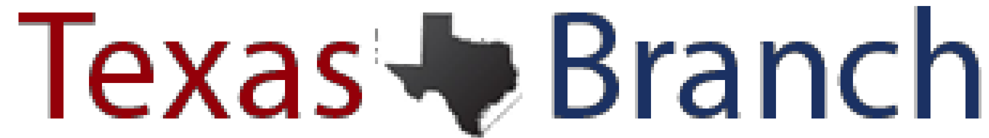

Abstract Book

2016 Spring Meeting

March 31 – April 2, 2016

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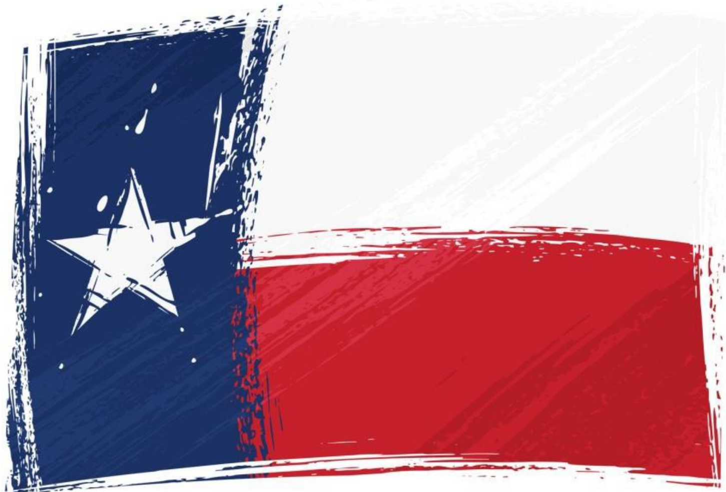

Undergraduate Student Oral Presentations

1. Antibacterial Activity of Plant Extracts on *Pseudomonas aeruginosa*
Christophe Chahine
St Edwards University

The bacterium, *Pseudomonas aeruginosa* is categorized as nosocomial and opportunistic, meaning that it frequently infects patients in hospitals especially those with some underlying condition. It has a relatively impermeable outer membrane making it less susceptible to many antibiotics. However, if a harmful molecule does enter the cell, then a unique efflux pump may quickly expel it. *P. aeruginosa* can also form a protective biofilm which further protects it from antibiotics. Approximately 2 million Americans become infected with antibiotic resistant bacteria annually, resulting in approximately 23,000 deaths. To provide new antibiotic alternatives or additives, the use of natural substances that have antibacterial properties would be a good choice. This research analyzed plant extracts for their possible antibacterial properties. In this research, 88 types of plant extracts were incubated in ethanol solution, used to saturate a paper disk, and the disks placed on Mueller-Hinton agar plates inoculated with a suspension of *P. aeruginosa*. None of the plant extracts tested resulted in the inhibition of *P. aeruginosa*. Two plant extracts that showed promise from a previous study were also tested for inhibition. *Virola sebifera* previously inhibited *Staphylococcus aureus* and *Daviesia quadrilateral* previously inhibited *Chromobacter violaceum*. When tests were performed to confirm these previous data, no inhibition was detected. It is possible that there were inconsistencies in the experimental methods or misinterpretation of the results. For further research, additional plant extracts should be tested. If any plant extract shows bacterial inhibition, then determining and isolating the chemical compound(s) responsible would be the next step.

2. Testing plant extracts for anti-quorum sensing activity.
Malaysone Chong Foung and Patricia Baynham, PhD
St Edwards University

There are approximately 2 million people infected with antibiotic-resistant bacteria in the United States, and at least 23,000 deaths reported annually. There is a necessity to develop new drugs as a solution to antibiotic resistance threats. Previous studies showed that targeting the chemical communication known as quorum sensing or QS, which is a bacterial networking behavior, could be an effective approach. In QS, bacteria are able to sense the density of their own species and can then undertake group behaviors when the bacterial population density is high. Processes controlled by QS include production of a protective biofilm or toxin production, which are activities that make bacteria more dangerous. If QS inhibitors or QSIs can be found, these may make bacteria less pathogenic. In this research, various unknown plant extracts from Peru were tested for their ability to inhibit QS activity using the pigment production in *Chromobacterium violaceum* as a model for detecting QSIs. Out of the 88 unknown plant extracts, one showed QSI, so the next step in this research will be to request an additional sample of these extracts, for further testing with pathogenic bacteria such as *Escherichia coli*, *Pseudomonas aeruginosa*, *Staphylococcus aureus* and *Enterococcus faecalis*. We can then find the minimum inhibition concentration and determine the mechanism of action. If this research is successful, this could lead to the creation of a new drug, whose application would be a new weapon against antibiotic resistant bacteria.

3. The Pursuit for Novel Antibiotics

Fashakin Sarah
Baylor University

According to the Centers for Disease Control and Prevention (CDC), almost two million people are infected with antibiotic resistant bacteria, and over 23,000 of those people die. This antibiotic resistance threat is becoming more prevalent, especially in the six multi-drug resistant bacteria known as the "ESKAPE" pathogens. These pathogens include: *Enterococcus faecalis, Staphylococcus aureus, Klebsiella pneumoniae, Acinetobacter baumannii, Pseudomonas aeruginosa,* and *Enterobacter cloacae*. One plan of action that the CDC suggested to combat this issue was to develop novel antibiotics. In light of this, Yale University, in 2012, established the Small World Initiative (SWI), an innovative research program that is committed to tackling this global health issue of the declining number of effective antibiotics by encouraging "crowdsourcing antibiotic discovery" through research. Statistics have also shown that two-thirds of antibiotics originated from the soil; evidently because that is where most of the microbial warfare occurs. So, the SWI spotlights the soil as the basis of its research. Using soil collected from Cameron Park, Waco, TX, we (my research partner, and I) successfully isolated a bacterial strain of the Pseudomonas genus that showed some antimicrobial action. We employed different techniques, like the Kirby-Bauer method, and different biochemical tests to garner more information, and understanding of this organism. The biochemical tests showed us that it was of the pseudomonas genus, and so did the Basic Local Alignment Search Tool (BLAST) results. What we were not able to figure out was the species it belonged to. Although four likely candidates that closely resembled this organism according to BLAST were: *Pseudomonas mosselii* strain CFML 90-83, which had one gap, and an identity score of 1266/1267(99%). This species was extracted from human blood in France. Another prospective match was *Pseudomonas monteilii*, which had zero gaps and an identity score of 1264/1267(99%). It was also isolated from clinical samples, but from Denmark. *Pseudomonas entomophila* strain L48 is also a possibility with zero gaps, and an identity score of 1264/1267(99%). It was isolated from soil in France, making it a better candidate than those that were mentioned previously. *Pseudomonas taiwanensis* strain BCRC 11751 had zero gaps, and an identity score of 1264/1267(99%). We also used the Automated Biometric Information System (ABIS) to compare the biochemical test results of our organism with those recorded for these candidates, and we found the similarity even more striking. This made it hard to narrow down our organism's species. From the Kirby-Bauer method results, our organism showed some resistance to antibiotics like penicillin, erythromycin, and sulfamethoxazole. The high antibiotic resistance observed in our soil isolate is characteristic of the *Pseudomonas* genus, which is known to be highly competent. However, we also noticed sensitivity to ciprofloxacin. This sensitivity, we believe, is a window to a breakthrough – final solution to this present health menace.

4. *Myxococcus xanthus* Social Motility-driven Colony Expansion is Dependent on Cell Density, Exopolysaccharide Deposition, and Nutrient Exposure

Kimberley Kissoon[1], Isabel Cornejo[2], Pintu Patra[3], Oleg Igoshin[3], and Heidi B. Kaplan[4]
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Social (S) motility is the type IV pili (TFP)-mediated flagella-independent group movement exhibited by the gram-negative soil bacterium *Myxococcus xanthus* on solid surfaces. The binding of TFP to an exopolysaccharide (EPS)-coated neighbouring cell is known to stimulate the pilus retraction required for this motility. Previous results examining the expansion of S-motile colonies at 24 h revealed a cell density dependence, in which colonies with higher initial

cell densities expanded more rapidly. We propose that there is a direct relationship between cell-generated EPS deposition and colony expansion. To test this hypotheses, we first developed a mathematical model based on Fischer's equation that predicts the effects of cell density, EPS deposition, and nutrient exposure on the colony expansion rate. Specifically, our model predicts that at low initial densities, more time is required for the cells to accumulate enough EPS to activate S-motility resulting in a longer lag period. Furthermore, our model makes the novel prediction that after the lag phase, the population will expand at a constant rate independent of the cell density. We experimentally tested the model by measuring the long term colony expansion dynamics of S-motile cells. Liquid cultures of *M. xanthus* DK1218, a strain exhibiting only S motility, were grown, diluted to different densities, and 3 µl drops were spotted onto agar plates. The plates were incubated at 32˚C in a humid chamber for up to 96 h. Each spot was imaged with a dissecting microscope, and the distance moved by the colony edge was measured at 2 h, 4 h, 6 h, 8 h, and least twice a day for up to four days. Under standard conditions (1% casitone; 0.5% agar), as expected, the higher density colonies had proportionally shorter lag periods, expanded more rapidly, and travelled farther. However, under these conditions at all densities the expansion rate decreased over time, deviating from the model's prediction of a constant expansion rate. To identify conditions resulting in a constant expansion rate, we tested plates with various agar (0.3%, 0.4%) and nutrient (0.5%, 1.5%, 2% casitone, 1% casitone with 0.2% yeast extract) contents. Only the highest nutrient plates (0.2% casitone, 1% casitone with 0.2% yeast extract) allowed for constant expansion. These data support and confirm our model's predictions that explain the dependence of colony expansion on cell-density, EPS deposition and nutrient exposure.

5. Investigating Unique Genomic Features of Rare Cluster M Mycobacteriophages in the Novel Phage 'Nanosmite'

Reavelyn M. Pray, R. Deborah Overath, Daiyuan Zhang, J. Robert Hatherhill
DelMar College

Phages are ancient but poorly understood and have large amounts of genetic diversity. Some phage genomes are better understood than others due to higher abundance of genomic sequencing. There are over 7000 sequenced Actinobacteriophage; 1,129 of which are known to infect *Mycobacterium* spp.. Mycobacteriophages are separated into clusters based on their sequence homology and then further segregated into sub-clusters. Cluster M currently has only nine members, with newly sequenced 'Nanosmite' being assigned to its own sub-cluster. Mycobacteriophage 'Nanosmite' was isolated from a public system waterway and sequenced in 2015. Cluster M phages are known for their remarkable collections of tRNA isotypes, which may be important to complete late lytic cycle growth. Cluster M phage also carry a collection of genes from *Mycobacterium abscessus,* a close relative to *Mycobacterium tuberculosum*. This rapidly growing bacterium persists in waterways and is known to cause chronic lung disease, surface infections, and disseminated cutaneous diseases. Like other Cluster M phages investigated so far, 'Nanosmite's' genome also includes a serine integrase, presumably involved in lysogeny. By investigating such integrases, which may be involved in phage-to-host genomic interactions and lateral gene transfer, as well as other novel genomic aspect of Cluster M phages, we gain greater understanding of viral evolution. In addition, understanding molecular mechanisms in these phages may lead to novel biological approaches to medical treatment and biotechnological advancement.

6. Exploration of Putative Riboswitches Involved in Bacteriophage Temperate Life Cycle

John F Ramirez
Del Mar College

Bacteriophages are viruses that survive by infecting and then replicating using a bacterial host's genetic machinery, leading to the destruction of the host cell. Bacteriophages and their hosts are locked in an evolutionary struggle that has led to the development of new mechanisms of defense and infection. Bacteria have regulatory structures known as a riboswitch, an ancient regulation mechanism composed of a single piece of RNA that alters its self-annealed structure in the presence or absence of cellular metabolites. The cellular metabolite interacts with the portion of the RNA structure forming a hairpin-loop and making the Shine-Delgarno sequence unavailable to ribosomes. We used an enrichment method to isolate phage that infect the host Mycobacterium smegmatis. Phage were purified and classification was assisted by TEM imaging. The quality and quantity of DNA harvested from 'Chupacabra' were measured through restriction enzyme digest. After genomic sequencing, bioinformatic analyses of the isolated phage genome were used to classify the phage and annotates its genome. Putative riboswitches were located in our isolated phage using the Denison Riboswitch Detector (DRD). In addition, FASTA files available from GenBank for four bacteria and 94 bacteriophage were analyzed using the DRD. During this project, the novel bacteriophage 'Chupacabra' was isolated. This phage belongs to the cluster A and subcluster A10 of bacteriophages. In culture, 'Chupacabra' exhibits a temperate life cycle and forms plaques approximately 3 mm in diameter. Its capsid and tail were 60nm in diameter and 140nm long, respectively, and its genome was 50,286 base pairs in length. Annotation of the 'Chupacabra' genome revealed genes that were atypical when compared to related lytic phages. Among the four bacterial species included in our study, we identified 322 total putative riboswitches. We also located 110 putative riboswitches across the bacteriophage genomes. Lysogenic and lytic phages appear to favor different types of riboswitches. To our knowledge, this is the first time that putative riboswitches have been located in an actinobacteriophage genome. These findings suggest that riboswitches may have functioned as metabolite sensors in primitive organisms and actinobacteriophage and modern cells still retain some of the ancient regulatory control systems. We postulate that riboswitches are able to regulate gene expression and are, therefore, able to control the transition from a lysogenic to a lytic lifestyle.

Graduate Student Oral Presentations

7. Effects of *Pediococcus acidilactici* probiotic on the human microbiome

Z Carroll[1], R Pandey[2], B McFarlin[3], R Azad[3], M Choudhary[1]
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Depts. of Biological Sciences[2], Kinesiology[3] University of North Texas, Denton, Texas 76203

The gut of a healthy human plays host to a precarious assemblage of commensal microorganisms. This community's composition, though dynamic, is often stabilized by an individual's genetics, environment, and to a greater extent, diet. Strong selective forces, such as a course of antibiotics or the introduction of pathogenic species, can lead to microbial imbalance, termed dysbiosis. can have direct physiological repercussions on metabolism and gastrointestinal health. Probiotic supplements, which inoculate the gut with viable bacteria, have been receiving attention for their potentially therapeutic applications. *Pediococcus acidilactici*, a well-studied lactic acid bacterium, has received attention for its ability to colonize the intestine, modulate immune function, and, most promising, excrete a family of narrow-spectrum antimicrobial polypeptides, pediocins. These proteins act strongly against many food-borne pathogens – particularly *Listeria* species. In this study, a seven-day course of probiotics

containing the lactic-acid bacterium *P. acidilactici* was administered healthy human subjects (n=4). Illumina sequencing was used to process the 16S ribosomal RNA present in fecal samples collected before and after the treatment. The bioinformatics programs PICRUSt and STAMP (Statistical analysis of metabolic profiles) were employed to postulate biologically-relevant explanations for the taxonomic changes observed. Future works might explore metagenomics samples in consonance with 16S rRNA samples, allowing for a deeper exploration of the functional dynamics that underlie the observed changes. Additionally, the effects of long-term use of this probiotic, perhaps as a prophylactic, may be investigated.

