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Abstract Book

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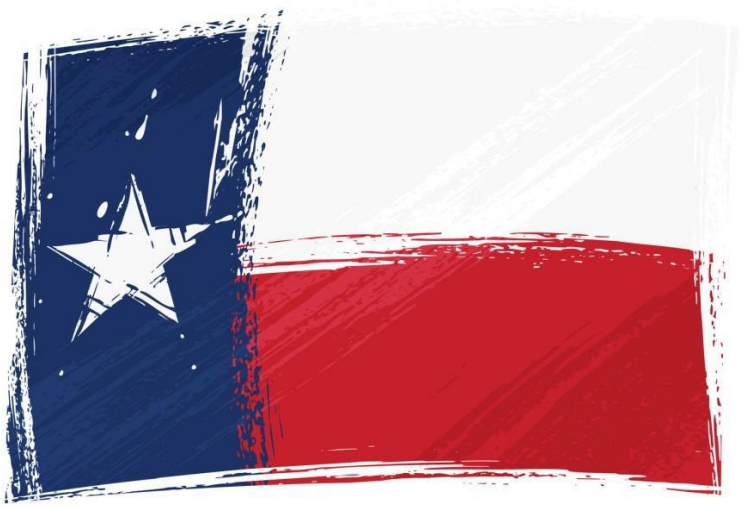

Eugene and Millicent Goldschmidt Graduate Student Award

Targeted metagenomics using housekeeping genes for priority pathogen identification

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Microbial forensics is an emerging field involved in the investigation and attribution of a biocrime or biological terror attack. Many technical challenges exist in the microbial forensics field, such as identification of select agents or other pathogens of interest within highly complex metagenomic samples, distinguishing select agents from near-neighbors or closely related avirulent strains, and the degree of confidence that can be assigned to a potential species/strain attribution. The complexity of metagenomic samples poses great challenges for full characterization of the organisms within that community. Current metagenomic approaches apply massively parallel sequencing (MPS) and target the 16S rRNA gene or use shotgun sequencing of whole genomes. There are limitations with both of these methods as they apply to microbial forensics (i.e., general inability to achieve species-level resolution and limited depth of coverage with susceptibility to stochastic effects, respectively). We are developing a novel MPS metagenomics approach that targets multiple bacterial housekeeping genes, containing conserved and variable regions, to more accurately characterize microorganisms of interest in complex microbial communities with a limit of detection that is superior to whole genome sequencing. The goal is to be able to achieve species- to strain-level identification of key pathogens of interest even when the target is at trace levels. We have identified 192 housekeeping genes as potential candidates for resolving bacteria down to species level identification. These genes are well-known and documented housekeeping genes and therefore offer the best set for pan-bacterial characterization. A manually curated bacterial whole-genome database was constructed, consisting of 2,580 whole-genome bacterial reference assemblies, for candidate gene selection for *in silico* development of our targeted metagenomic gene panel. The database was constructed from 2,769 bacterial genomes downloaded from NCBI. Manual curation checked for and corrected inconsistencies and errors between the sequence files and genome reports, in order to reduce the potential for data misrepresentation and phylogenetic misinterpretations in downstream analyses. The most commonly encountered errors were naming inconsistencies and erroneous files contained within genome folders in the database, and these were subsequently removed. Future efforts will focus on using the bacterial housekeeping gene panel to identify select agents from synthetic metagenomic datasets developed *in silico* and validate the bacterial housekeeping gene panel by generating empirical sequence data from synthetic metagenomic communities and spiked metagenomic samples. Our targeted metagenomics panel will significantly improve select agent identification from complex samples, aid in the development of novel microbial forensics tools and provide utility in other important areas, such as infectious disease diagnostics and public health investigations.

