

G5. Identifying Mechanisms of Simian Immunodeficiency Virus Restriction in Baboons

Amanda Marek*,[1,2], Veronica Obregon-Perko[1,2], Vida Hodara[2], Laura Parodi[2], Jessica Callery[2], Jason Ladner[3,4], Bridget Fisher[5], Donald Sodora[5], Jeremy Glenn[2], Shelley Cole[2], Gustavo Palacios[4], and Luis Giavedoni[2]
[1]University of Texas Health Science Center, San Antonio, TX; [2]Texas Biomedical Research Institute, San Antonio, TX; [3]Northern Arizona University, Flagstaff, AZ; [4]US Army Medical Research Institute of Infectious Diseases, Frederick, MD; [5]Seattle Children's Research Institute, Seattle, WA

In its natural African non-human primate (NHP) hosts, simian immunodeficiency virus (SIV) infection does not result in immunodeficiency, despite chronic active viral replication. Conversely, SIV infection in Asian rhesus macaques often progresses to AIDS. Baboons, an African NHP, do not naturally harbor SIV, despite exposure through co- habitation and predation on infected NHP. It has been demonstrated baboons are resistant to chronic SIV infection *in vivo*. From previous work, we observed SIV replication is restricted in baboon PBMC compared to rhesus. However, infection in isolated baboon CD4 cells is equally permissive as in rhesus CD4 cells and progresses faster than in baboon PBMC. Baboon PBMC produce higher levels of MIP-1α, MIP-1β, and RANTES than rhesus PBMC. Inhibition of these chemokines in baboon PBMC increased SIV levels. We generated baboon-adapted SIV by serial passage of virus in baboon PBMC or isolated CD4 cells and analyzed changes to the genome by deep sequencing. Non-synonymous mutations appeared early in PBMC passages; a significant portion of changes were in Env. This could be an indicator of adaptation to more efficiently bind CCR5, compensating for higher levels of interfering chemokines. Changes to CD4-passaged SIV were gradual and few became fixed. These findings suggest SIV faces stronger selective pressure in baboon PBMC versus isolated CD4 cells. Furthermore, we observed intracellular restriction factors do not play a significant antiviral role against SIVmac in baboons in single-cycle infections. However, some restriction factors act on subsequent rounds of replication. Single nucleotide variation analysis suggests an accumulation of G-to-A mutations across passages. These data could indicate involvement of baboon intracellular factors, such as APOBEC3, in SIV restriction. We are identifying the biological role of significant mutations by generating viral clones containing different mutation variations. These clones will be used to infect baboon PBMCs and isolated CD4 cells *ex vivo*. Investigating the relevance of these mutations on virulence could elucidate mechanisms of baboon restriction contributing to the selection of these variants.

G6. CRISPR-based Detection of *Enterococcus* in Texas Coastal Waters using DETECTR

Carrie Maryak*, Dr. Jeff Brady, Dr. Janice Speshock, Stephanie Brady Department of Biological Sciences, Tarleton State University, Stephenville, TX

Public access water bodies are susceptible to *Enterococcus* fecal contamination from various sources and require periodic, accurate monitoring methods to ensure public health. Texas water quality monitoring agencies utilize IDEXX trays, which indicate fecal contamination via color development following incubation, or quantitative Polymerase Chain Reaction (qPCR) with

increasing frequency, which detects target organisms by hydrolysis of a fluorescent probe during thermal cycling (1). IDEXX results require at least 24 hours and qPCR results can be influenced by the presence of inhibitors in saltwater.
A recently established method, termed DNA Endonuclease Targeted CRISPR *Trans* Reporter (DETECTR) utilizes Clustered Regularly Interspaced Short Palindromic Repeats (CRISPR) machinery to detect target nucleic acids in a solution (2). DETECTR reaction components include DNA primers, RNA activator (crRNA), CRISPR associated protein 12a (Cas12a), and single-stranded DNA (ssDNA) reporter molecules. Primers and crRNA are specific and modifiable, allowing rapid adaptation of the method for detection of *Enterococcus*, and can be used for host species distinction.
DETECTR was used to identify the presence of *Enterococcus* in water samples collected from the Texas Gulf Coast in summer 2018. The detection limits of DETECTR were similar to IDEXX and qPCR, and DETECTR has benefits such as rapid results, minimal instrumentation requirements, and the potential for filter paper-based testing on site.