8. The Role of the Leader Peptide in the Biosynthesis of the Lanthipeptide Mutacin 1140.

Jerome Escano, Byron Stauffer, Monica Bullock, and Leif Smith.
Texas A&M University

Mutacin 1140 belongs to a class of ribosomally synthesized peptide antibiotics called lanthipeptides. Lanthipeptides are composed of an N-terminal leader peptide that is cleaved to yield the antibacterial peptide called the core peptide. It has been proposed that the leader peptide is important for recognition of the post-translational modification (PTM) enzymes and for inhibiting the core peptide activity. Herein, we further our understanding of sequence and structural requirements of the leader peptide for the biosynthesis of the lanthipeptide mutacin 1140. We have determined that the length of the peptide and a novel four amino acid motif are important for the biosynthesis of mutacin 1140. The leader peptide cleavage site was found to be promiscuous in its amino acid composition with the exception of arginine at the -1 position. Furthermore, the leader peptide was found to have an additional cleavage site at the -9 position, which may be required for the release of the lanthipeptide from the PTM enzymes prior to being transported out of the cell. Our study on the leader peptide of mutacin 1140 provides a basis for future studies aimed at producing novel peptide based therapeutics that contain post-translational modifications found in mutacin 1140. Furthermore, our study demonstrates that mutacin 1140, which is produced by *Streptococcus mutans JH1140*, is a useful model system for the study of lanthipeptide biosynthesis.

9. The HTLV-1 latency-maintenance factor $p30^{II}$ induces aberrant lymphoproliferation and mitochondrial antioxidant-signaling in retroviral carcinogenesis

Tetiana Hutchison[1], Aditi Malu[1], Averi White[1] and Robert Harrod[1]
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The human T-lymphotropic virus type-1 (HTLV-1) is a hematotropic oncoretrovirus that transforms and promotes the aberrant proliferation of infected CD4+ T-cells. Approximately 3-5% of HTLV-1 cases will develop adult T-cell leukemia/lymphoma (ATLL) –an aggressive lymphoproliferative malignancy that is generally resistant to anticancer therapies. Most ATLL patient isolates contain a highly-conserved nucleotide sequence, known as pX, which encodes several non-structural/regulatory proteins, including Tax, $p8^{I}$, $p12^{I}$, $p13^{II}$, $p30^{II}$, Hbz, and Rex. The HTLV-1 $p30^{II}$ protein is a latency-maintenance factor that deregulates host signaling pathways associated with cell cycle progression and cell survival. Our lab has previously shown that $p30^{II}$ cooperates with the oncoprotein c-MYC and enhances its oncogenic potential through molecular interactions with the MYST-family acetyltransferase, TIP60. The overexpression of

oncogenes, such as c-MYC, can induce the accumulation of cytotoxic metabolic byproducts and reactive oxygen species (ROS) which may cause DNA-damage and apoptosis. Our lab recently demonstrated that the HTLV-1 p30^{II} protein induces the *Tp53-induced glycolysis and apoptosis regulator* (TIGAR). TIGAR is a metabolic effector that increases NADPH levels and scavenges c-Myc-induced ROS. Furthermore, our studies have revealed that the TIGAR protein is highly expressed in HTLV-1-infected lymphoma T-cell lines. In addition, under conditions of mitochondrial depolarization, the E3 ubiquitin-ligase Parkin is targeted to mitochondria for the polyubiquitylation of damaged or unfolded proteins as a mitochondrial quality-control mechanism. Lentiviral HTLV-1 p30^{II} induces Parkin expression in transduced cells and causes the Parkin protein to become localized in the mitochondria in response to oxidative stress. Importantly, the roles of mitochondrial antioxidant-signaling in promoting oncogene-activation during viral carcinogenesis are not well understood. The induction of TIGAR and Parkin by HTLV-1 p30^{II} could provide a survival mechanism to protect ATLL cells against mitochondrial damage induced by oxidative damage. These studies allude to a pivotal role for the p30^{II} latency-maintenance factor in promoting oncogene-activation through the induction of p53-dependent metabolic effectors, and will contribute to our understanding of how host cellular factors cooperate with transforming viruses in retroviral carcinogenesis.

10. Effects of dust exposure on the opportunistic bacterial co-cultures

Mariam Konate
Texas Southern University

PGEs naturally occur in relatively small amount in the earth crust (~ 0.0005 ppm, (USGS, July 2014)). However, there are several anthropogenic sources of PGEs (Sebastien Rauch, 2008). Beginning in the1970s, automobile catalysts were progressively used for the amelioration of the air quality. Unfortunately, those catalytic converters released a great amount of PGE in the environment (Fathi Zereini, 2015). On the others hand, chemical and mining facilities are also emitters of PGE which can be transmitted in dust particles throughout the air and eventually settle on our road sides, within our houses, and even on our produce grown in fields near roadsides or near such chemical facilities. This high concentration is more likely to pose some health problems. The purpose of this study is to show the physiological effects of dust particles (containing PGEs as well as other contaminants on co-cultured opportunistic bacterial pathogens that associate with the human microbiome). Bacteria are constantly facing ever-changing environments that threaten their existence. This is true of both environmental bacteria as well as opportunistic bacteria that inhabit the human microbiome. The gastrointestinal tract is one such human microenvironment where commensal and opportunistic bacteria can be found. With such a plethora (10^{14}) of bacteria coexisting, the introduction of any new element into the ecosystem could disturb the “equilibrium. Recent studies conducted in our lab have shown that dust exposure influences bacterial growth, oxidative stress resistance and virulence potential when in pure culture (Suraju et al., 2015).Little is known about the physiological effect of dust particles on bacterial co-culture. To highlight those effects, we evaluated the impact of dust exposure on co-culture Growth kinetics (in both liquid and selective solid medium), on biofilm formation of various co-cultures, and on collective oxidative stress resistance. Also, a co-cultured bacterial mixtures and HT 29 cell line (exposed to dust and purified dust contaminants) have been used to gauge bacterial competition indices as well as eukaryotic responses to those exposures.

11. Identifying the Molecular Target of a Novel Antifungal Named Occidiofungin

Akshaya Ravichandran and Dr. James L. Smith

Texas A&M University

Since the 1950s, the antifungal compounds that have been developed for clinical use fall under three broad families: azoles, polyenes and echinocandins. These compounds target fungal cells by disrupting cell wall biosynthesis or inhibiting ergosterol production. Widespread resistance to these mechanisms of action has been reported and this has created an urgent need to identify antifungal compounds that target a novel cellular component. Occidiofungin is a non-ribosomally synthesized glycolipopeptide, produced by the soil bacterium *Burkholderia contaminans* MS14. Occidiofungin has a base mass of 1200 Da and consists of eight residues which include standard and non-standard amino acids. The compound is rapidly fungicidal against a wide spectrum of fungal species and has been shown to cause minimal toxicity in murine models. We have previously reported that occidiofungin causes fungal death by a mechanism that is different from the three aforementioned classes of antifungals. Occidiofungin is rapidly fungicidal and has been observed to trigger apoptosis in the cell leading to cell death at 1 hour following exposure. In order to determine the molecular target of the compound, click chemistry was performed using an alkyne functionalized variant of occidiofungin and a fluorophore with an azide group. Localization studies and time course experiments indicated the binding of occidiofungin to bud tips in *S. cerevisiae* and eventual internalization into the mature cell, leading to the formation of a unique cellular distribution. Similar studies in the cells of *Schizosaccharomyces pombe* demonstrated localization of occidiofungin near the poles of the cells and along the division septum. Affinity purification assays followed by trypsin digestion and LC-MS/MS were carried out to identify the proteins that were pulled down by occidiofungin. These assays indicate binding of occidiofungin to actin patches in the fungal cell leading to cell cycle arrest, starvation and death.

12. A Study of the Spatial and Temporal Features of the Human Face Microbiome during Decomposition in Southeast Texas

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Human decomposition is a process marked by events categorized into five stages. These stages occur as a fluid procession rather than through precise demarcation of events and may lead to cadavers experiencing multiple stages of decomposition at once. Previous studies have investigated biodiversity of necrophagous bacteria and insects at predefined stages of decomposition with a focus on taphonomy. One area yet to be explored is fine-scale temporal and spatial influence on our findings. Our pilot studies and early data have shown that there are shifts in the microbiome present on the skin of human cadavers which also follow the shifts in stages of decomposition, or what events most commonly correlate with these stages. In this study, two human cadavers were placed outdoors at the Southeast Texas Applied Forensic Science (STAFS) facility in Huntsville, Texas. These cadavers were sampled at eighteen locations on the face every six hours over the course of four days. The samples were subjected to 16S rRNA gene sequencing using the Illumina platform and then analyzed using the QIIME software. Results show that the two cadavers follow different initial trends, but follow the distinct overall trends that have been seen in other similar studies. Cadaver STAFS 2012.035 follows the trend of human associated bacteria (*Staphylococcus* and *Pseudomonas*) shifting to insect associated bacteria (*Ignatzschineria* and *Wohlfhartiimonas*) that then shifts to soil associated

bacteria (*Sporosarcinia*). Cadaver STAFS 2013.026 follows a similar trend with a surprising interval of *Clostridium* dominating the sample set between the initial samples and the time where insect associated bacteria is dominant in the samples. While these two cadavers do not follow the same trend of shifting bacterial communities, the data helps to bring insight into our overall goal of the microbial study. Over the course of a single day, these data show that time of day likely plays a factor in the bacterial community composition in a sample. Between sample locations, there is also indication of differences in bacterial communities. The significance of these differences will determine future sampling techniques as well as help to standardize the way in which the microbiome of human decomposition is studied and used as a predictive model for estimating the time since death.

13. Development and Evaluation of Bacteriophage Cocktail against *Salmonella* Isolated from the Beef Feedlot Environment

Yicheng Xie[1], Jason J. Gill[1,2]
Texas A&M University

Asymptomatic *Salmonella* carriage in beef cattle is a significant food safety concern and the beef feedlot environment is a reservoir of this pathogen. Bacteriophages may play a role in the ecology of *Salmonella* in the feedlot environment and may also prove useful as a means of controlling this pathogen in beef. The objective of this study is to determine patterns of phage resistance in *Salmonella* in order to formulate an effective phage cocktail capable of overcoming bacterial mutation to phage resistance. Phages will then be evaluated for efficacy alone and in combination in an in *vitro* assay.
Panels of *Salmonella* bacterial isolates and *Salmonella* phages were collected in 2014 from South Texas beef feedlots and from municipal wastewater. A *S*. Kentucky strain isolated from cattle fecal sample and a subset of 16 phages capable of infecting the feedlot-isolated *S.* Kentucky strain were selected for further study. Phage-resistant mutants of *S.* Kentucky were selected by co-culturing the bacterium with each phage individually and isolating surviving bacterial colonies. Phage-resistant strains were assessed for their sensitivity to an 18-phage panel to determine if resistance to one of the test phages conferred resistance to other phages in the collection. Phages with independent resistance were combined and evaluated for their efficacy of suppressing *S.* Kentucky in *vitro* assay. Briefly, a standardized bacterial inoculum was placed into broth medium in 96-well microtiter plates and challenged with varying concentrations of single phages or phage mixtures, and growth was observed at various time points or at an overnight endpoint. These assays provide a measure of phage virulence and also assess the development of phage resistance in the bacterial host.
While phenotypically resistant S. Kentucky colonies were observed for all 16 phages, genetically stable S. Kentucky mutants were only obtained for six phages. Fully independent and partially independent resistance patterns were observed. Resistance independence is defined as resistance to one phage that does not confer resistance to a second phage; partial independence is resistance to one phage also conferring resistance to the other. Based on this liquid assays, the combination of two phages with fully independent resistance were able to suppress bacterial growth for the 12-hour experiment and the combination of two phages with partially independent resistance does not result in strong growth inhibition compared each phage used alone. In this study, phage cocktail developed against *Salmonella* showed improved efficacy which implies that phage cocktail maybe used to overcome phage resistance when applying phages as an antimicrobial intervention.

14.

Undergraduate Student Posters

U1. Comparing Culture-based and Molecular Methods in Microbiome Research

John B. Pinard[1,2,3], Chelcy E. Brumlow[1], and Todd P. Primm[1,3]
[1]Department of Biological Sciences, [2]Department of Agricultural Sciences & Engineering Technology, [3]Honors College, Sam Houston State University

Classic microbiology culturing methods are utilized in many different applications for medicine and research. Countless methods have been studied for the isolation of collections of microorganisms as well as individual selection. Our model vertebrate organism *Gambusia affinis* serves as a tractable system for study of antibiotic effects on the natural microbiota of a mucosal surface. Mucosal surfaces in humans, including lung, gut, and oral, have the highest counts of bacteria and also the most intimate interactions with the immune system, and therefore major health effects. A major advantage of our fish model is that the skin is mucosal, giving a system that is easy and non-lethal to sample. The microbiome of humans and fish is a complex community with several dominant organisms and hundreds of rare ones. This project analyzes how different methods quantify the microbiome community, which is essential for effective research in manipulating the microbiome for health benefits, such as the use of probiotics.

The skin microbiome community was extracted from fish into liquid and then; 1) spread onto media plates for colony counts, 2) added into broth media, and 3) DNA extracted and analyzed by RISA (Ribosomal Intergenic Spacer Analysis) or ERIC (Enterobacterial Repetitive Intergenic Consensus sequence analysis). Following both the #1 count and #2 culture, RISA or ERIC was performed on the organisms that were cultured. In one experiment, to gain a full exploration of the community members for comparison, a 16S gene profile was performed. Preliminary data suggests that many community members are not represented during the solid media colony counts, which was predicted. However, the liquid culture was able to grow a broader array of the skin community. We hypothesize that this is because bacteria in the microbiome have evolved to grow in community, and share metabolic intermediates with neighboring organisms. Liquid culture would allow that, while solid does not. With the fish microbiome, RISA does not represent the community composition well when compared to a full 16S gene profile. Future work will reveal the limitations and strengths of each method in understanding the microbiome community.

U2. Social motility-driven *Myxococcus xanthus* colony expansion can be modeled using Fisher's equation

Isabel Cornejo[1], Kimberley Kissoon[2], Pintu Patra[3], Oleg Igoshin[3], and Heidi B. Kaplan[4]
[1]University of Houston-Downtown, Houston, TX; [2]Del Mar College, Corpus Christi, TX[3]; Rice University, Houston, TX; [4]The University of Texas Medical School, Houston TX

Myxococcus xanthus is a Gram-negative soil bacterium that moves exclusively on surfaces using two genetically distinct types of motility. Social (S) motility uses type-IV pili (TFP), which retract in the presence of exopolysaccharides (EPS) produced by neighboring cells and as a result require cells in close proximity. In previous experiments social motility was studied by depositing different concentrations of cells onto agar and measuring the rate of colony expansion after 24 h. An important observation from these studies is that the colony expansion rates increases with increasing initial cell densities. Although the mechanism responsible for such dependence is unknown, these results suggested a relationship between social motility and EPS deposition. We developed a mathematical reaction-diffusion model to study this relationship by describing the effect of cell density, EPS deposition, and nutrient exposure on colony expansion. Specifically, our model can explain the effect of initial cell-density on a lag phase prior to expansion and proposes that the EPS must reach a critical threshold concentration before it stimulates S-motility. Thus, at low density there is an extended lag phase, as more time is needed for EPS accumulation. The model also predicts that after the lag phase, the colonies expand at a constant rate that is independent of initial cell density. To test our model predictions, we collected colony expansion data of S-motile cells for ten days. Liquid cultures of *M. xanthus* DK1218, a strain exhibiting only S-motility, were grown, diluted to different densities ($2x10^7$ to $4x10^9$ cells/ml), and 3 µl spots of each density were placed onto 0.5% agar plates with different nutrient contents (1% casitone, 2% casitone, and 1% casitone with 0.2% yeast extract). The plates were incubated at 32˚C in a humid environment for up to 240 h. Each spot was imaged with a dissecting microscope, and the colony diameter was measured at 2

h, 4 h, 6 h, and 8 h and twice daily after 24 h. Under all the nutrient conditions tested, the high-density colonies exhibited a shorter lag, expanded more rapidly, and traveled farther compared to the lower-density colonies. However, only the highest nutrient plates (0.2% casitone and 1% casitone with 0.2% yeast extract) resulted in a constant expansion rate after the initial lag phase. These results confirm our model and show that at steady state the colony expands as a traveling wave with a speed determined by the interplay of cell motility and growth, a well-known characteristic of Fisher's equation.