Undergraduate Student Oral Presentations

1. Programmed Cell Death is Distinct from Necrosis in *Chlamydomonas*

Matthew R. Breuer, Allyson R. Keathley, and Anne R. Gaillard
Sam Houston State University

Programmed cell death (PCD) is a form of cell death that is activated by a genetic program. In multicellular organisms, this process plays many crucial roles, such as the destruction of pre-cancerous cells or the shaping of digits during development. However, PCD has also been observed in many single-celled organisms. This phenomenon is inherently more difficult to explain because no obvious benefit is granted to the organism upon its death. Using the model organism *Chlamydomonas*, a well-studied unicellular alga, we hope to shed light on the seemingly illogical notion of a benefit to PCD in the unicellular world. When faced with a mild stressor, *Chlamydomonas* have been shown to undergo a PCD distinctly different from that of necrosis. Previous experiments also indicate that there exists a benefit to surviving cells following a mass PCD event, but no benefit to survivors following a mass necrotic event.

Because of this, we hypothesize that in *Chlamydomonas*, PCD has evolved as amechanism for kin selection. Kin selection can be defined as an evolutionary strategy in which the fitness of a genetically related population is favored above all else, even at the cost of the individual's life. To test this hypothesis, our laboratory is focusing on 1) further differentiation between PCD and necrosis, 2) independently verifying the contrasting effects of PCD and necrosis on surviving organisms, 3) quantifying the amount of PCD in an axenic culture compared to that of a mixed culture. The focus of this presentation will be on the first and second goals. To differentiate between PCD and necrosis, a comet assay is being used to detect DNA damage in cells that have been subjected to mild and severe heat-stresses, respectively. We are currently attempting to optimize the comet assay for use with *Chlamydomonas* cells. To confirm that PCD increases the fitness of surviving kin, supernatant from cells that were mildly heat-stressed and supernatant from cells that were intensely heat-stressed were taken from two species of *Chlamydomonas* and used to grow new cultures. Preliminary results indicate that *Chlamydomonas* releases beneficial compounds during PCD and detrimental compounds during necrosis.

2. Molecular Analysis of *recA* Mutant in *Rhodobacter sphaeroides*

Michelle Harrel, Veronica Rodriquez, Amber Neal, Hannah Johnson, Madhusudan Choudhary
Department of Biological Sciences, Sam Houston State University

RecA mediates the regulation of the SOS response, a DNA-damage repair system in prokaryotes. While LexA binds to the promoter of the SOS regulon under normal conditions and represses transcription of over 40 genes, RecA is activated by UV-damaged DNA and de-represses the transcription of the SOS regulon through the cleavage of LexA. The RecA protein also regulates error-prone DNA synthesis that bypasses DNA lesions. An abundance of gene duplications present in the *Rhodobacter sphaeroides'* fully-sequenced genome are the result of RecA-mediated homologous recombination. The above characteristics make *R. sphaeroides* a model bacterium to study the SOS response and homologous DNA recombination. This study employs the construction of an in-frame deletion (Δ*recA* mutant strain) and comparisons of the extent of DNA damage and repair under UV-induced conditions. We have successfully constructed and confirmed the Δ*recA* strain of *R. sphaeroides,* which has similar growth characteristics as the wild type under aerobic conditions. Results revealed that as UV exposure increased, survival rates of the wild type and the Δ*recA* strain differentially decreased with the Δ*recA* strain being more affected than the wild type. However, neither strain grew under aerobic conditions beyond 75 mJ of UV exposure. Finally, total RNAs from wild type and Δ*recA* strains will be isolated and sent away for total RNA sequencing, and expression patterns of selected genes suspected to be involved in the SOS response will be further validated by RT-PCR analysis.

3. Insights on Rhomboids in *Trichomonas foetus*.

Katherine Arriola
Lamar University

Cattle Trichomoniasis is the number one sexually transmitted disease caused by *Tritrichomonas foetus* leading to a measurable loss to the cattle industry worldwide and particularly in Texas. Following adherence to host cells, parasite cells are transformed from flagellate to amoeboid forms. We hypothesize that intramembrane rhomboid serine-proteases play a crucial role in the amoeboid transformation by cleaving substrates that aid in the cytoskeletal rearrangement of trichomonads.

In this current project, we used the Trichomonad genome database (TrichDB) to identify the rhomboid proteases-like sequences. BLAST, ClustalW, TargetP and TMHMM servers were used to analyze the sequences. Gene specific primers were synthesized based on the sequence analysis. Genomic DNA was isolated from a fresh clinical isolate (Tf-31). PCR amplification was performed and the amplified products were re-sequenced. Total RNA was isolated and mRNA was purified. RT-PCR analysis was performed to confirm gene expression.