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G7. Characterizing the role of rapid intestinal epithelial cell loss and immune surveillance in promoting pathogenic infection using zebrafish (*Danio rerio*) as a model host

*Anh K Trinh Nguyen[1,2], George T. Eisenhoffer[1], and Anne Marie Krachler[2]
[1]Department of Genetics, the University of Texas MD Anderson Cancer Center, Houston, TX 77030 [2]Department of Microbiology, the University of Texas Health Science Center at Houston Graduate School of Biomedical Sciences, Houston, TX 77030

Cellular extrusion is a mechanism by which the epithelium eliminates dying or crowded cells to maintain homeostatic conditions. The dysfunction of extrusion may be relevant in understanding intestinal disorders such as inflammatory bowel diseases and diarrheal diseases. Previous observations in cultured cells have shown that the rapid loss of multiple cells by extrusion can lead to breaches in cell-cell contacts, potentially exposing structural weaknesses or paths to luminal pathogens, immune cells, and small molecules entering the basolateral compartment[1].
The objective of this study is to determine how compromised barrier function may drive pathogenesis and inflammation in epithelial tissues. The interactions between immune cells and luminal bacteria during intestinal epithelial extrusion represent some of the earliest events during infection, yet have not been well characterized, as an in vivo model has yet to be established that would facilitate dynamic high-resolution imaging. To overcome this limitation, we have developed tools and imaging methods for the zebrafish (*Danio rerio*) to develop an in vivo intestinal epithelial extrusion model and address questions of how pathogens may capitalize on transient loss of barrier function during induced cell loss. Further, our novel approach revealed an increased presence of neutrophils at sites of epithelial extrusion. Our preliminary data suggests that increased amounts of cell elimination by extrusion may disrupt barrier function and allow pathogens to invade.
Given that zebrafish have a similar intestinal structure to mammals, and many epithelial signaling processes and developmental pathways are conserved between fish and mammals[2], this

work will uncover novel mechanisms pathogens use to invade host tissues and promote intestinal disorders.

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G8. Assessment of Virulence Gene Prevalence in *Escherichia coli* from Environmental Water Samples using qPCR

Reagan Patterson, Dr. Jeff Brady and Dr. Janice Speshock
Department of Biological Sciences, Tarleton State University, Stephenville, Texas

Freshwater bodies throughout the United States attract many patrons during the warmer months of the year. However, there is a continuing concern of illness resulting from fecal contamination. Fecal indicator bacteria like *Escherichia coli* are known to reside within the gastrointestinal tracts of both humans and mammals. *E. coli* is a coliform regularly utilized by regulatory agencies to examine the safety of public recreational freshwater (1, 3). These regulatory agencies utilize detection assays such as the Colilert IDEXX tray to determine the quantity of coliforms and *E. coli* present within a given sample. Unfortunately, these culture-based presence-absence tests are unable to determine strain specifics such as virulence factors and toxins that could potentially cause human health risks.

Quantative Polymerase Chain Reaction, or qPCR, has been utilized for the detection, quantification, and typing of different microbial agents by identifying specific target DNA sequences or genes (2). qPCR measures the momentary amount of DNA amplicons within each cycle (2), thus determining the presence and prevalence of target genes within a given sample. Therefore, by utilizing qPCR and specific virulence gene primers *E. coli* strains from water samples that pose a direct human health risk can be identified quickly and efficiently.