U3. The Effect of Hepatitis C Virus Non-structural Protein NS5A on the Activation of Transcription Factor NF-κB

Elli Hoge
Texas Christian University

Hepatitis C Virus (HCV) evades the immune system by blocking the production and release of Interferon-β, allowing the virus to chronically infect liver cells. Elucidating the means by which HCV interferes with the cell's antiviral response will allow for the development of better treatment options for those inflicted by Hepatitis C. Previous research has shown that the HCV non-structural protein NS5A plays a necessary role in blocking Interferon-β expression. However, the specific mechanism by which HCV blocks Interferon-β expression is unknown. There are three transcription factors that bind to and activate the Interferon-β promoter: ATF-2, IRF-3, and NF-κB. When the cell detects viral infection, a signal transduction pathway activates the transcription factors and induces their translocation from the cytoplasm into the nucleus. The focus of our research is the effect of NS5A on the nuclear translocation and activation of NF-kB.

To visualize the translocation of NF-κB, HeLa cells expressing HCV NS5A were infected with Sendai Virus (SV) then stained with fluorescent antibodies specific to NF-κB. Fluorescent microscopy was then used to visually determine whether NF-κB is located in the nucleus or cytoplasm. To quantitatively measure the activation of NF-κB, a Dual Luciferase Assay was performed by transfecting 293HEK cells with the promoters of interest—interferon-β and NF-κB—attached to the luciferase reporter gene. Then, the cells were co-transfected with NS5A and infected with SV. The activation of NF-κB was analyzed using a luminometer to measure the luminescence given off by luciferase. We observed that NS5A inhibits the SV-induced activation of NF-κB and interferes with the nuclear translocation of NF-κB, possibly explaining how Interferon-β expression is inhibited by HCV.

U4. Effect of deleting genes involved in copper homeostasis *on S. cerevisiae* cells with a functional and non-functional nonsense-mediated mRNA decay pathway.

Roxanne Martino
Baylor University

The Nonsense-mediated mRNA decay (NMD) pathways exist in all eukaryotic cells. This pathway regulates and degrades both mRNAs with premature termination codons as well as some naturally occurring mRNAs. The regulation of natural mRNAs by this pathway has been observed in multiple organisms ranging from yeast to humans. Global expression profiling studies of the effect of NMD on mRNA levels in *Saccharomyces cerevisiae*, *Drosophila melanogaster* and humans show that approximately 10% of mRNAs are affected when NMD is inactivated. However, the regulation of natural mRNAs by NMD has been most extensively studied in *S. cerevisiae* and has been shown to have physiological consequences. In *S. cerevisiae*, one physiological consequence of the NMD pathway is seen in tolerance to high copper environments. When the NMD pathway is non-functional, *S. cerevisiae* cells can withstand high levels of copper. We have found that the pathway targets certain mRNAs that are involved in the process of copper metabolism such as *CTR2*, *COX19*, *COX23*, *FRE2*, *CRS5*, *PCA1* and *MAC1*. We have also shown that the ability for NMD mutants to withstand the higher levels of copper is dependent on the presence of *CTR2*. Here we would like to figure out the physiological consequences that result from deleting the other genes involved in copper metabolism and regulated by the pathway from *S. cerevisiae* cells with a functional and non-functional NMD pathway. We can then test the copper tolerance through drop tests onto different levels of copper. By looking at the outcome we can figure out how the genes and the pathway impact the cells ability to metabolize copper.

U5. Investigating the connection between the ClpXP protease and FtsZ in antimicrobial peptide resistance

in *Bacillus anthracis*
Diem Ngo
Texas Christian University

Anthrax is caused by *Bacillus anthracis*, a deadly gram-positive bacteria that could be used as a potential bioterrorist weapon. *B. anthracis* has virulence factors that contribute to its lethalness by helping the bacterium evade destruction by the host immune system. One of these factors is a protease subunit called ClpX which was shown to be essential for virulence in animal models. Loss of ClpX also resulted in decreased resistance to antimicrobial peptides, an essential part of innate immunity. However, it is not yet understood why the loss of ClpX results in a loss of resistance to antimicrobial peptides in *B. anthracis*. More recently, we have seen that *B. anthracis* lacking ClpX has aberrant cell division resulting in mini-cell formation. Mini-cell formation can occur when there is too much expression of cell division proteins such as FtsZ. Interestingly, ClpX regulates FtsZ expression in bacterial species such as E. coli. For those reasons, we hypothesized that loss of ClpX would lead to overexpression of FtsZ and to mini-cell formation. These mini-cells may be contributing to decreased resistance to antimicrobial peptides. To test this hypothesis we overexpressed FtsZ using an inducible vector in *B. anthracis* and exposed the cells to increasing concentrations of an antimicrobial peptide called LL-37. However, we found that after exposure to antimicrobial peptides with increased levels of FtsZ *B. anthracis* did not exhibit the same phenotype as the ClpX mutant. Thus, our results suggest that the rise in FtsZ levels may not be a key factor in the loss in *B. anthrac*is resistance to antimicrobial peptides in the ClpX mutant.

U6. Tolerance to gold stress is mediated by cellular adaptation in *Rhodobacter sphaeroides*
Caroline Obkirchner, Hannah Johnson, M. Choudhary
Sam Houston State University

Heavy metal contamination is a worldwide problem that can be handled with the use of bioremediation using microorganisms, including *Rhodobacter sphaeroides*. *R. sphaeroides* has a wide range of metabolic capabilities, and has shown a tolerance to gold contaminated conditions. A series of growth kinetics and colony forming units (CFU) were analyzed under the aerobic and photosynthetic growth conditions to determine if the survival is due to mutant-selection or to cellular adaptation mechanism. A range of gold concentrations were used including 0.0 μM, 0.1 μM, 0.5 μM, 1.0 μM and 10.0 μM $AuCl_3$ contaminated Sistrom medium to evaluate the response of the bacteria under the stressed conditions. A growth curve was analyzed using optical density at a wavelength of 600 nm. A significant lag phase was seen in the samples exposed to the higher concentrations of the gold. To determine if the extended lag phase is attributed to the spontaneous-random mutation-selection or to cellular adaptation, cells from the liquid culture were grown on solid plates at the same concentration of gold, as well as plates without the presence of gold. If a mutant is being selected, it is expected that the mutant cells will grow higher numbers on the gold exposed plates as well as the non-exposed plates. If the cells are surviving the high concentrations as a result of cellular adaptation, smaller number of cells will be seen on both plates with or without gold contamination. The implications of this hypothesis will allow better understanding of the cellular mechanisms for gold tolerance in bacteria, and will advance the use of microorganisms in bioremediation of gold.

U7. UV induced DNA damage-repair Analysis of the *recA* Mutant of *Rhodobacter sphaeroides*
Veronica Rodriguez, Wanji Banda, Berra Koskulu, Hannah Johnson and M. Choudhary
Sam Houston State University

RecA regulates the SOS response, a UV induced DNA damage-repair system in bacteria. *Rhodobacter sphaeroides* is a model organism for studying the SOS response, since it is found in diverse environment including a niche with high UV radiation. This current study examines the effect of UV exposure to both wild type and Δ*recA* mutant strains. Results exhibit the severe effect of an increasing dosage of UV radiation on the cell survival and the extent of DNA damage. However, both strains do not show any difference in colony morphology when grown under different growth conditions.

U8. Purification and Crystallization of HTLV-1 HBZ Protein

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Human T-cell leukemia virus type 1 (HTLV-1) is a complex retrovirus that is the causative agent of two major human diseases: adult T-cell leukemia (ATL) and HTLV-1-associated myelopathy / tropical spastic paraparesis (HAM/TSP). Globally, an estimated 20 million people are infected with HTLV-1. There are currently no successful treatments for these diseases. The HTLV-1 genome encodes for several proteins that regulate virus replication and mediate immune evasion, with one of the most influential being HBZ. By determining the structure of HBZ, novel therapeutics could be developed to inhibit its function, and which could prevent HTLV-1-associated disease. We hypothesize that HBZ protein can be purified alone as a monomer and is able to form protein crystals, which can later be used to determine protein structure by X-ray crystallography or nuclear magnetic resonance methods. HBZ protein contains a leucine zipper motif and is able to bind to cellular transcription factors. Other leucine zipper-containing proteins have been successfully purified and crystallized and their structure determined. To determine whether HBZ protein could be purified alone and crystallized, DH5α *E. coli* cells were transformed with plasmid DNA for the purpose of cloning the His-GST-tagged HTLV-1 HBZ gene inserted within the plasmid. Following amplification, the plasmid DNA was extracted and purified from the bacteria through a standard lysis method. Isopropyl β-D-1-thiogalactopyranoside (IPTG)-inducible BL21 *E. coli* cells were then transformed with the cloned and purified plasmid to produce HBZ protein in sufficient quantity. Proteins will be extracted from the bacteria with the purpose of enrichment and purification through columns using high performance liquid chromatography (HPLC) to obtain the HBZ protein. The chromatography method consists of multiple purification steps, which include binding of the His/GST tags with a HisTrap column and GSTrap column and elution by PreScission protease. Once purification is complete, the fractions of protein will be dialyzed with the use of a dialysis cassette to remove excess salts from the protein purification process. Proteins will be resolved by gel electrophoresis and stained using coomassie stain to determine the enrichment of the protein. Other qualitative tests such as immunoblot assays will be performed to further demonstrate the identity and enrichment of the HBZ protein. The enriched protein will then be crystallized with the use of the NeXtal crystallization kit in 96 different buffering conditions and dehydrated by the hanging drop method. Once formed, the crystals will be observed through microscopy. Successful completion of this project is a critical step in determining the structure of this important viral protein. After determining the protein crystallization conditions, we plan to next focus on preparing the crystals for X-ray crystallography.

U9. Role of HTLV-1 HBZ Variability in Virus Replication

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Human T-cell leukemia virus type-1 (HTLV-1) is a retrovirus that causes two major diseases: adult T-cell leukemia (ATL) and HTLV-1-associated myelopathy/tropical spastic paraparesis (HAM/TSP). Only 3-5% of the estimated 20 million infected individuals worldwide will develop either ATL or HAM/TSP after several years of being infected. It is unknown what determines whether HTLV-1 will cause disease or why one disease develops instead of the other. However, after disease symptoms occur, ATL patients have higher virus levels than patients with HAM/TSP or asymptomatic carriers. Tax, the HTLV-1 oncoprotein upregulates viral gene expression and can dysregulate cellular gene expression to initiate disease progression. When patients develop disease symptoms, Tax and other viral proteins are no longer always expressed. The one exception is the viral protein HBZ, which is able to abrogate the effects of Tax by competing for binding to cellular factors that are needed for activation of viral and cellular gene expression. As such, we hypothesize that HBZ contains genetic variability that could affect virus replication and disease outcome. We analyzed whole virus genome DNA sequences from 90 HTLV-1-infected patients. Using MEGA6 sequence alignment and phylogenetics software, HBZ variability was identified in 70% of patients with a mean of 5.5 mutations per patient. Silent mutations, which are possible indicators of specific amino acids requirements for proper protein function, occurred at 31 conserved amino acid positions. Phylogenetic analysis linking HBZ variability and disease outcome was inconclusive. Through DNA sequence alignment we determined a new HBZ consensus DNA sequence for the virus variants that are endemic to Brazil, which differs from the virus variants that are endemic to Japan. Currently, we are investigating whether changes in the silent mutations affect HBZ stability,

localization, and function as compared to the Brazilian and Japanese consensus sequences.

U10. Isolation and screening of potential halophilic crude-oil degraders from a brine lake

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Truscott Brine Lake located in Knox County, Texas is a part of the Red River Basin Chloride Control Project which diverts millions of gallons of natural brine emissions to modulate chloride levels in Lake Kemp. Truscott lake provides a unique habitat for halophilic organisms, and a potential reservoir of halophilic bacteria suitable for bioremediation in high salt conditions as found in hydraulic fracturing. In this work we analyze the diversity of aerobic bacteria in Truscott Lake. Bacteria were isolated from surface water, and species were identified using 16S PCR DNA analysis. The salt tolerances of six isolates were determined to be between 5-15%, and they were screened on media supplemented with crude-oil.

U11. Understanding Community Interactions in a Fish Model of Mucosal Microbiomes

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Mucosal microbiomes are complex microbial communities that have major effects on the host organism's health. Typically in microbiomes, when external bacteria are introduced, they fail to colonize or are outcompeted. This suggests specific control of community members, which likely comes from both host factors and the microbial members themselves. To better understand member interactions, we use a fish (*Gambusia affinis*) skin model, which is a mucosal microbiome that is easy to manipulate and sample.

Acinetobacter is a native organism often found as one of the dominant genera in the skin microbiome. To determine microbiota persistence of *Acinetobacter*, fish are exposed to high concentrations in the water column. Then the microbiome is extracted and bacteria quantified using selective, differential media. We predict that the native organism will persist at a higher rate than what is normally found in the microbiome. This model system should allow future explorations on mechanisms behind microbiome community selection, and understanding of community interactions with other genra present.

U12. Prevalence of Pathogenic Isolates in Migratory Birds and Transfer to Local Populations

Qianying He
Midwestern State University

Like humans, birds serve as hosts for microorganisms. In some cases, these microorganisms can cause diseases in humans. Migratory birds, such as seagulls, can transport these potentially pathogenic microorganisms across long distances and introduce them into new communities by contaminating local water supplies. The introduced pathogens may become a permanent part of these communities if taken up by local waterfowl. The goal of this research project is to determine if the annual appearance of seagulls in Wichita Falls affects the composition of existing bacterial populations in local waterfowl. Fecal samples were collected from seagulls and resident waterfowl at a small lake on the university campus. Selective media were used to enrich for *Escherichia coli* and *Staphylococcus aureus*. Thus far we have collected samples from 56 birds including Ring-billed Gulls, Mallards, Canada Geese, Double-crested Cormorants and Common Coots. From these samples we obtained twelve *E. coli* isolates and one *S. aureus* isolate. Susceptibility to four antibiotics (oxacillin, imipenem, cefotaxime, and tetracycline) was determined for each isolate. We identified one *E. coli* isolate resistant to tetracycline. Five other *E. coli* isolates showed intermediate resistance to tetracycline. The single *S. aureus* isolate was susceptible to all antibiotics tested. Our current results suggest that antibiotic resistance is not widespread in these bird populations. However, continuing and expanding the surveillance of these bird populations will help us better understand the possible role of wildlife as vectors and as reservoirs where resistance genes can accumulate.