PCR from genomic DNA resulted in amplification of four of the eight predicted rhomboid genes. Sequence analyses revealed that except for the conserved catalytic amino-acid residues and signature transmembrane residues, the gene sequences considerably varied within the eight trichomonad rhomboids as well as among rhomboids from other eukaryotic organisms. Analysis of transmembrane domains showed that only four of the eight genes had 7 TMs consistent with other eukaryotic rhomboids.

Interestingly, RT-PCR amplified transcripts of only three of the eight genes. We are currently performing the localization studies of these rhomboids using Halotag reporter gene.

4. Expanding Phage Insight: A High throughput Method to Cluster Archived Mycobacteriophage

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Phage are ubiquitous in the environment. Due in large part to the efforts of the HHMI SEA-PHAGES program, it has become apparent that mycobacteriophage, the viruses that infect mycobacteria, are extremely diverse and have highly mosaic genomes; however, these data only come from a small sampling of phage. The greatest source of biological information concerning mycobacteriophage is in the thousands of isolated, characterized, and archived phages that institutions have gathered through the years. To date a total of 5848 phage have been isolated and have yet to be sequenced. These phage are unclustered and hold the key to many evolutionary and ecological mysteries. Pulse Field Gel Electrophoresis (PFGE) is a technique used by molecular biologist to determine to the molecular weight of large nucleic acid sequences. By using PFGE in combination with Polymerase Chain Reaction (PCR), we purpose a high throughput and cost efficient method that allows for further characterization of the archived phage. We hypothesize that the clustering of yet to be sequenced phage may be inferred via PFGE in combination with PCR.

5. Engineered, Affordable Glycans as New Antimicrobials

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Gastroenteritis is a common cause of morbidity and mortality worldwide, especially in infants in the developing world, but vaccines against such enteric infections are largely ineffective. This poses a need to produce an effective, economical therapy for this disease. Human milk oligosaccharides such as 2'-fucosyllactose could serve as antimicrobials, because they mimic the interactions between histoblood group antigens and viruses, and therefore prevent pathogen-host binding. This project aims to prepare a carbohydrate analog for viral binding and inhibition containing a 2'-fucosyllactose-like linkage. Multiple experimental pathways have been previously explored with unsuccessful results, to synthesize 2'-fucosyllactose through the use of a *lacZ- lacY+ lacI-* strain of MG1655 *E. coli* with serial deletion of the *purR, fucI,* and *wcaJ* genes. In this study, we harvest and investigate the viral inhibitory capacity of the O-polysaccharide component of lipopolysaccharide (LPS) on O128:B12 *E. coli*, which contains a Fucα1,2Galβ linkage that is similar to 2'-fucosyllactose. We have engineered and optimized O128:B12 to overexpress LPS, through altered growth conditions or bacterial transformations with the *ypdI, rcsB,* or *lpxC* genes. Our results will point to a powerful method for large-scale synthesis of complex carbohydrates and a new therapeutic approach for gastroenteritis.

Graduate Student Oral Presentations

6. Chemotaxis Contributes to *Pseudomonas aeruginosa* Virulence in a Thermally Injured Mouse Model of Infection

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Pseudomonas aeruginosa (PA) is a gram-negative, opportunistic pathogen that is exceptionally well adapted at establishing systemic infections in thermally injured patients. PA utilizes a unique strategy to promote systemic infections by forming biofilms around the damaged, leaky vasculature within burn wounds. This phenomenon, referred to as perivascular cuffing, acts as a direct portal of entry for PA to

gain access to the bloodstream; however, at present, the mechanisms necessary to conduct this form of behavior are unclear. Here, we employed a high-throughput sequencing-based technique, Tn-Seq, which identified a number of chemotaxis (CTX)-related genes that are required for full fitness of PA in thermally injured mice. *In vitro* directional motility assays demonstrated that PA exhibits a predilection to chemotax towards an, as yet, unidentified small molecule in murine serum. To verify the importance of CTX *in vivo*, we infected burn mice with individual transposon insertion mutants representing genes in PA CTX gene clusters I, II, and V and evaluated their ability to cause sepsis and mortality. Burn mice infected with Δ*cheR1*, Δ*cheB*, and Δ*cheA* mutants did not develop sepsis and exhibited prolonged survival compared to burn mice infected with wild-type PA, suggesting that bacterial chemotaxis may play a significant role in PA virulence in burn wounds and aid in the systemic spread of infection.