Colilert trays containing samples from the Lampasas and Bosque River were obtained from TIAER. Positive wells were selected, diluted, and then plated on selective media for colony selection. *E. coli* colonies were then selected and streaked for isolation. Isolated *E. coli* was then grown in a broth stock to be utilized for DNA extraction via silica-binding plates. qPCR was performed with specific virulence gene primers that were selected based upon known virulent strains. This study aims to utilize Colilert trays in order to examine the human health risk *E. coli* isolates may pose by identifying specific virulence gene presence and prevalence.

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G9. Screening and characterization of monoclonal antibodies against m*ycoplasma pneumoniae c*ommunity acquired respiratory distress syndrome toxin

Ileana Pedraza*, Te Chang, Kumaraguruparan Ramasamy, Lavanya Pandranki and T.R. Kannan
Department of Microbiology, Immunology and Molecular Genetics, The University of Texas Health Science Center at San Antonio, San Antonio, TX

Mycoplasma pneumoniae (Mp) is the leading cause of bacterial community-acquired pneumonia (CAP) estimating to about 2 million cases per year in the United States. Mp is an atypical bacterium that causes a wide range of airway clinical manifestations resulting in acute and chronic respiratory infections, such as bronchiolitis and chronic obstructive pulmonary disease, as well as extrapulmonary conditions such as dermatitis and meningoencephalitis. In general, Mp-related clinical manifestations can be controlled through macrolide antibiotic treatment; however, with an alarming rise in macrolide-resistant Mp infections and the absence of a vaccine, Mp infections are becoming increasingly difficult to treat. Consequentially, Mp has become a ubiquitous bacterial respiratory pathogen. Mp expresses a unique toxin, Community-Acquired Respiratory Distress Syndrome (CARDS) toxin, as a major virulence determinant, exhibiting both ADP-ribosyltransferase and vacuolating activity in target mammalian cells. CARDS toxin can recapitulate the symptomology and pathology seen with Mp-infected individuals and animal models. Further CARDS toxin is highly expressed during infection and has been shown to be immunogenic, functioning much like other known bacterial toxins. Previously, bacterial toxins such as the diphtheria, pertussis, and tetanus toxins have been successfully used for vaccination. Interestingly, these bacterial toxins also resemble symptoms seen with the pathology and exhibit similar functional and immunogenic properties to that of CARDS toxin, suggesting CARDS toxin could serve as a potential vaccine candidate. In this study, we raised monoclonal antibodies (mAbs) against CARDS toxin and screened for their sensitivity and specificity through dot blot and ELISA analysis. We performed epitope mapping through immunoblot and peptide array analysis and identified N- and C-terminal CARDS toxin region-recognizing mAbs. Further, mAbs were analyzed for neutralization activity in human cell lines through immunofluorescence and live-cell imaging analysis. These studies establish the foundation for vaccine development and therapeutic approaches to combat Mp infection. Furthermore, these studies can provide a basis for the advancement of new strategies using toxin derivatives in the treatment of human disease.

G10. Toxicity and limitations of glycoside hydrolases in dispersing poly-microbial biofilms

Whitni K. Redman*, Derek Fleming, Kendra P. Rumbaugh
Department of Surgery, Texas Tech University Health Sciences Center, Lubbock, TX

85% of all bacterial infections are biofilm-associated impacting 2% of the United States population at some point in their life. Biofilms are communities of microorganisms with a self-synthesized extracellular polymeric substance (EPS). EPS not only makes it difficult for immune cells to enter the biofilm, but also creates a challenge for antimicrobial agents to reach the infection. Previous studies have shown glycoside hydrolases (GHs) are effective in breaking the glyosidic linkages found within the EPS, dispersing the bacterial cells, and allowing antimicrobial agents in contact with the microbes. This study focuses on determining the safety of using GHs as well as comparing the efficacy of GHs in biofilm dispersal. Various concentrations of GHs were used to treat normal colonic epithelial (CoN) cell line CCD841. Cell