U13. Succession of soil bacterial communities during human cadaver decomposition in southeast Texas

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Decomposition of humans can be described as a dynamic ecological process in which the features of a cadaver change in response to environmental changes in a relatively predictable pattern. Throughout the process of decomposition, a cadaver exhibits several major phases of tissue change ranging from wet (fresh-bloat) to dry decomposition (decay, mummification, or skeletonization). The main source of this decomposition is due to the presence of bacterial communities on the skin. Understanding bacterial succession during decomposition is crucial to understanding the decomposition process as a whole. The study of succession during decomposition is emerging as an important field in forensics and serves as a tool for modern forensic research. It provides the ability to accurately estimate the post mortem interval based on the presence of certain bacterial communities. Preliminary studies have already shown that a shift in bacterial communities occurs on the skin across the varying stages of decomposition. However, this study intends to show the succession of bacterial colonies within the soil surrounding a decomposing cadaver during the spring and fall seasons. A total of three pairs of cadavers were placed outdoors in alternating seasons at the Southeast Texas Applied Forensic Science (STAFS) facility located at the Center for Biological Field Studies (CBFS) in Huntsville, Texas. Soil core samples were taken near the groin and control sites during each of the major stages of decomposition. To assess the presence of bacterial succession, the samples were processed through16S rRNA gene amplification on the Illumina MiSeq platform and analyzed using the QIIME software, version 1.7.0. Early results have indicated that there is a shift in the microbial communities within the soil throughout the process of decomposition. Overall, this data has helped to illustrate how communities can shift in composition throughout the course of decomposition. In order to provide a more definitive model of succession, more tests will have to be conduced.

U14. Effects of Postmortem Storage Conditions on Shifting Skin Bacterial Communities during Human Cadaver Decomposition in Southeast Texas

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Human decomposition is a dynamic process whereby features of a cadaver change in a relatively predictable pattern over time. As a cadaver decomposes, it passes through several major stages of tissue change leading from wet decomposition (fresh, bloat) to dry decomposition (decay, mummification, and/or skeletonization). Early stages of decomposition are wet and marked by discoloration of the flesh and the onset and cessation of bacterially-induced bloat. Intrinsic bacteria begin to digest the intestines from the inside out, eventually digesting away the surrounding tissues. During putrefaction, bacteria undergo anaerobic respiration and produce gases as by-products, the buildup of which creates pressure, inflating the cadaver, and eventually forcing fluids out (purge). In the trunk, purge is associated with an opening of the abdominal cavity to the environment. The microbiome of human decomposition is an emerging aspect of forensic research and holds the potential of providing a collaborative estimate of the post mortem interval. While bacteria are credited as a driving force of decomposition; relatively little is known about bacterial succession during decomposition. Understanding the bacterial basis of decomposition is crucial to understanding decomposition as a whole and may help explain the variation of decomposition seen between cadavers. Preliminary studies have shown a shift in the communities across the varying stages of decomposition. To investigate the effects of storage conditions on the microbiome of human cadavers, six human cadavers were placed outdoors to decompose under natural conditions at the Southeast Texas Applied Forensic Science (STAFS) facility at the Center for Biological Field Studies (CBSF), Sam Houston State University, Huntsville, Texas. The cadavers were sampled during winter, spring and summer months. Three of the cadavers were stored frozen prior to placement while the other three were

not. The cadavers were sampled by externally swabbing the various body locations over the course of decomposition. To assess alpha and beta diversity, sample processing, 16S rRNA gene amplification, and Illumina sequencing were performed following protocols benchmarked as part of the Human Microbiome Project. 16s data were processed and analyzed using QIIME version 1.7.0. Samples were grouped according to body site, cadaver of origin, and storage condition. Initial results suggest differences in microbial community structure between frozen storage and non-frozen storage. Ultimately, bacterial data such as these can be refined to develop a model of microbial succession that can be used to estimate the postmortem interval, or the time since death.

U15. Study of shifting oral and fecal skin bacterial communities during human cadaver decomposition in southeast Texas

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Decomposition is dynamic and continuous process whereby features of a cadaver change in a relatively predictable pattern over time. As a cadaver decomposes, it passes through several major stages leading from wet decomposition (fresh, bloat) to dry decomposition (decay, mummification, and/or skeletonization). Early stages of decomposition are wet and marked by discoloration of the flesh and the onset and cessation of bacterially-induced bloat. Intrinsic bacteria begin to digest the intestines from the inside out, eventually digesting away the surrounding tissues. During putrefaction, bacteria undergo anaerobic respiration and produce gases as by-products, the buildup of which creates pressure, inflating the cadaver, and eventually forcing fluids out (purge). In the trunk, purge is associated with an opening of the abdominal cavity to the environment. While bacteria are credited as a driving force of decomposition; relatively little is known about bacterial succession during decomposition. Understanding the bacterial basis of decomposition is crucial to understanding decomposition as a whole and may help explain the variation of decomposition seen between cadavers. To investigate community structure of the skin, human cadavers were placed outdoors to decompose under natural conditions at the Southeast Texas Applied Forensic Science (STAFS) facility (a willed body facility) at the Center for Biological Field Studies (CBSF), Sam Houston State University, Huntsville, Texas. The skin of 14 cadavers,was sampled by swabbing the fecal, and oral sites through the stages of decomposition. To assess alpha and beta diversity, sample processing, 16S rRNA gene amplification, and Illumina sequencing were performed following protocols benchmarked as part of the Human Microbiome Project. 16s data were processed and analyzed using QIIME version 1.9.1. Samples were grouped according to site, cadaver of origin, and accumulated degree hours. Initial results suggest different trends of microbial communities before and after the purge stages of decomposition.

U16. Competitive exclusive in the microbiome of *Gambusia affinis.*

Rebecca Pittser
Sam Houston State University

The *Gambusia affinis* is a common river fish, it is a surface feeder and survives on small insect that come in contact with the rivers surface. Like most other living things the *Gambusia's* skin has a diverse microbiome made up mostly of gram negative bacteria(1). *Pseudomonas spp.* are one of these many types of bacteria that litter the skin and scales. By altering the amount of *Psuedomonas putida* by exposing seven fish is sterile water with 5 ml of concentrated *Psuedomonas* the levels of bacteria on the skin will increase. After a 24 hour period one fish will be tested and the rest will be moved to a fresh bucket of sterile water. Every 24, 48 and 96 hours the fish are tested again to see how quickly the levels of bacteria drop. Through several trials where many of the fish died before the 24 hour exposure was finished the levels of bacteria do increase after 24 hours. It Is still unclear how long it takes for the levels to decrease.

U17. Evaluation of Germicidal Effectiveness of Personal UV Products

Matthew Ramirez
Victoria College

Commercial, portable UV scanners have been a popular choice for consumers to sanitize everyday objects. The IDEAWORKS™ UV scanner utilizes a UV wavelength range from 1850-2600Å, with a peak of 2537Å and claims to kill 99.8% - 100% bacteria following 20 seconds exposure. We have exposed *Serratia marcescens* ATCC 8100 to the UV scanner under various times and surfaces to determine the effectiveness of the device. Although the IDEAWORKS™ UV scanner claims to a 100% kill rate within 20 seconds, the results show the product had an average 62.04% kill rate at 20 seconds, but 92.74% kill rate within 60 seconds, and 100% kill rate within 90 seconds of exposure of UV light to *S. marcescens.* The Oral SteriClean™ toothbrush sanitizer uses similar technology to sanitize toothbrushes using UV light and claims to kill 99.9% of bacteria within a fixed 6 minute timed exposure. We have exposed a toothbrush contaminated with *Streptococcus sanguis* ATCC 10556 to the UV apparatus to simulate application of this product. Surprisingly, our data would appear to confirm this claim as we could not recover any bacteria after 6 minutes of exposure. However, our results question the accuracy of the IDEAWORKS™'s claim regarding the UV scanner. Our results with *S. marcescens* indicate a 62.04% kill rate at 20 seconds rather than 100.00%. Overall, these data generally support the effectiveness of personal UV disinfecting devices.

U18. A Study of Shifting Bacterial Communities during Human Cadaver Decomposition in Southeast Texas: A Male and Female Comparison

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Human decomposition is a dynamic ecological process whereby features of a cadaver change in a relatively predictable pattern over time relative to environmental factors. As a cadaver decomposes, it passes through several major stages of tissue change leading from wet decomposition (fresh, bloat) to dry decomposition (decay, mummification, and/or skeletonization). While bacteria are credited as a driving force of decomposition; relatively little is known about bacterial succession during decomposition. Understanding the bacterial basis of decomposition is crucial to understanding decomposition as a whole and may help explain the variation of decomposition seen between cadavers. The microbiome of human decomposition is an emerging aspect of forensic research and holds the potential of providing a collaborative estimate of the post mortem interval. Preliminary studies have shown a shift in the communities across the varying stages of decomposition. This study will aim to compare two male cadavers placed in the spring to two female cadavers placed in the following spring. Four human cadavers were placed outdoors at the Southeast Texas Applied Forensic Science (STAFS) facility at the Center for Biological Field Studies (CBFS) in Huntsville, Texas. All of the cadavers were allowed to decompose in a natural setting while external samples were taken at various locations on the body over the course of decomposition. These samples were processed using 16S rRNA gene amplification on the Illumina MiSeq platform. The data was analyzed through the QIIME software, version 1.7.0. An overall analysis reveals a general trend from human associated bacteria to insect associated bacteria to soil associated bacteria. The comparison between male and female shows differences in bacterial community structure. While no direct conclusive trends can be drawn from this limited study, this comparison can serve as an initial report on the differences seen between male and females during decomposition. Overall, this data set can serve as an early indication of community structure and trend differences based on gender to which future comparisons can be made. Through the extended acquisition of data in this manner, it is predicted that a model for the trends of bacterial community shifts can be developed including a subset of models for comparisons such as the one in this study.

U19. Comparative methods for culturing and isolation of ciliates in the rhizosphere.

Chris Fratus, Josie Minick, Jennifer Wilson
Baylor University

To develop a procedure for ciliate collection, isolation, culturing, and characterization, we have explored the relative value of different isolation methods and culture medias and found that sterilized soil extract or cerophyll and picking individual organisms were most effective. As part of the curriculum design

of an introductory inquiry-based biology lab, we plan to have students collect soil samples from which they will extract, culture and characterize protists. In order to be able to implement a curriculum of this style, we need to master and evaluate the efficacy of several methods of isolation and characterization sourced from existing literature. We first compared two isolation techniques: centrifugation and micropipetting individual organisms. Our second goal was to find a media that promoted protozoan replication, especially ciliate growth. For isolation, we found that while the picking yielded more reliable results than centrifugation, it required more time and skill. We discovered three media that were effective at promoting ciliate life - cerophyll, ciliate mineral medium (CMV), and sterilized soil extract. The cultures using sterilized soil extract and cerophyll consistently contained ciliates. Our work in these initial experiments will provide a foundation for the continued development of an interactive, discovery-based biology laboratory for freshmen-level students.

U20. A seasonal comparison of shifting bacterial communities during human cadaver decomposition in southeast Texas

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Decomposition is dynamic and continuous process whereby features of a cadaver change in a relatively predictable pattern over time relative to temperature and specific ecological scenario. As a cadaver decomposes, it passes through several major stages of tissue change leading from wet decomposition (fresh, bloat) to dry decomposition (decay, mummification, and/or skeletonization). Early stages of decomposition are wet and marked by discoloration of the flesh and the onset and cessation of bacterially-induced bloat. Intrinsic bacteria begin to digest the intestines from the inside out, eventually digesting away the surrounding tissues. During putrefaction, bacteria undergo anaerobic respiration and produce gases as by-products, the buildup of which creates pressure, inflating the cadaver, and eventually forcing fluids out (purge). In the trunk, purge is associated with an opening of the abdominal cavity to the environment. While bacteria are credited as a driving force of decomposition; relatively little is known about bacterial succession during decomposition. Understanding the bacterial basis of decomposition is crucial to understanding decomposition as a whole and may help explain the variation of decomposition seen between cadavers. To investigate the effect of seasonality of the community structure during decomposition, six human cadavers were placed outdoors to decompose under natural conditions at the Southeast Texas Applied Forensic Science (STAFS) facility (a willed body facility) at the Center for Biological Field Studies (CBSF), Sam Houston State University, Huntsville, Texas during winter, spring and summer months. The eight cadavers were sampled by externally swabbing the various body locations through the stages of decomposition. To assess alpha and beta diversity, sample processing, 16S rRNA gene amplification, and Illumina sequencing were performed following protocols benchmarked as part of the Human Microbiome Project. 16s data were processed and analyzed using QIIME version 1.9.1. Samples were grouped according to body site, cadaver of origin, and season placed. Initial results suggest season has an effect on the microbial communities during decomposition. Ultimately, bacterial data such as these can be refined to develop a model of microbial succession that can be used to estimate the postmortem interval, or the time since death.

U21. Phosphorylation of *Pseudomonas aeruginosa algR* is required for virulence in a *Drosophila melanogaster* oral feeding infection model.

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Pseudomonas aeruginosa is an opportunistic pathogen known to cause acute infection in immune-compromised hosts, such as those suffering from burn wounds or cancer patients.
Notably, chronic infection by *P. aeruginosa* is the leading cause of morbidity and mortality in individuals

diagnosed with the genetic disorder cystic fibrosis. Research into the two-component signal transduction system AlgZR has demonstrated its role in the regulation of several virulence factors, including twitching motility, cyanogenesis, and alginate production. However, characterization of the histidine kinase sensor AlgZ and the phosphorylation state of the response regulator AlgR in virulence has been limited.
In this study, various strains of *P. aeruginosa* were utilized to determine the impact of AlgZ and the phosphorylation state of AlgR on virulence in a *Drosophila melanogaster* chronic infection model. Similar to strains lacking *algR* (PSL317 and PSL317Z) , those expressing either a phosphorylation defective AlgZ (*algZ* H175A) or AlgR (*algR* D54N) were attenuated in the virulence model, showing an approximate 85% and 65% survival rate , respectively, whereas the isogenic wildtype experienced a significantly lower survival rate. Moreover, a strain expressing a phosphomimetic isoform of AlgR (*algR* D54E) was similar to the wildtype, further demonstrating the importance of the AlgR phosphorylation state in pathogenesis.