7. Interactions Among Environmental Variables and Microbial Communities in Reservoirs of the Upper Colorado and Brazos Rivers, Texas, as a Potential Proximate Cause of Golden Alga Blooms

Tirhas Hailu[1*], Randall Jeter[1], John Zak[1] and Reynaldo Patino[1, 2]
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Understanding how environmental variables and microbial communities interact to create conditions favorable to toxic blooms of *Prymnesium parvum* (golden alga) can lead to strategies for managing blooms. Water samples were collected from 5 reservoirs of the Upper Colorado River (one sampled twice) and one from the Brazos River, and the composition of microbial communities in each sample was determined by 454 pyrosequencing of eDNA. Principal Component Analysis indicated that temperature (negative) and dissolved oxygen (positive) showed the strongest associations with golden alga presence among the environmental variables, in combination with higher relative abundances of the bacterial classes Oscillatoriales and Planctomycetacia and lower abundance of Spirochaetia. Using taxonomic levels lower than class in the analysis failed to separate sampling events according to alga presence or absence, suggesting that the presence or absence of entire taxonomic microbial groups rather than a suite of specific species may contribute to the growth or decline of golden algal populations.

8. Characterization of the Roles of Tigar and Parkin in Preventing Oncogene-Induced Mitochondrial Damage during Retroviral Carcinogenesis

*Tetiana Hutchison, Megan Romeo, Rebecca Brady, Janice Kim, Averi White, Catherine Hazen, Rachel Gardner, Mary Hancock, Rao Neha, Nguyen Olivia, Chu Jessica and Robert Harrod, Ph.D., Department of Biological Sciences and the Dedman College Center for Drug Dicovery, Design and Delivery, Southern Methodist University, 6501 Airline Drive, 334-DLS, Dallas, TX 75275-0376. Tel: (307)240-1602. Email: tyhutchison@smu.edu

The human T-cell leukemia virus type-1 (HTLV-1) is an oncogenic retrovirus that transforms $CD4^+$ T-lymphocytes and is the etiological agent of adult T-cell leukemia/lymphoma (ATL), an often-fatal hematological malignancy that is resistant to most anticancer treatments. The HTLV-1 proviral genome contains a highly-conserved 3' nucleotide sequence, known as *pX*, which encodes at least seven nonstructural proteins (Tax, Rex, $p8^I$, $p12^I$, $p13^{II}$, $p30^{II}$,and Hbz) and is conserved in the majority of ATL clinical isolates. The *latency maintenance factor*, $p30^{II}$, suppresses viral gene expression and also promotes proviral replication through cooperation with the c-Myc oncoprotein and the induction of aberrant lymphoproliferation. Previous studies by our lab have shown that $p30^{II}$ enhances c-MYC-dependent transactivation and oncogenic potential by stabilizing recruitment of the TIP60 acetyltransferase to $p30^{II}$/c-MYC nuclear complexes. The MYST-family TIP60 protein is a transcriptional cofactor for both c-Myc and p53. The overexpression of oncogenes, such as c-Myc, can lead to the intracellular accumulation of reactive oxygen species (ROS) associated with single-stranded DNA breaks and cytotoxicity. The generation of ROS within mitochondria can lead to mitochondrial damage or mitophagy – a process of autolytic mitochondrial destruction. Our lab has found that the induction of TIGAR (*Tp53-induced glycolysis and apoptosis regulator*) by HTLV-1 $p30^{II}$ prevents the accumulation of c-Myc-induced and inhibits oncogene-associated cellular senescence and apoptosis. Intriguingly, under

conditions of oxidative stress, the RING-HECT hybrid E3 ubiquitin ligase, Parkin, has been shown to localize in mitochondrial-derived vesicles and participates in mitochondrial "quality control" by preventing mitochondrial damage. We therefore hypothesize that $p30^{II}$ may activate antioxidant-signaling pathways to induce mitochondrial targeting of the TIGAR and Parkin proteins to prevent oxidative mitochondrial damage associated with aberrant oncogene-activation in HTLV-1-infected leukemic T-cells. *These studies reveal a pivotal role for the $p30^{II}$ protein in promoting c-myc oncogene-activation through the induction of p53-dependent metabolic effectors, and will advance our understanding of how host cellular factors cooperate with transforming viruses during carcinogenesis*.