toxicity was determined by completing a colorimetric assay using AlamarBlue. At a maximum concentration of 20%, 2x concentration used *in vivo*, $2x10^4$ cells were seeded 48 hours before one 90-minute treatment. Amylase and cellulase exhibited cell toxicity at 10% or higher and 5% or higher concentrations, respectively. 48-hour *ex vivo* biofilms were treated with 500 units/gram of various GHs for 4 hours to compare efficacy in biofilm dispersal. The efficacy of dispersing *ex vivo Pseudomonas aeruginosa* and *Staphylococcus aureus* biofilms varied greatly depending on the GH. E*x vivo* clinical samples were treated with amylase or cellulase to determine targeted species as well as restrictions for each GH. Cellulase was effective in dispersing *Klebsiella oxytoca* while it did not effectively disperse bacteria from the phyla Firmicutes and Bacteroidetes. Amylase was not effective against *Corynebacterium striatum* but was effective in dispersing Proteobacteria phyla. In conclusion, GHs may show potential toxicity *in vitro* and each GH has their own limitations on efficacy depending upon which bacteria are present in the infection.

G11. gPCR assessment of fecal-indicator organism, Enterococcus

Jordan Reeder*, Dr. Jeff Brady, Dr. Janice Speshock
Department of Biological Sciences, Tarleton State University, Stephenville, Texas

Enterococcus is the marine water, fecal-indicator organism that is currently used to assess water quality in Texas. Micro-organisms, such as *Enterococcus* and *Escherichia coli* (*E. coli*), have traditionally been used to detect fecal and/or pathogen contamination in water systems. The information attained from these indicator organisms helps organizations to regulate water systems, regarding fecal contamination in recreational waters. The goal of this study is to analyze isolates of Enterococci, from IDEXX water testing, using qPCR. Primarily, qPCR will be utilized to confirm the isolate is *Enterococcus*, but will also be used to assess various antibiotic resistance genes these isolates may possess. While *Enterococci* are generally not pathogenic to humans, these bacteria readily acquire antibiotic resistance and can transfer genes to other species, and the transfer of antibiotic resistance genes to other pathogenic bacteria could pose potential hazards.

G12. Gain-of-function variants of FtsA form diverse oligomeric structures on lipids and enhance FtsZ protofilament bundling

Kara Schoenemann*, Marcin Krupka, Veronica Rowlett, Steven L. Distelhorst, Bo Hu and William Margolin. Department of Microbiology and Molecular Genetics, University of Texas Health Science Center at Houston, Houston, TX

Escherichia coli requires the proteins FtsZ, FtsA and ZipA for early stages of cell division. FtsA and ZipA act as inner membrane tethers for cytoplasmic FtsZ. Gain-of-function mutants of FtsA such as FtsA* (R286W) map to the FtsA self-interaction interface and can bypass the need for ZipA. Purified FtsA forms closed minirings on lipid monolayers that separate and prevent the bundling of FtsZ protofilaments, whereas FtsA* forms smaller oligomeric arcs that promote bundling. Here, we examined three additional FtsA*-like mutant proteins for their ability to form oligomers on lipid monolayers and bundle FtsZ filaments. All three formed distinct structures at lower concentrations of protein, ranging from mostly arcs (T249M), a mixture of minirings, arcs and straight filaments (Y139D), or short straight double filaments (G50E). Large filament sheets were formed by high concentrations of the three mutant proteins with added

ATP. Despite the diversity of structures formed, all three mutant proteins allowed FtsZ protofilament bundling on lipid monolayers, much like FtsA*. Expression of the FtsA*-like proteins *in vivo* suppressed the toxic effects of a bundling-defective FtsZ, exacerbated effects of a hyper-bundled FtsZ, and rescued some thermosensitive cell division alleles. Together, the data suggest that conversion of FtsA minirings into any type of non-miniring structure can promote the correct balance of FtsZ protofilament bundling needed for cytokinesis.