U22. Prevalence of Reticuloendotheliosis Virus in Wild Galliformes in Texas
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Reticuloendotheliosis virus (REV) is an avian retrovirus that infects galliforme birds, such as domestic and wild chickens, turkeys, pheasants, and quail. The virus causes suppression, neoplasia, runting and feathering abnormalities. The virus infects B-cells, a blood component of the immune system. There is some evidence that blood-feeding insects and arthropods may act as vectors to harbor the virus, and potentially contributes to the spread of the virus. However, the pathogenesis, mode of transmission, and prevalence in nature is unknown. We hypothesis that a significant number of wild turkeys are infected with REV, which could permit wild turkeys to serve as a reservoir for transmission by insects to other closely related birds, such as endangered Attwater's prairie chickens. By better understanding the sources of new virus infections, additional measures could be implemented to protect at-risk bird populations. To determine the prevalence rate of REV-infected wild turkeys, we propose to analyze whether B-cells in blood taken from wild turkeys contains virus DNA. Blood samples donated by the Texas Parks and Wildlife Department and other sources, including hunters, will be used to determine if an individual bird is positive for REV. We have collected blood samples from 139 individual wild turkeys from 11 counties in Texas (15 samples from Bell, 3 from Blanco, 33 from Cottle, 11 from Gillespie, 3 from Kendall, 13 from Lampasas, 19 from Llano, 5 from Martin, 26 from Mason, 5 from Scurry and 7 from Wise). Samples were collected between 15 January 2016 and 10 March 2016. Drops of blood were placed on Whatman qualitative grade 3 filter papers, air-dried for two hours, sealed in protective plastic bags, and mailed to Tarleton State University. Blood samples were removed from the filter papers and DNA extracted using Tris-EDTA buffer. We next plan to screen the DNA samples for presence of viral DNA. Five microliters of the extracted DNA will be amplified by polymerase chain reaction (PCR) using REV-specific primers. Amplified PCR products we will be resolved in agarose gel electrophoresis and presence or absence of amplified REV DNA will indicate whether an individual bird is infected or not.

U23. Investigating the Role of *S. aureus* in Necrotizing Soft Tissue Infections
Cody Fell
Texas Tech University

Cases of skin and soft tissue infections (SSTIs) have more than tripled in the US over the last decade and are now the second most common type of infection seen in the clinic. *Staphylococcus aureus* is widely known to be one of the leading causative agents of SSTIs in the world. Clinicians are beginning to recognize that SSTIs represent a spectrum of infection that ranges from superficial and localized, such as abscesses, to necrotizing and life-threatening. Due to emerging antibiotic-resistant strains of *S. aureus*, such as community-associated multidrug-resistant *S. aureus* (CA-MRSA), the number of people admitted to hospitals for SSTIs, including those for necrotizing soft tissue infections (NSTI), increased from 1.2 million in 1996 to 3.28 million in 2005. Very little is known about why *S. aureus* causes limited, self-resolving abscesses in some individuals and life-threatening NSTIs in others. There are *S. aureus* virulence factors believed to contribute to NSTI formation; however, most of these have not been thoroughly investigated. Here we use a mouse model of SSTI and dermonecrosis to investigate the potential role that specific virulence factors play on abscess formation versus NSTIs of different strains of

S. aureus. We hope that our data will help shed light on the factors that predispose some patients to devastating NSTIs.

U24. Characterization of Silver Nanoparticles Antibacterial Effects and Toxicity to Mammalian Cells
Maliha Dhunani*, Cassandra Grizer*, Abel Joseph, Daniel Korir, Dr. Sreekar Marpu, Dr. Mohammad A. Omary, and Dr, Laura Hanson
Texas Woman's University

Silver nanoparticles (AgNPs) are increasingly being used for a wide variety of purposes making use of their optical, sensing, and stability properties. AgNPs have documented antimicrobial properties including antibacterial, antifungal, and antiviral. AgNPs are being looked at for possible therapeutic uses such as scrubs, masks, topical creams (i.e. vaginal), and wound dressings, but can also have toxicity issues with mammalian cells, especially phagocytes. AgNp's effects on both mammalian cells and microorganisms may be affected by many factors including shape, size, and modifications to their surface. The purpose of this project is to analyze the toxicity, antibacterial and antiviral, activity of a panel of novel AgNPs photochemically-synthesized by the Omary group at the University of North Texas. For our experiments we are using IC-21 mouse macrophages and NIH3T3 mouse fibroblasts as our cell models, with *Escherichia coli* and mouse cytomegalovirus (MCMV) (of the herpesvirus family) as our initial pathogens. Fibroblasts were chosen as they play an important role in wound healing, and macrophages were chosen as they are important in response to infection as well as involved in clearance of particulates. Thus macrophages are often particularly sensitive to NPs. We are evaluating both acute toxicity and inhibition of normal cellular functions such as migration and phagocytic activity. AgNO3 precursors were mixed with a polyacrylic acid polymer and photochemically activated at either 10 mM AgNO3 or 1 mM AgNO3. The photochemically synthesized nanoparticles had higher activity than the control samples of the same mixtures without activation. For the E. coli the MIC is between a 1:40 and 1:100 dilution of the 10 mM AgNO3 preparation. For the NIH3T3 fibroblasts 100% of the cells were detached within 24 hours after exposure to a 1:100 dilution, while only about 40% of the cells were detached after exposure to a 1:1000 dilution. The IC-21 macrophages had an interesting phenotype with the majority of the cells rounded and detached with the 1:1000 dilution of the nanoparticles, but only about 10% of the cells detached at the 1:100 dilution, suggesting that there might be some saturation of binding/ uptake affecting toxicity in these cells. Because of the toxicity in the mammalian cells, we have not yet tested antiviral activity. Understanding the mechanisms of action by testing different sizes, shapes, and concentrations will be important to improve the results for relative toxicities.

U25. Investigating dual-species interactions in an *in vitro* wound environment
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TTU HSC Department of Surgery

Chronic, non-healing wounds are an increasingly relevant medical issue, affecting upwards of 6.5 million patients a year in the US alone and burdening our economy with treatment costs estimated to be as high as 25 billion dollars in 2010. Chronic wounds are often polymicrobial, whereby two or more species of bacteria can interact cooperatively, antagonistically or synergistically within the same environment. However, very little is understood about the nature of these interactions, or whether they influence the severity of the infection. One reason for this deficiency is the difficulty of growing multiple bacteria together *in vitro*. Therefore, *in vitro* models that simulate the chronic wound environment and support polymicrobial growth are needed. Thus, we produced a novel *in vitro* polymicrobial 'wound-like' assay in order to investigate the interactions between several opportunistic pathogens that are commonly found in chronic wounds. Utilizing this assay, our goal is to uncover and characterize interactions between bacteria in wound infections and determine the role of these interactions in pathogenesis, with the hope that new treatment options for debilitating chronic wounds may be uncovered. To date, we have uncovered an antagonistic relationship between *Pseudomonas aeruginosa* and *Acinetobacter baumannii*in both aqueous and semi-solid environments. Through co-culture studies of morphology, phenotypic variation and biofilm production, we are beginning to understand the antagonistic relationship between these two opportunistic pathogens commonly isolated from clinical chronic wound samples, which could make them more difficult to treat in human wounds.

U26. Examining Antimicrobial Activity of Plant Extracts toward *Escherichia coli*

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Antibiotic resistance in bacteria is one of the most important issues for human health since these drugs will no longer be effective in treating harmful microorganisms. In this study, the main focus is examining the possible antimicrobial activity of plant extracts from Peru and Vietnam toward *Escherichia coli.* This type of bacterium can lead to possible death in humans by causing bloody diarrhea and kidney failure. Furthermore, *E.coli* also develops antibiotic resistance so it is important to find new drugs to treat these infections. In this project, the extracts were tested using the Kirby Bauer Disk diffusion method. Each of the extracts was resuspended in methanol, applied to a paper disk and then placed on an agar plate inoculated with *E. coli*. Zones of inhibition indicated that 2 out of the 178 extracts tested showed antimicrobial activity against *E.coli.* Previously, a plant named *Malpighiaceae Byrsonima arthropoda* from Peru showed antimicrobial activity against the bacterium *Chromobacterium violaceum.* This was further explored by determining the minimum inhibitory concentration (MIC) of this extract. To do this, the bacterium was exposed to different concentrations of extract in a microbroth dilution assay. However, the results were inconclusive and more trials will be required in order to determine the MIC. Further study can be applied to the plant extracts to determine if they can be developed into suitable antimicrobial treatments. This would give the medical community additional tools to fight infection.

U27. Use of *Galleria mellonella* as a model organism to study wound infections

Helen Scott, Rebecca Gabrilska and Kendra Rumbaugh
Texas Tech University

The impact of wound infections on healthcare is enormous. Infections of the dermis, including burns, surgical-site infections and non-healing diabetic foot ulcers affect a large population and cost over $18 billion in direct medical costs in the United States annually. The microbial populations of these infections are typically polymicrobial and often highly resistant to antibiotics. It is known that members of polymicrobial communities often display synergistic interactions that can enhance virulence, persistence and/or antimicrobial tolerance. However, their pathogenesis has been significantly understudied. One major focus of our research is to understand the influence that polymicrobial interactions have on wound infections, especially those caused by the ESKAPE pathogens (*Enterococcus faecium, Staphylococcus aureus, Klebsiella pneumoniae, Acinetobacter baumannii, Pseudomonas aeruginosa,* and *Enterobacter* species), a group of bacterial species that are often responsible for a substantial percentage of nosocomial and wound infections, and are infamously known for their antibiotic resistance One strategy we use to study the polymicrobial interactions is to measure the impact of co-infection versus monoinfecton on virulence in mouse wound infection models. However, these experiments can require many animals depending on the number of interactions studied. The invertebrate, *Galleria mellonella* (Greater Wax Moth) has been proven to be a representative model for *Pseudomonas aeruginosa* wound infections in mouse models. For this study, we hypothesized that *G. mellonella* larvae can be effectively used as a surrogate model to study polymicrobial interactions in wounds. To test this hypothesis, *G. mellonella* were infected with ESKAPE pathogens then measured for mortality and bacterial growth to evaluate virulence and polymicrobial synergy. These data suggest that the underlying mechanisms of infection are similar in mice and wax moth larvae, making them a suitable model to study wound infections.

U28. Screening Costa Rican and Vietnamese Plant Extracts for Antibacterial Properties

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Pseudomonas aeruginosa is a common and pervasive nosocomial pathogen associated with significant morbidity and mortality among infected patients. This bacterium is resistant to many currently available antibiotics necessitating development of new and novel antimicrobial strategies. Plant substances are a known source of compounds demonstrating medicinal, antimicrobial and broadly

physiologic properties; a variety of plant extracts, obtained from the National Cancer Institute, were studied in order to identify their effect on the growth of *P. aeruginosa.*

176 Vietnamese and Costa Rican blinded plant extracts, suspended in ethanol, were tested using the Kirby Bauer Disk Diffusion Method. Of the 88 plant extracts from Costa Rica, zero (0/88) demonstrated inhibition of *P. aeruginosa.* Of the 88 plant extracts from Vietnam, 13.75% (11/88) demonstrated inhibition. Previously, an extract of the plant *Gochnatioa polymorphia* (*G.p.*) demonstrated inhibitory properties. In this study, differing concentrations of the plant extract were used and the effects on the growth
of *P. aeruginosa* and *Staphylococcus aureus,* another common pathogen, were measured.
P. aeruginosa demonstrated zones of inhibition at concentrations of 750μg, 450μg, 150μg, while *S. aureus* demonstrated a zone of inhibition only at 750μg. The minimum inhibitory concentration (MIC) was determined to be 64μg/mL for *P. aeruginosa* and 16μg/mL for *S. aureus*. It is noteworthy that the *G.p.* extracts gave larger zones of inhibition at lower concentrations for *P. aeruginosa*, but a lower MIC for *S. aureus*.

If the above plant extracts prove effective in vivo and non-toxic for humans, they could be used alone or in conjunction with antibiotics as a new antimicrobial treatment. The development of new antimicrobial substances or enhanced antibiotics will aid in the fight against antibiotic resistance in global health care.

U29. Analyzing the Antimicrobial Properties of Plant Extracts Against *Staphylococcus aureus*
Kenya Su
Saint Edwards University

There is a growing problem of antibiotic resistant bacteria which is impacting human health on a global scale. This creates the need for the development of new antimicrobial substances, which is the focus of this investigation. *Staphylococcus aureus* is an especially formidable pathogen and this project tested plant extracts from Colombia and Thailand for the presence of antimicrobial properties. In order to accomplish this, two different methods were used, one that uses small amounts of the extracts in an initial screen and another that examines a larger sample more closely. The Kirby Bauer Disk Diffusion method was used to test 176 plant extracts from Colombia and Thailand for inhibition of *S. aureus*. These extracts were resuspended in methanol and compared with a methanol control. Results indicated that 1 out of 88 extracts from Colombia and 9 out of 88 extracts from Thailand displayed zones of inhibition against *S. aureus*. A second technique was employed using extracts of *Ilex aquifolium* (also known as European Holly). A previous researcher found that extracts of this plant inhibited bacterial growth. The Minimum Inhibitory Concentration method was employed to determine the minimum amount of this plant extract needed to inhibit bacterial growth using a series of dilutions. The MIC indicated that *S. aureus* was not inhibited even by the highest concentration of the extracts. These findings indicate that 11 extracts can be further examined for their antibacterial properties. If successful, these extracts could serve antimicrobial treatments either alone or in combination with known antibiotics.

U30. Antibacterial activity of Guatemalan and Vietnamese plant extracts against *Staphylococcus aureus*
Emily Truong and Dr. Patricia Baynham
Saint Edwards University

Staphylococcus aureus can infect the blood stream, bones, joints and the respiratory system, by breaching the skin and mucous membranes. Possible effects of this bacterium can include skin infections or pneumonia. Contaminated hands and medical devices in hospitals aid in the spread of this bacterium to patients with a high susceptibility of infection due to a decrease in the immune system. Different antibiotics have been used to treat this bacterium, however, due to the overuse and misuse of these antibiotics, strains of this bacterium have become resistant. There is a growing need for new medicines to treat *S. aureus* and this study examines plant extracts for antibacterial properties. Plant extracts from Guatemala and Vietnam were obtained from the National Cancer Institute and tested to determine their potential antibacterial properties using the Kirby-Bauer Disk Diffusion Method. None of 88 Guatemalan extracts and eleven of 88 Vietnamese plant extracts inhibited the growth of *S. aureus.* Previously, an

extract from a Brazilian plant, *Ratonia elaeagnoides*, exhibited bacterial inhibition. This was retested using 75ug, 225ug and 375ug of the plant extract against the following bacteria: *S. aureus, Chromobacterium violaceum, Enterococcus faecalis*, *Escherichia coli*, and *Pseudomonas aeruginosa*. This extract inhibited the growth of *C. violaceum.* Further development of these extracts could lead to new medicines to treat bacterial infections.

U31. Analysis of Bacteriophage Cocktail Efficacy Against Pathogenic *Salmonella*

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Salmonella, a common foodborne pathogen, is responsible for an estimated 1.2 million illnesses each year in the United States. Bacteriophages, viruses that specifically kill bacteria, have been studied as an alternative to antibiotics as resistance to antibiotics in bacteria has become more prevalent.
However, bacteria are often able to mutate and become resistant to individual phages. Through use of a phage mixture (or “cocktail”), multiple phages attacking at different receptors of the bacterial cell decreases the chances for the cell to develop a resistance against all phages. In this study, phage resistant mutants were isolated and characterized in order to develop and evaluate a set of phage cocktails. A panel of 11 phages were co-cultured individually with *S. typhimurium* LT2 to select and isolate surviving phage-insensitive mutants. The same panel of phages was spotted to lawns of these mutants to determine bacterial sensitivity to all phages in the panel. Based on plaque formation on each mutant, phages were paired and grown in liquid media in 96-well plates in the presence of the wild-type LT2 at multiple dilutions to observe the efficacy of the combinations for inhibiting bacterial growth. The growth of LT2 mutants developing from the wild-type LT2 culture against the phage combinations was monitored and compared to single phage infection through optical density readings at 30-minute intervals over a 12-hour period. It was found that the phage combinations used were more effective and suppressed the growth of mutants compared to the use of single phages.
These findings indicate that phage combinations can be more effective antibacterials than single phages, an important consideration when attempting to use phage to control pathogens.