9. Isolation and Characterization of Gold (III) Resistant Mutant of *Rhodobacter sphaeroides*

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Sam Houston State University
Department of Sciences[1], Department of Computer Science[2]

Rhodobacter sphaeroides belongs to α-3 subdivision of the *Proteobacteria* that is metabolically capable to tolerate high levels of toxic heavy metals. These heavy metals constitute a major pollution that was contributed to by a variety of sources, such as industrial effluents, leaching out metal ions from the soil, and acid rain. These pollutions pose a serious problem to human health and require bioremediation of such toxic metals from our streams, lakes, and soils. Previous studies have shown that some bacterial species tolerate varying levels of heavy metals in their environments. The heavy metal tolerance in bacteria is mediated through spontaneous mutation and selection of mutant in the continuing bacterial culture. Strains of *R. sphaeroides* were continually selected on minimal medium with varying concentrations of $AuCl_3$ in both aerobic and photosynthetic growth conditions. A growth curve was performed on both conditions to support the mutation hypothesis. Strains grown under aerobic and photosynthetic growth conditions were analyzed for reduction of gold in membrane and cytosolic fractions of cells using ICF-Analysis. Gene homologs of previously identified genes involving metal tolerance in *Pseudomonas putida* were identified in the genome of *R. sphaeroides*; the genes include sensor kinases, membrane bound transporters, etc. It is suspected that a number of these genes might have altered expression patterns under selective growth condition, which will be measured by reverse transcriptase polymerase chain reaction (rtPCR) analysis. Results of the current study will have an array of applications to scavenge heavy metals from polluted environment at a larger scale.

10. Deciphering Horizontal Gene Transfer Events in *Galdieria sulphuraria* 074W

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Unicellular alga *Galdieria sulphuraria* is an interesting species owing to its characteristics like surviving on more than 50 different types of carbon media and adaptation to hostile environments like high heat, toxicity due to heavy metals, high acidity and hot spring water. These environmental adaptations can be attributed to the genes that were transferred horizontally to *G. sulphuraria* 074W during the course of evolution. Horizontally transferred genes are the genes inherited from the genome of a donor organism other than the parental genome. Genome of *G. sulphuraria* was analyzed by an integrative methodology of recursive segmentation and agglomerative clustering. The clusters thus formed revealed that almost 30% genome of *G. sulphuraria* is composed of alien genes. Protein BLAST of these genes revealed the donor organisms, most of which are proteobacteria and archaea. Most of these donor organisms are known to thrive in various hostile conditions due to their harboring genes that can metabolize various toxic elements.

11. Schistosomicidal Oxamniquine Derivative Drug Activity against Human Schistosomiasis

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Human schistosomiasis is a disease caused by species of the genus *Schistosoma,* which globally affects over 200 million people. The major species effecting humans are *S. mansoni, S. haematobium,* and *S. japonicum.* There is currently only one method of treatment (monotherapy), the drug Praziquantel (PZQ). Constant selection pressure through mass chemotherapy - this year alone will see the administration of over 250 million doses - has yielded evidence of resistance to PZQ. This has been observed in both the laboratory and field. The purpose of this research is to develop a second drug for use in conjunction with PZQ. Previous treatment of *S. mansoni* included, among others, the use of oxamniquine (OXA), a prodrug that is enzymatically activated in *S. mansoni* but is ineffective against *S. haematobium* and *S. japonicum*. The OXA activating enzyme was identified, described, and crystallized by our laboratory as being a sulfotransferase (SmSULT). The focus of this research is to reengineer OXA to be effective against *S. haematobium* and *S. japonicum*. 5 generations of OXA derivatives have been synthesized and tested against *S. mansoni*, with a total of 8 derivatives discovered that may potentially be used to treat schistosomiasis. *In vitro* test of these 8 derivatives against *S. haematobium* and *S. japonicum* have also yielded positive results. This iterative generational process of using structural data to inform chemical synthesis of derivatives, which are then tested *in vitro*, continues to provide us with novel compounds with improved antischistosomal activity. The information gleaned from these early studies will be used to optimize OXA derivative design.