G13. Constraints to horizontal gene transfer

Soham Sengupta[1], Rajeev K. Azad[1,2]
[1]Department of Biological Sciences, University of North Texas, Denton, TX
[2]Department of Mathematics, University of North Texas, Denton, TX

Horizontal gene transfer (HGT) is the exchange of genetic material between organisms from different lineages with a profound impact on prokaryotic evolution. Disparate evolutionary histories of genomic fragments complicate the "tree of life" metaphor of organismal evolution and bring new challenges in understanding evolutionary relatedness or speciation, specifically for microorganisms. HGT is a major source of phenotypic innovation and a mechanism of niche adaptation in prokaryotes. Antibiotic resistance and pathogenicity are often the consequences of HGT; however, the scope of HGT goes far beyond this. Quantification of HGT is critical to deciphering its myriad roles in microbial evolution and adaptation. Beyond quantification and association of HGT with novel phenotypes, currently the focus of most studies in the field, understanding the factors or mechanisms that constrain or facilitate HGT is central to understanding the differential gene flow within and between different lineages and the relationships that are based on shared similarity. Recent studies in this direction have focused on the impacts of ecology, phylogeny and geography on horizontal gene flow. On the other hand, some studies have attempted to associate the mobility of genes or gene clusters to their biological functions. Here, we focus on understanding the constraints on HGT by analyzing over 2,000 completely sequenced genomes of prokaryotes that also have meta-information available particularly on their ecological niches, geographical locations, lifestyle, and phylogeny. We are currently assessing various factors for their impact on horizontal gene flow by first constructing a gene-sharing network. The network is decomposed into gene sharing modules using Markov clustering algorithm. These modules provide information on potential gene donors and recipients, as well as the information on the preferential donors and recipients for different lineages. The meta-information is overlaid on the gene-sharing network and then the differential constraints on transfers of single genes or clusters of genes are assessed.

G14. Evaluation of slow migrating forms of sterol regulatory element-binding protein 2 (SERBP2) in cells infected with mouse cytomegalovirus (MCMV)

Mari A Tayyar and Laura Hanson, Ph.D.
Department of Biology, Texas Woman's University, Denton, Texas

Human cytomegalovirus (HCMV) is an enveloped virus and a member of the herpesvirus family. Infection with HCMV has high prevalence in the adult human population ranging between 60 – 100%. Clinical studies have detected HCMV proteins and DNA in atherosclerotic lesions. The detection of HCMV protein and nucleic acid in these atherosclerotic lesions suggesting a possible direct role of HCMV infection in developing atherosclerosis. Studies using an apo-E

deficient mouse model of atherosclerosis showed an accelerate process of developing atherosclerosis when the mice were infected with MCMV, and MCMV was detected in these lesions. Occurrence of atherosclerosis is a multifactorial etiology, but it is correlated with the presence of high lipid levels. Infected cells with CMV showed induction of lipogenesis regulation factors including SREBP1 and SREBP2. SREBP2 is a transcription regulation factor involved in controlling cholesterol synthesis. SREBP2 normally is present as an inactive precursor localized in the endoplasmic reticulum. When SREBP2 is activated it is cut into two parts, one is relocalized in the nucleus where it activates the transcription of genes involved in cholesterol synthesis. Infected cell with MCMV showed slow migrating forms of the SREBP2 precursor. Also, these slow migrating forms of SREBP2 were detected in the nucleus. However, there was no induction of two genes downstream of SREBP2. To examine these modifications on SREBP2, we will use a plasmid expressing an epitope-tagged human SREBP2 (amino acids 14-1141), this plasmid expression is driven by the HSV TK promoter. The product of pTK-HSV-BP2 will be detected with HSV-Tag monoclonal antibody. Cells will be transfected with pTK-HSV-BP2 and then will be mock infected or infected with MCMV. Then cells lysate will be examined with western blotting for the detection of pTK-HSV-BP2 product. The outcomes of this experiment will show whether these modifications are occurring for both of human and mouse SREBP2, or just restricted to the mouse SREBP2. Also, this will open the way for further analysis for the functions of these modified SREBP2.