U32. Antibacterial Properties of Common Household Spices.

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Multi-drug resistant bacterial species are a major problem in the modern society. As the well-known antibiotics are no longer effective, it is important to explore new sources of anti-bacterial compounds. Some of everyday spices have antimicrobial properties that may act as natural remedies for treating sickness and killing bacteria. In this study, we have used disc diffusion assays and contact-dependent killing assays to test the effects of clove, black pepper, cumin, and turmeric oil against various bacteria such as *Escherichia coli*, *Enterobacter aerogenes*, *Staphylococcus epidermidis*, *Streptococcus pyogenes* and drug resistant Staphylococci. Acetone was used as an organic solvent in order to ensure maximum extraction of the active ingredient within the spices, with the exception of turmeric oil. Results with the most clarity regarding antimicrobial effect was determined using optical density of each bacterial culture containing varied spice concentrations. Furthermore, plate counts for each of the treatment were done in order to improve accuracy of data. Although there were varied levels of antimicrobial activity depending on the spice used, most of the spices tested exhibited strong antimicrobial effects on the test bacterial cultures. Moreover, some of the spices had anti-bacterial effect on the drug-resistant staphylococci as well. Further details on the concentrations of the active ingredients present in these spice-formulations have to be elucidated.

U33. Assessing the correlation between *agr* polymorphisms and *Staphylococcus aureus* sensitivity to blue light inactivation

Sarah Yuen

Baylor University

Staphylococcus aureus is a Gram-positive pathogen responsible for minor skin infections, deep tissue infections, and even death. Antibiotic resistance of strains such as Methicillin-Resistant *Staphylococcus aureus* (MRSA) leads to an increased difficulty in treating "staph" infections caused by this bacterium. Due to the increasing resistance of staph strains to antibiotics, it is important to investigate and develop alternative modes of treatment for bacterial infections. Photodynamic therapy using 470 nm blue light has been identified as a viable alternative to antibiotic treatment, however significant differences in the sensitivity of strains to blue light have been found. This project investigated the role of a genetic component, the Accessory Gene Regulator (agr), found to be down regulated in *S. aureus* isolates exposed to blue light. While no correlation was found between *S. aureus* strains and the various *agr* types with their sensitivity to blue light, there is a statistical difference between the response of differing isolates which requires further investigation.

U34. Isolation of Phage 'Chupacabra' and Comparative Annotation of Gene 46

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Del Mar College

Bacteriophages are a significant factor influencing the amount of genetic variation in their hosts. In this project a novel phage was isolated from a soil sample taken from a chicken coop located in Corpus Christi, Texas. The soil sample was enriched using *Mycobacterium smegmatis* as a host. After incubation, the soil lysate was filtered, diluted and plated to test for the presence of bacteriophage. Using spot and streak tests, we continued purification of the phage sample, and identified a new temperate phage named 'Chupacabra'. A TEM image was obtained via uranyl acetate staining of our high titer lysate. to predict the cluster of the phage. Using DNA that was extracted and purified from the phage, a restriction enzyme digest assay performed. The 'Chupacabra' genome was sequenced via ion-torrent method and determined to have a genome length of 50,836 base pairs. The phage 'Chupacabra' has over 99% homology with another subcluster A10 bacteriophage 'Goose'. The genome was annotated using the bioinformatics programs "DNA Master" and "Phamerator". Gene product 46 is putatively identified as thymidlate synthase. This enzyme is vital to DNA replication.

U35. Isolation of Wheelbite phage from Arthrobacter

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In 2015, Arthrobacter phage Wheelbite was isolated by direct plating from a soil sample obtained from Argyle, Texas. Throughout the semester I gathered information on the virus including the size of plaques formed when cultivated with the Arthrobacter bacteria and relative size of the genome which may be approximated using visual information derived from the gel electrophoresis. After undergoing DNA sequencing the Wheelbite genome was annotated using the DNA master program along with data from GeneMark, Starterator and Phamerator. After identifying the best candidate starts we attempted to identify as many functions as possible using the blast tools on phagesdb.org as well as the NCBI blast done through DNA master. We also used Phamerator to identify conserved domains and HH pred as another blasting tool. The information acquired has not yet been uploaded to Gen Bank.

U36. Isolation, purification, and characterization of a '*wunderphul*' phage and the use of bioinformatics in genomic analysis in order to annotate the genome

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Phage WunderPhul, a Mycobacteriophage of Subcluster A6, is one of the 27 bacteriophages isolated at LeTourneau University. WunderPhul was isolated from a soil sample that was enriched with 7H9 complete medium along with AD supplement, CHX stock, $CaCl_2$, and *M. smegmatis* mc^2155 cells. Streak plates were created in order to purify the phage. Ten-fold serial dilutions were performed to determine the

titer of the WunderPhul phage stock. Web plates were then created from the purified stock in order to produce a high titer phage stock to be used in DNA extraction.
The DNA extracted from WunderPhul was sequenced using Illumina Platform Sequencing, revealing a GC content (61.5%), the genome length (48724 bp), the overhang length (10 bp), and the overhang sequence (CGGTCGGTAA). The phage was determined to belong to morphotype Siphoviridae, and is a member of Cluster A (Subcluster A6). The genome of WunderPhul was then annotated using software such as DNA Master and Phamerator in order to compare and contrast its genes with other phages of its subcluster.
During the second phase of the research, bioinformatics software was used to analyze genes and determine gene function. The auto-annotated gene calls were examined independently by multiple research groups. The auto-annotation software missed several genes and marked several improbable open reading frames as genes. Following independent genomic analysis of phage WunderPhul, the analysis of each group was compared with the others and the reasoning assessed in order to construct a final completed genome.
The most notable observation was the identification of an immunity repressor gene that was interpreted as two different genes by the computer due to a premature stop codon introduced by a mutation at codon 87. The premature stop codon results in the immunity repressor gene being split into two genes which are predicted to produce gene products of 87 and 90 amino acids, respectively.

U37. Analysis and characterization of AQ phages: Boersma and Ichor
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Baylor University

Bacteriophages, highly specialized viruses that infect bacteria, are found in most environments and are considered the most prolific entities in the biosphere. Arthrobacteriophages, a class of viruses specific to Arthrobacter species, include both temperate and virulent types. They are bacteriophages that infect Arthrobacter species, a genus of bacteria found most abundantly in the soil. This study entailed the isolation, purification,and characterization of Arthrobacteriophages from a variety of geographic locations by Baylor University students. Each soil sample underwent enrichment and plaque purification on plates containing Arthrobacter. The viral morphology was determined using transmission electron microscopy. The subsequent phage DNA was analyzed by restriction digestion and gel electrophoresis. In the fall of 2015, 24 Baylor University biology students isolated, purified, and characterized 21 Arthrobacteriophages from soil samples. There were 2 AQ phages found, Boersma and Ichor. These samples were then sequenced using Illumina sequencing at the Pittsburgh Bacteriophage Institute's sequencing facility. Using bioinformatics tools such as Glimmer, GeneMark, BLAST, HHPred, Starterator, and Phamerator, potential genes were identified and assigned putative functions. Comparison of these genomes with other sequenced Arthrobacteriophages resulted in a draft of the annotated genome with predicted gene products. Further research into the genetic diversity of Arthrobacteriophages may lead to the development of new molecular tools for research. These may include novel bacterial strains able to reduce the accumulation of harmful inorganic compounds and agricultural pesticides in the soil. More research into the genomes of bacteriophages may lead to the development of new therapeutics, discoveries in genetics and biotechnology, and a useful model for studying gene regulation and evolution.

U38. Comparing tRNA Genes Identified In Different Siphoviridae Phages Isolated from the Environment On The LeTourneau University Campus
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LeTourneau University, Longview, TX

Three Siphoviridae phages isolated at LeTourneau University contained at least one tRNA gene. The research presented is a comparative analysis of these phage genomes and the tRNA encoding genes within them. The Phage WunderPhul genome codes for a total of four tRNA genes. Phage WunderPhul is a member of mycobacteriophage cluster A. Specifically it is a member of subcluster A6. The first two tRNA genes in WunderPhul, based on order within the genome, were determined to be almost identical in 2-D structure. The 2-D structure of the third and fourth tRNA genes were slightly different from the previous two. Interestingly, the fourth tRNA gene in WunderPhul contains an intron. All tRNA genes in WunderPhul are located near the beginning of the left arm of the phage genome. After examining the

other five phages for the presence of tRNA genes, we established that Phage Dublin contained a single tRNA gene and Phage Peanam contains two tRNA genes. Phage Dublin is a member of the mycobacteriophage cluster A, subcluster A5. The tRNA gene found in Dublin was identified by both DNA master and Aragorn. The tRNA gene in Dublin is located near the far left arm of the genome, between genes 4 and 5. The third phage, Peanam, is a member of the cluster K, subcluster K1. The only tRNA gene identified in Phage Peanam, by DNA Master, is also located between genes 4 and 5. Similar to Phage WunderPhul, the Phage Peanam genome also has an additional tRNA gene that was only identified by Aragorn. This study will present additional structural comparative analysis of these seven phage tRNA genes.

U39. Comparative Analysis of Streptomyces Bacteriophages OlympicHelado and Rima

Isabel Delwel
University of North Texas

Bacteriophages are viruses which infect bacteria. The isolation of bacteriophage (phage) and its subsequent study is imperative for the furthering of genomic sequencing and phage therapy. Through the SEA-PHAGES program at the University of North Texas, two unique phages were isolated from the same soil sample using two different host bacterium. OlympicHelado was isolated on host *Streptomyces griseus*; Rima was isolated on host *Streptomyces azureus*. Both bacteria were sequenced, annotated, and organized into the BI cluster. A comparative analysis of OlympicHelado and Rima, which make up two of three phage in the BI cluster, was conducted. OlympicHelado has a total genome length of 56189 basepairs (bp) and a total CG content of 59.5%. Rima has a total genome length of 56168 bp and a total CG content of 59.6%. OlympicHelado and Rima both have 9 bp 3' sticky overhang length containing the following sequence: CGCCCGCCT.

U40. Effect of GntR Family Transcriptional Regulator and Immunity Repressor Proteins in Actinobacteriophage Infections

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Due to increasing cost for treating medical conditions, preventative medicine is quickly overtaking more traditional, invasive methods of treatment. The result of this new research direction is an increasing demand for scientists to understand some of the regulatory mechanisms of pathogenic organisms. Regulatory mechanisms can operate in various pathways within the cell of an organism such as gene transcription. Our research project included the *in vitro* isolation and characterization of the Actinobacteriophage 'SeaMonayy' followed by the *in silico* annotation of phage 'Oogway'. In the course of our research, we have putatively identified two genes within 'Oogway' that appear to influence gene regulation. These genes encode for GntR family transcriptional regulator and the immunity repressor proteins. The GntR family transcriptional regulator contains a N-terminal DNA-binding domain consisting of a 3-helical bundle core with a small beta-sheet. Immunity repressor proteins assist in repressing the transcription of genes involved in the phage lytic cycle. With additional research, it may be possible to generalize our findings to the mechanisms of human pathogen actions. We hypothesize that the presence of these genes can contribute to the ability for a bacteriophage to successfully infect host bacterial cells. Future work is planned to elucidate the structure and activity of these gene products.

U41. An Investigation into Recombinant Mechanisms of Bacteriophage Oogway Through Genomic Annotation

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Bacteriophage are among the most numerous and dynamic organisms in the biosphere. With an estimated 10^{31} phage particles present on earth at any given time, the phage genome is staggeringly vast, intricately complex in its mosaicism, expansive in its diversity, and evolutionarily ancient. One of the defining characteristics of phage is the ability to incorporate DNA into a specific bacterial host via genetic recombination. Because of a perpetual arms race between these viruses and their bacterial hosts,

bacteriophage have adopted a number of molecular mechanisms that are antagonistic to bacteria which can be used to develop valuable techniques in the fields of genetic recombinational techniques and gene therapy. The bacteriophage 'Oogway' was discovered in Corpus Christi, Texas in 2014 by students at Del Mar College. A member of the A cluster, 'Oogway' has a genome of 51,745 bp and contains 88 coding regions. To examine the genomic conservation and protein homology of this novel phage, the bioinformatics programs 'DNA Master', 'Phamerator', and 'Starterator' were utilized. Additionally, 'HHPRED' was used in identifying pairwise comparisons of putative proteins to potential matches in various databases. The putative genes products of 1 and 37 have been identified as HNH homing endonuclease and integrase, respectfully. These putative gene products are employed in the lysogenic cycle of bacteriophage and are vital components of the processes by which phage successfully integrate their genome into that of the host through DNA strand cleavage, insertion, and sight-specific recombination.

U42. Phage Gideon: The Real OG of Cluster G

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In this study, we took a closer look at *Mycobacterium* phage Gideon and how its genome compares with other phages within *Mycobacteriophage* cluster G, subcluster G1. Using the enrichment plate method with *Mycobacterium smegmatis* mc² as the host, phage Gideon was isolated from a soil sample found on the LeTourneau University campus in the fall of 2015. Gideon is one of 27 phages currently in subcluster G1 and was found to possess the *Siphoviridae* morphotype. It is 41,903 bps in length and contains 62 genes, however 61.3% of these genes had unknown functions. Using data collected from DNAMaster, Phamerator, Starterator, HHPred, Local Blastp, and NCBI Blast, phage Gideon was compared to fully annotated phages Halo, Phreak, and Angel, as well as other phages in subcluster G1, all of which have strong homologies with it. Phage Halo was isolated in Pittsburgh, PA at Central Catholic High School in 2003. It is 42,289 bps long and contains 64 genes. Phage Phreak was isolated in Spokane, WA at Gonzaga University in 2012. It is 41,901 bps in length and contains 63 genes. Phage Angel was isolated in O'Hara Township, PA at the University of Pittsburgh in 2007. It is 41,441 bps long and contains 61 genes. All of these phages have the *Siphoviridae* morphotype and were isolated using *Mycobacterium smegmatis* mc²155 as the host during the isolation and purification stages of the research. Similarities, differences, and unique characteristics possessed by phage Gideon as compared to phages within the same subcluster will be discussed.

U43. Faulty Immunity Repression Gene in Phage WunderPhul

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Phage WunderPhul was among numerous bacteriophages isolated in the fall of 2015 from an enriched soil sample gathered near wood chips and grass. The ambient temperature at the time of collection was 30°C. Phage genomic DNA was submitted for DNA sequence analysis on the Illumina Platform at the Pittsburgh Bacteriophage Institute in order to characterize and analyze their genomic organization. Initial characterization following DNA sequence analysis indicated that Phage WunderPhul is a member of the A6 subcluster. Genomic annotation was completed using bioinformatics software including DNAMaster, BLASTp, and Phamerator. A total of 89 typical protein coding genes were identified. In addition, three genes encoding tRNAs were identified. Genomic investigation of Phage WunderPhul revealed the presence of two genes bearing homologies to the same immunity repressor gene in other phages within subcluster A6. HHpred also showed high homology with helix-turn-helix motifs that contribute to gene regulation and to DNA binding proteins within each of these two genes. The current gene structure would lead to the production of two partial immunity repressor proteins. When considered together, these bear a more complete homology with other phages. However, because the genes are separated by a stop codon, it appears that the stop codon was generated as a result of a transitional point mutation which results in a thymidine replacing a cytidine in a codon which normally would have coded for Arginine. This point mutation converts the Arginine codon to a TGA stop codon. In fact, there is a 99% identity match with homologous phages in subcluster A6 when the genes are combined, where the sole identity mismatch occurs at the stop codon. We hypothesize that the introduced stop codon would impair the

function of the immunity repressor protein. Thus, immunity to superinfection would be inhibited by the impaired repressor function and allow for homoinfection.

U44. Isolation and Characterization of the Novel Actinobacteriophage ‘Draco’ and Annotation of the Phage ‘Oogway’

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Del Mar College

Bacterial resistance to antibiotics has become a global epidemic that is steadily increasing. Research on what causes resistance and ways to stop it are in high demand. Bacteriophages are viruses that are being studied to help combat antibiotic resistant bacteria because they are target-specific, can lyse the bacteria, and can be used to produce treatments for bacterial infections. The ability to find a bacteriophage that is able to infect and then destroy target bacteria that are nonpathogenic for humans would be of great importance in the biomedical field. We studied the genes of the Actinobacteriophage ‘Oogway’ using bioinformatics software. We were able to discover a novel Actinobacteriophage isolated from the host bacteria *Mycobacterium smegmatis*, mc^{2}155, ‘Draco.’ By comparing the novel phage ‘Draco’ with other novel phages isolated from the same host, such as ‘Oogway,’ we may be able to find genes that have similar functions. By comparing phage genomes, we are able to probe the function of the individual genes that have been studied, sequenced, and tested. Of particular interest was gene 32 in the novel phage ‘Oogway,’ which codes for a putative D-ala-D-ala-carboxypeptidase, that may have originated from lateral gene transfer from its host. Carboxypeptidase enzymes are known for penicillin-binding proteins and may serve as possible binding sites to lyse bacterial strains that have become antibiotic resistant. We hypothesize that with future research it may be possible to show similar antimicrobial gene products in the novel phage ‘Draco.’

U45. PhageNotes: A Collaboration Tool for Improved Phage Annotation

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Abstract: Phage annotations for the HHMI SEA-PHAGE initiative requires formatting that can easily become convoluted by a large scale annotation team. In order to allow for effective annotations to be completed for many different phage genomes, by a large annotation team, an excel spreadsheet was designed with a series of “concatenate” and “if/then” logic commands. The excel spreadsheet template, uploaded as a Google Sheet file, allowed for consistent annotations that were all formatted properly and could easily be copied and pasted into a final DNAmaster file. The creation of this excel organization tool allowed undergraduate students to completely annotate 6 Arthrobacter phage in the course of 1 semester. The addition of the excel spreadsheet to the annotation process resulted in a user-friendly annotation format, a quick and viable annotation check system, and a consistent format for all annotation forms.

U46. Isolation, Purification, and Analysis of Arthrobacteriophage genomes

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Bacteriophages, highly specialized viruses that infect bacteria, are found in most environments and are considered the most prolific entities in the biosphere. Arthrobacteriophages are bacteriophages that infect Arthrobacter species, a genus of bacteria found most abundantly in the soil. This study entailed the isolation, purification, and characterization of Arthrobacteriophages and their genomes from a variety of geographic locations by Baylor University students. Each soil sample underwent enrichment and plaque purification on plates containing Arthrobacter. The viral morphology was determined using transmission electron microscopy. The subsequent phage DNA was analyzed by restriction digestion and gel electrophoresis. Ten phage genomic DNA samples were submitted for sequencing. Here we compare four cluster AN Arthrobacteriophages, KylieMac, Elkhorn, Taj14, and Saphira. These samples were sequenced using Illumina sequencing at the Pittsburgh Bacteriophage Institute’s sequencing facility. Using bioinformatics tools such as Glimmer, GeneMark, BLAST, HHPred, Starterator, and Phamerator,

potential genes were identified and assigned putative functions. Comparison of these genomes with other sequenced Arthrobacteriophages resulted in a draft of the annotated genome with predicted gene products. Further research into the genomes of bacteriophages may lead to the development of new therapeutics, discoveries in genetics and biotechnology, and a useful model for studying gene regulation and evolution.

U47. A Study of Improved Phage Genome Annotation Over the Years 2006-2016

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Annotation is the process of analyzing a raw DNA sequence, describing different regions of the genome, identifying protein-coding regions (genes), and adding this pertinent information to a genome database. The recently isolated *Mycobacterium* phage Gideon demonstrates the changing process of phage genome annotation. Over the years, there have been improvements in how particular genes are called, with newer methods favoring certain annotation guiding principles over others. In this particular example, homologues of phage Gideon's relatively short gene product 52 have only been called in more recently annotated genomes. Older genome annotation approaches favored the longer product for homologues of gene product 53, in effect calling two genes (52 and 53) as one gene. The current paradigm shift favors the selection of operons, and the ribosome-preferred 4 base pair start-stop codon overlaps of genes, instead of just favoring the longest possible reading frames and excluding putative genes for being smaller in size. Basically, in the past, entire upstream or downstream genes were discounted just to allow for one gene to be as long as possible. With the newer calls, genes in phage Gideon form an 8-gene operon, comprised of genes 48 through 55. Older annotations broke up this operon by excluding gene 52 and extending 53 upstream, leaving a 1 base pair gap from gene 51. The older annotations were called between 2006 and 2011. These include phages BPs, Angel, Hope, Annihilator, Frosty24, and Avrafan. Newer annotations have been called since 2012, and include phages FlagStaff, Cambiare, Phreak, and Cedasite. Once phage Gideon's annotation is complete, it will join the latter group. For future annotations, it is important to choose a reference genome that is the most up-to-date and reliable, and also compare new genomes to multiple references so that inconsistencies can be identified and eliminated.

U48. Genomic Comparison of Bacteriophages in Cluster BF

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Bacteria in the Genus *Streptomyces* are prolific in most soil environments and are known to produce an abundance of secondary metabolites, which often have antibiotic or other biological activity. The study of phages regularly lends to the development of genetic tools for manipulation within the host. In the Streptomycetes, this interest lies primarily in the biosynthesis of antibiotics or the development of genetic techniques to produce novel secondary metabolites. The study of generalized transducing phages and the mechanisms by which these types of bacteriophages are able to incorporate their genetic material into the host genome are of great interest. While the species within *Streptomyces* vary greatly in their susceptibility to genetic manipulation, phage-derived tools have made major impacts in the ability to transfer DNA from one species to another by genetic manipulation. Bacteriophage Immanuel3 was isolated using *Streptomyces griseus ssp. griseus in 2012* and was believed to be a singleton, not fitting into any *Streptomyces* bacteriophage clusters, until the discovery of bacteriophage Manuel in 2014 at Wilkes University. Manuel was isolated using *Streptomyces lividans* as host. Once sequencing of Manuel was completed, it along with Immanuel3, was designated to Cluster BF based on Genetic similarities. This study compares the only two phages of the BF cluster, Immanuel3 and Manuel. In this study, the phages are examined for similarities in both genomics and proteomics.

U49. The Isolation, Purification, and Analysis of Bacteriophage OverAchiever

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Bacteriophages are interesting and unique numbering at about 10^31 individuals in the world.

This work is a documentation of experimental bacteriophage research using the host bacteria *Streptomyces griseus.* OverAchiever is the phage discovered through soil enrichment. This phage was enriched and isolated from a soil sample found next to a pond in a local park in Denton, TX on August 22nd, 2015 at 4:03 pm at a local temperature of 97˚F. Once isolated, OverAchiever was multiplied in lab to result with a High Titer Lysate of 8.9x10^9 phage forming units per milliliter, and its genome was analyzed using six different restriction enzymes (HaeIII, KpnII, PmlI, SacI, SfiI, and StyI) on a restriction gel. Though OverAchiever has been determined to be identical to previously isolated bacteriophage Chymera, these experimental results may lead to more characteristics of Chymera and phages like it. Due to the specific characteristics of bacteriophages, OverAchiever/Chymera and others, this work could potentially be a vital force in fighting pathogenic bacteria in the future.

U50. The Annotation of the Actinobacteriophage ‘Oogway’ and Exploration of Portal Proteins and Methyltransferase

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Del Mar College

Bacteria play major roles in global energy and biogeochemical cycles and in the production of industrial goods, including important drugs. Bacteriophages, however, outnumber bacteria by a factor of 10 to 1 and have the ability to affect bacterial genomes. Despite having so many bacteriophages in our environment, their genomes are the least known and studied. We annotated the genome of the Actinobacteriophage ‘Oogway’ with bioinformatic programs to compare its genome to those of similar phages (Cluster A, Subcluster A1). Gene product (GP) 12 is likely a portal protein and GP58 is likely a methylase protein. These two proteins potentially act as the attack and defense mechanisms of the phage. The portal protein is located on the capsid of the virus where the entire DNA of the phage is stored and controls the injection of the viral genome into the host cell. This happens through the coiling and uncoiling of the portal protein, forming an opening that allows the genome to transfer into the bacterial host. Once the bacteriophage genome enters a bacterium, it comes under rapid attack from the host cell, especially from restriction enzymes. Methyltranserferase plays an important role in bacteriophage defending their genomes from restriction enzyme attack. These gene products are great tools to help prevent and modify the bacteria genome, including applications to combat bacterial diseases that affect humans. We hypothesize that that the presence of a portal protein and methyltransferase enzyme contribute to the ability for a bacteriophage to survive and successfully infect its host.

U51. Discovery, Characteristics, and Basic Genomic Analysis of Bacteriophage PapayaSalad

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In the lab of Dr. Lee Hughes I worked to isolate from topsoil originating from my home town in Keller, TX a Streptomyces griseus infecting bacteriophage I named PapayaSalad using the detailed Streptomyces Resource Guide provided by the SEA PHAGES Program. Following the isolation of PapayaSalad we observed that it creates clear, circular plaques roughly 0.6mm in diameter, suggesting a lytic phage. Additionally an electron microscopy later revealed that it’s most likely a part of the myoviridae class of viruses. After its genome was completely sequenced we observed that PapayaSalad is a part of the BC3 sub cluster with a genome size of 38411 base pairs containing an approximated 54 genes.

U52. Isolation and Characterization of the Novel Actinobacteriophage ‘Nitzel’ and the Annotation of ‘Oogway’

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Bacteriophage researchers make use of comparative genomics to address questions regarding the form and the function of a bacteriophage. The ever-increasing number of sequenced phage genomes allows us to better understand the mechanisms by which phage genomes function. We isolated the novel phage ‘Nitzel’ via enrichment of a soil sample obtained from a manure pile in Corpus Christi, TX. Analysis and

annotation of the Actinobacteriophage 'Oogway' genome was accomplished using DNA Master, Phamerator, and the National Center for Biotechnology Information (NCBI) Blast. The 'Oogway' genome is 51745 base pairs (bp) in length with 88 possible gene features. Our bioinformatic analyses also revealed highly conserved sequences of phage tail-related proteins. Most bacteriophages are characterized by a tail that may be used to inject viral DNA into their hosts. The tape measure protein received its name because its corresponding gene, which is usually the longest in the genome, is proportional to the length of the bacteriophage's tail. In the genome of 'Oogway,' gene 24 was putatively identified as the tape measure and gene 25 was putatively identified as a minor tail protein. We hypothesize that different tail sizes perform different functions and aid to infect the bacteria host.

U53. Tompkins Phage

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The bacteriophage Tompkins was isolated from a soil sample collected in Haslet, Texas. After enrichment, the bacteriophage was isolated and brought to a high titer lysate concentration. Tompkins formed plaques with sharp edges. Tompkins can be classified as a lytic phage. Genetic material was isolated and purified. After purification, the extracted genetic material was quantified using a spectrometer and ran on an electrophoresis gel to check for genomic DNA.

U54. Comparative Analysis of Mycobacteriophage Wunderphul and Other Cluster A6 Phages With Respect to Genome Size and the Absence of a Series of Phams at the 3' End of Phage Wunderphul

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Phage WunderPhul is a member of the Mycobacteriophage subcluster A6. The genome of WunderPhul, at 48,724bp in length, is notably the shortest of all identified A6 phages. The second shortest A6 phage, Jeffabunny, has a genomic size of 48,963bp. The genomes of all other A6 phages range between 50,341bp and 52,879bp in length. BLASTN analysis on the phage WunderPhul genome against the Mycobacteriophage genome database on PhagesDB reveals that it is most similar to phage Zaka. Measuring 52,122bp, the Zaka genome is significantly longer than that of WunderPhul. The second, third, and fourth best matches, phages Wiks, Zula, and ToneTone respectively, are all drafts and therefore not heavily relied upon for purposes of this comparison. However, it is interesting to note that these three phages are 52,044-52,499bp in length, which are all at least 3KB longer than the genome of phage WunderPhul. Comparative analysis of Zaka and WunderPhul using Phamerator showed that WunderPhul has genes which are in the same phamilies as genes 89 and 102 in Zaka. However, genes 90-101 observed in Zaka had no equivalent homologs in WunderPhul. In Zaka, these 12 genes encompass a total of 2,202bp and are therefore equal to approximately 65% of the difference in size between the two phage genomes. Phamerator analysis also indicates that these 12 genes in the same set of phamilies were present in the same location in Jeffabunny and many other A6 phages.

U55. WunderPhul Discoveries: Isolation, Characterization, and Comparative Genomic Analysis of Mycobacterium Phage WunderPhul

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Phage WunderPhul was isolated in the fall of 2015 from a soil sample at LeTourneau University (32.466073 N, 94.72684 W) using the enrichment plate method with *Mycobacterium smegmatis* mc^{2}155 as the host. Following purification of the virus, genomic DNA was extracted, and sent for sequencing at Pittsburgh Bacteriophage Institute. We analyzed the genomic DNA sequence of Phage WunderPhul and we are interested in expanding our knowledge of the subcluster of which it is a member. Many similarities were observed as Phage WunderPhul was compared to other A6 subcluster members. The 24 members of subcluster A6 have an average genomic size of about 51,838 bp, an average GC content of 61.5%, an

average of 98.2 genes, and 3 tRNAs. Phage WunderPhul has a genome length of 48,724 bp with a GC content of 61.5%. The length of WunderPhul is the farthest from the average of the subcluster and has the least number of genes compared to the currently annotated phages within the A6 subcluster. The Phage WunderPhul genome includes eighty-nine genes with about one-third of the open reading frames in a forward direction and an additional three tRNA genes similar to other annotated A6 phages. WunderPhul is most closely related to Phage Zaka. The second most closely related A6 phage is Phage Wiks.