G15. Comparison of near full-length 16s rRNA sequencing to enterolert and quantitative PCR (qPCR) for water quality testing

Bianca D. Willis*, Dr. Jeff Brady, Dr. Janice Speshock, and Stephanie Brady
Department of Biology, Tarleton State University, Stephenville, TX

Water is a valuable resource commonly used for everyday consumption and recreational, agricultural, and commercial purposes. Because of the threat to human health, it is important to test for the quality of both fresh and marine water in terms of the presence of pathogenic bacteria that may harm the public. Current methods used include dated culture methods, IDEXX Enterolert system, and quantitative polymerase chain reaction (qPCR) which rely on the presence of fecal indicator bacteria (1).

16s rRNA genes are ubiquitous in bacteria; therefore sequencing has become a widely used method for identifying pathogenic bacteria within a microbial community (2). Near full-length sequencing of 16s rRNA genes can be accomplished using a Tn5 transposon-tagging protocol to prepare libraries for sequencing on MiSeq (2, 3). Using computer software, full-length and near full-length sequences can be constructed and metagenomic analysis on data obtained can be done to identify bacterial presence in the water samples, specifically pathogenic bacterial strains, and can potentially determine probable host sources (2).

This method can detect pathogenic bacteria that are missed by traditional methods like IDEXX and qPCR, offering a higher level of sensitivity. Since many pathogenic bacteria such as *Vibrio*, *Yersiniae*, *Salmonella*, and *Shigella* are not detected by IDEXX Enterolert trays, sequencing will provide detection of pathogens in water samples, and allow an evaluation of bacterial health risk compared to the IDEXX assessment. The objective is to compare near full-length 16s rRNA sequencing of water samples collected from the Texas Gulf Coast using Tn5 tagmentation to Enterolert and qPCR results; and assess the ability to predict risk from pathogenic bacteria in water samples from numbers of enterococci.

1. EPA. 2015. Method 1609.1: Enterococci in Water by TaqMan® Quantitative Polymerase Chain Reaction (qPCR) with Internal Amplification Control (IAC) Assay. EPA-R-15-099.

G16. Regulation of *MAC1* mRNA by Nonsense-Mediated mRNA Decay (NMD) depending on the environmental conditions

Xinyi Zhang and Bessie W. Kebaara
Department of Biology, Baylor University

The Nonsense-Mediated mRNA Decay (NMD) pathway is a highly conserved mRNA degradation pathway with a dual function. The mRNAs with Premature Termination Codons (PTC) are degraded by this pathway. In addition, natural mRNAs that encode fully functional protein may also be regulated by NMD pathway. Thus, this is also an important way to regulate gene expression. mRNA levels can be increased by stabilizing the mRNA. We are interested in understanding how the NMD pathway regulates mRNAs involved in copper homeostasis. An example of an mRNA involved in copper homeostasis and regulated by NMD is *MAC1*. *MAC1* is a copper-sensing transcription factor that regulates high affinity copper transport and is activated under low copper conditions. Mac1p has been shown to bind the copper responsive element (CuRE) consensus sequence (TTTGCTC) in *FRE1*, *CTR1* and *CTR3* under low copper conditions. Therefore, we would anticipate the *MAC1* mRNA would be stabilized in wild-type yeast strains under specific growth conditions. Previous studies have shown that regulation of *MAC1* by the pathway is dependent on environmental copper levels. We aim to understand how the regulation of *MAC1* mRNA by the pathway changes with the change of environmental conditions. Specifically, the response of *MAC1* mRNA to copper availability.