Graduate Student Posters

G1. Factors Impacting Recolonization after Antibiotic Exposure of a Vertebrate Model Mucosal Microbiome

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Disrupting homeostasis of the human gut microbiome caused by antibiotic treatments can promote the emergence of opportunistic infections (i.e. *Clostridium difficile*). Underlying mechanisms of the microbiota's community structure is thought to be mainly host driven, but human studies are unclear given wide patient variability. Deciphering which factor between environment, host mucosal components, and/or bacteria antagonism, has the greatest impact on the recovering microbiota structure is our research focus. The skin mucus layer from a small freshwater fish, *Gambusia affinis*, serves as a tractable model vertebrate microbiome. The experimental procedure will consist of sampling the fish skin microbiome initial structure, after exposure to a broad spectrum antibiotic, and after a recovery phase. Fish groups will be separated during the recovery phase, sterile water or original aquarium water, to comparatively assess effect of environment. The skin microbiome samples will be analyzed in a variety of ways: the compositional change within the microbiota via 16S rRNA profile sequencing, semiquantitative PCR to estimate total number of bacteria, ERIC-PCR fingerprinting to capture community diversity, biochemical activity profiling of community metabolic function, and skin mucus quantitation by an Alcian Blue colorimetric assay. Results obtained from this study will provide an in-depth assessment on factors impacting the recolonization of the microbiome after antibiotic-induced disruption. Findings from this research may suggest novel implications for preventing and restoring the adverse effects associated with antibiotics in aquaculture, agriculture, and human health.

G2. Glycoside Hydrolase Degrades the Extracellular Polymeric Substance of Polymicrobial Bacterial Biofilms

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The persistent nature of chronic wounds leaves them highly susceptible to invasion by a variety of pathogens that have the ability to construct an Extracellular Polymeric Substance (EPS). This EPS makes the bacterial population, or biofilm, up to one-thousand percent more antibiotic tolerant than planktonic cells, and makes wound healing extremely difficult. Thus, chemicals which have the ability to degrade the EPS of mono- and co-culture biofilms are highly sought-after for clinical applications. In this study, we examined the efficacy of two glycoside hydrolases, α-amylase and cellulase, which break down complex polysaccharides, to eradicate *Staphylococcus aureus* (*Sa*), *Pseudomonas aeruginosa* (*Pa*), and *Sa*+*Pa* co-culture biofilms in clinically relevant *in vitro* and *in vivo* models. We hypothesized that, in both mono- and co-culture *in vitro* and *ex vivo* biofilms, glycoside hydrolase therapy will significantly reduce EPS biomass, converting bacteria to their planktonic state and leaving them more susceptible to conventional antimicrobials. Treatment of *Sa, Pa,* and *Sa*+*Pa in vitro,* and *ex vivo* biofilms with solutions of both α-amylase and cellulase, and with α-amylase and cellulase together, resulted in significant reductions in biomass, particularly when a combination of both enzymes was utilized. Additionally, treatment of biofilms grown in wound-like media with both enzymes separately, and together, resulted in the dissolution of the biofilm and an increase in the effectiveness of subsequent antibiotic treatments. Current studies are focused on adapting the glycoside hydrolase therapies to the *in vivo* environment, with and without concurrent antibiotic treatment. Also we are in the process of optimizing the ratio of α-amylase to cellulase in dual-enzyme treatments.

G3. Assessing life span and aging of *Chlamydomonas reinhardtii* in different growth conditions

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The mechanism of lifespan and aging has been a mystery to us. Replicative aging and chronological aging has been studied extensively in *S. cerevisae*. However, *C reinhardtii* has not been used as model

organism extensively for aging assays although it has been used for understanding several fundamental processes of cells. The objective of this project is to assess lifespan and aging using *C. reinhardtii* as a model. This green alga has a capability to undergo different modes of life: mixotrophic, autotrophic, or heterotrophic, depending upon the type of growth conditions. For mixotrophic conditions, *C. reinhardtii* will be grown in 24 hour light conditions at 25˚C using R media. For autotrophic growth, algae will be grown in high-salt minimal medium providing 5% CO_2 under continuous light conditions at 25˚C. Whereas, in heterotrophic conditions, the algae will be grown in complete darkness in modified Sager and Granick media. Growth curve analysis will be performed for each growth condition to observe the differences in lifespan. Percentage motility and swimming speed analysis will be done to examine aging as a factor of how motility is affected with every generation of the cell cycle. In addition, stress analysis on *C. reinhardtii* will appraise the rate of aging, measuring the expression level of HSP 90 among different growth conditions. This study will help to determine which mode of growth conditions has a longer lifespan and lower aging rate among three basic modes of nutritional uptake in *Chlamydomonas*. We will also examine how cell number varies with respect to cell cycle in each of the growth conditions as a tool to measure lifespan. Thus, this project will appraise lifespan and aging in *C. reinhardtii* and provide an excellent basis for future genomic studies.

G4. Engaging students in class research to improve their learning experience

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Sam Houston State University

A core concept to the study of genetics is the wild type phenotype, yet there is a widespread, common misconception that the wild type is only represented by the dominant phenotype. The wild type is represented by the most common phenotype within a population; this trait may be dominant or recessive. To address the common misconception, thirteen traits were analyzed from an Introductory Genetics class of 63 undergraduate students. Some of the traits included were the presence of dimples or the absence of dimples, rolling tongue or non-rolling tongue, oval face shape or square face shape, separated eyebrows or joined eyebrows, free or attached earlobes, broad or thin lips, polydactly or non-polydactly, right over left hand clasping or left over right hand clasping, cleft chin or no cleft chin, the presence or absence of middle digit hair, long or short eyelashes, eye shape as almond or round, and widow's peak or no widow's peak. The data was collected and then further divided into groups, including gender and ethnic groups to analyze any patterns found across the subgroups. A chi-square test was used to test the null hypothesis for each trait where a p value <0.05 is indicative of a significant departure from a random distribution of alternate phenotypes. Results revealed the wild type is represented by either dominant or recessive phenotypes, supporting the hypothesis that the wild type is not only the dominant phenotypes. When all of the traits were analyzed, four wild type phenotypes were found to be dominant in the population, including rolling tongue, oval face shape, separated brows, and almond eye shape. However, four of the traits were found to be recessive wild type phenotypes, including the absence of dimples, non polydactly, no cleft chin and no widow's peak were shown prevalently in the student population. Of the thirteen total traits, five of the traits are commonly found dominant and recessive phenotypes are equally frequent, therefore both phenotypes depict the wild types. This type of study can be implemented into classrooms to incorporate research to clarify misconceptions about many other core concepts and some misconceptions in biology.

G5. Investigating *Staphylococcus aureus* Bacteriophages that are Present in Swine Production Environments

Abby Korn
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Methicillin-resistant *Staphylococcus aureus* (MRSA) poses a major risk to human health, with approximately 94,000 cases occurring annually in the United States. Recent surveys in the United States on the prevalence of MRSA in swine production have found various sequence and spa types of MRSA that are harbored by both the animals and the production workers. This increased colonization with MRSA may not represent a significant health concern for the animals, but it does raise concerns for worker safety and overall community health in areas where swine production is prominent. With antibiotic usage being gradually restricted in livestock production, there is a renewed interest in the application of

phage therapy, the practice of using bacteriophages, to decolonize swine and production environments of MRSA. Three novel *S. aureus* phage were isolated from swine environments. P4 was isolated from a research swine barn, TP1 was also isolated from a different research swine barn and RP1 was isolated from pig skin in an abattoir during swine processing. The host of range of P4, TP1 and RP1 was examined against a panel of 20 MRSA and MSSA clinical isolates. Using an efficiency of plating (EOP) of greater than 0.1 as a cutoff, it was determined that 20% of strains were sensitive to TP1, 50% to RP1 and 10% to P4. Transmission electron microscopy images revealed that RP1 and TP1 are both myophages, while P4 is a podophage. Genome analysis of all three phage genomes is currently ongoing. Preliminary data indicates that P4 has a 18kb genome and is most related to another *S. aureus* phage, P68. Additionally, RP1 has a 142kb genome and is most similar to *S. aureus* Phage K. Both P68 and Phage K are lytic *S. aureus* bacteriophages. This work is the beginning of expanding the knowledge that surrounds *S. aureus* phage that inhabit swine production environments, as well as information regarding *S. aureus* phage in general.

G6. Synergistic Mechanism for the Lantibiotic Mutacin 1140 and Kanamycin
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Streptococcus mutans JH1140 is a strain of bacteria which produces a lantibiotic product, named mutacin 1140. Mutacin 1140 has been shown to be effective at inhibiting Gram-positive bacterial infections caused by *Staphylococcus aureus* and *Streptococcus pneumoniae*. Mutacin 1140 is a ribosomally synthesized peptide antibiotic that undergoes extensive posttranslational modifications (PTM). Microdilution plate assays show that when combined, mutacin 1140 and kanamycin, an aminoglycoside, display synergistic behavior against *Staphylococcus aureus*. This was confirmed through dose dependent kill curves and colony forming unit (CFU) counts. Propidium iodide (PI) staining and confocal microscopy shows that disruption of the bacterial membrane is more prominent in dose combination of mutacin 1140 and kanamycin than individually against *Staphylococcus aureus* and *methicillin-resistant Staphylococcus aureus* (MRSA). Supplementing *S. aureus* media with 10% sorbitol caused a decrease in the number of cells stained by propidium iodide supporting the notion that mutacin 1140 and kanamycin synergy is due to membrane disruption. The results determined in this study provide a starting point for further analysis into the mechanism of action for lantibiotics and aminoglycoside combination treatment.

G7. Mouse cytomegalovirus increases markers of neurodegeneration in cell culture
Prapti Mody, DiAnna Hynds and Laura Hanson
Texas Woman's University

Cytomegalovirus is a species-specific herpesvirus infecting 60-100% of people worldwide. Human CMV (HCMV) is a major infectious cause for congenital birth defects, especially mental retardation and hearing loss. Recently, HCMV has been implicated in neurodegenerative diseases like Alzheimer's disease (AD). AD has two hallmark pathologies i.e. extracellular aggregates of beta-amyloid peptides (Aβ42) and intracellular phosphorylated tau aggregates. Amyloid fibrils interfere with normal synaptic functioning and tau bundles hamper normal cellular function, leading to cell death and consequent loss of neurons. Clinical correlation has been shown between CMV seroprevalence and increased risk of AD and HCMV proteins have been found co-localized with plaques in AD patients' brain tissue. Additionally, in culture, HCMV infection has been reported to increase levels of beta-amyloid in fibroblasts at 6 days post infection. Since HCMV is species-specific, studying mechanisms of pathogenesis has limitations. Mouse CMV (MCMV) is an important tool as its genetics and pathologies are similar to HCMV. We have demonstrated that MCMV increases levels of amyloid precursor protein (APP) and tau in fibroblasts and neuronal cells by 24 hours post infection with levels remaining elevated through 72 hours post infection. Inhibition of viral DNA replication inhibits a subset of viral proteins known as late gene products. Using such an inhibitor (Foscarnet) showed abolition of the increase of APP but tau was still upregulated. This indicates that different mechanisms are involved in upregulation of APP and tau.
Presenilin-2 is a transmembrane enzyme that processes APP to produce Aβ peptides. Initial westerns for Presenilin-2 levels in fibroblasts show no difference due to infection. This negates the possibility of APP increase due to a decrease in its' processing enzyme steady-state levels. Using an antibody specific for phosphorylated tau at serine 396, we found that phosphorylation is increased starting 24 hours post

infection. Although this antibody recognizes only one of many phosphorylation sites, the sizes of detected forms compared to unmodified isoforms indicate presence of additional modifications that may be upregulated also. CMV infection increases levels of phosphatases so it is likely that the increased phosphorylation is due to increased kinase activity. Glycogen synthase kinase 3 beta (GSK3β) is one known kinase that phosphorylates tau at serine 396. Preliminary western blots for levels of GSK3β showed no difference in levels or size in infected cells suggesting that GSK3β may not be involved in the increased phosphorylation. Thus, our data support a definite role of CMV infection in increasing amyloid, tau and tau phosphorylation at serine 396 and we have started determining mechanisms of these upregulations.

G8. The of Effect Lipogenesis Inhibitory Blue Berry Polyphenol Extract on Murine Cytomegalovirus Infection.

Mari Tayyar and Dr. Laura Hanson.
Texas Women's University

Human cytomegalovirus is a common herpesvirus, infecting 60-100% of people worldwide which exhibits clinical correlation with atherosclerosis. Several recent studies have shown that cytomegalovirus infected cells have increased synthesis of fatty acids and cholesterol. The viral regulators involving lipogenesis induction have not been clearly identified. Induction of lipid synthesis is important for virus production since it is an enveloped virus and it needs lipids for the final step of envelopment using a cell phospholipid bilayer and cell egress. Thus, materials that inhibit lipid synthesis would be expected to have antiviral activity. Previous studies showed that blueberry extracts rich in polyphenol could inhibit normal lipogenesis. We hypothesized that these blueberry polyphenols extracts would inhibit production of cytomegalovirus. To test this hypothesis we used the mouse cytomegalovirus (MCMV) model in cell culture. We have found that MCMV, like HCMV, induces specific lipogenesis factors, and the inhibition of viral DNA replication, which prevents expression of a subset of viral proteins known as late gene products prevents the viral induction. In our studies with the blueberry extracts we got results contrary to our hypothesis. Not only was there no evidence of a reduction in production of infectious virus but the blueberry polyphenol extracts enhanced induction of the lipogenesis factors specifically in the virally infected cells. Investigating the mechanism behind this altered response may lead to a better understanding of how both the virus and the blueberry polyphenol extracts function in altering lipid synthesis.

G9. The role of anaerobic bacteria in polymicrobial infections

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Skin and soft tissue infections can manifest in a variety of ways, ranging from a self-resolving abscess to a rapidly spreading necrotizing soft tissue infection (NSTI). While an abscess may require only minor medical intervention, if treatment is needed at all, an NSTI is a medical emergency, requiring immediate surgical intervention. NSTIs can either be monomicrobial or, more commonly, polymicrobial. Monomicrobial NSTIs generally involve well-studied pathogen such as *Streptococcus pyogenes* or *Clostridium perfringens*. Polymicrobial NSTIs, however, are not as well understood. *Staphylococcus aureus* (*Sa*), a facultative anaerobe, is the most commonly isolated pathogen in polymicrobial NSTIs, but it is also the most commonly isolated pathogen in abscess infections. The other microbial and host contributions that contribute to the development of NSTIs are not well studied. We hypothesize several different causes, one of which is the involvement of anaerobic bacteria, which are difficult to culture and thus often missed in pathogen isolation studies. In order to further elucidate the contribution of anaerobic bacteria in the development of NSTIs, we introduced a commonly isolated wound anaerobe, *Bacteroides fragilis* (*Bf*), with *Sa* into *in vitro* and *in vivo* wound models. We asked how does *Bf* affect the growth and virulence of *Sa*. Using an *in vitro* wound-like model, our preliminary data showed that in aerobic conditions, *Bf* required *Sa* to survive. Using our *in vivo* mouse abscess model, we show that the presence of *Sa* and *Bf* caused dermonecrosis 100% of the time while *Sa* alone only caused dermonecrosis about 50% of the time. Future research efforts will be directed towards solidifying current data, elucidating the underlying mechanisms of this potential synergy in virulence, and understanding if a similar phenomenon occurs with other common wound co-infections such as those involving *Finegoldia magna*.