Undergraduate Student Posters

U1. Transcriptional Regulation of the ICE R391 RumA'$_2$B DNA Polymerase V by SetR

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The integrating conjugative element (ICE) R391 codes for the error-prone DNA polymerase V homolog, RumA'$_2$B. DNA polV homologs have been shown to factor in cellular levels of spontaneous and DNA damage-induced mutagenesis. In order to address the regulation of the *rumAB* operon, spontaneous mutagenesis assays and western blot analysis were performed to characterize the R391 encoded putative repressor designated SetR. *Escherichia coli* expressing SetR, a λcI repressor homolog, demonstrated a reduced level of RumA'$_2$B–mediated spontaneous mutagenesis relative to *E. coli* cells lacking SetR. In addition, SetR was shown to repress the *rumAB* operon, while not the homologous *mucAB* or *umuDC* operons. Similar to the λcI repressor, *in vitro* studies with putative SetR show that under alkaline conditions, as well as in the presence of activated RecA, SetR will undergo autocleavage. To further validate this finding, we created a non-cleavable mutant version of SetR. Mutagenesis assays in an activated RecA strain demonstrated nearly equal levels of RumA'$_2$B-mediated spontaneous mutagenesis in *E. coli* with and without SetR; however, *E. coli* expressing the non-cleavable version of SetR demonstrated a substantial reduction in spontaneous mutagenesis. While ICE R391 already carries resistance to the antibiotic kanamycin, understanding the regulation of the RumA'$_2$B-mediated mutagenic activity is essential to minimizing mutation induced antibiotic resistance.

U2. Significant Microbiome Alteration from a Broad-Spectrum Antibiotic Induces Host Effects in a Vertebrate Fish Model

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The administration of antibiotics can significantly alter the intestinal microbiome in humans, with a diversity of clinical outcomes. Numerous studies have suggested that less diverse gut microbial communities can induce the incipient stage of metabolic disorders such as obesity, diabetes, and inflammatory bowel disease. A depleted microbiota can also lead to development of antibiotic-associated enterocolitis (AAE). To demonstrate effects following a broad-spectrum antibiotic exposure, we use a model vertebrate fish *Gambusia affinis* to portray host outcomes. Our previous work utilizing our well established system provides evidence that fish exposed to the antibiotic rifampicin for 3 days exhibit pre-exposure culturable numbers but the community composition for the skin and gut microbiomes have significantly lost taxonomic and metabolic diversity. In this study, fish were examined for five different effects on the host of this altered microbiome. Comparative results show that the antibiotic-exposed fish exhibit; less weight gain over time compared to controls when fed a standardized diet, increased susceptibility to infection using the established septic pathogen *Edwardsiella ictaluri* (mean time to death following exposure shifted from 98 to 56 hours), and inhibited osmotic regulation (88% death in high saline conditions as compared to 42% in control). No significant differences between treated and untreated fish were observed when fish were challenged with water containing high polymicrobial counts (from soil or feces) or the toxin nitrate. This study provides preliminary data suggesting antibiotic-disturbed skin and gut microbiomes effect negative outcomes to the host *G. affinis*. Future work entails examination of other host factors in the gut, including histological stains for mucus levels and neutrophil stains to evaluate inflammation. An assay for intestinal motility rate is also in development. Understanding host and microbiota alterations may lead to improvements for patients who suffer from AAE, and probiotic or prebiotic remedies.

U3. Antimicrobial Properties of Anthracyclines and Aziridinomitosenes

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Department of Chemistry and Biochemistry, Boise State University, Boise ID 83705

The frequent misuse of antibiotics has led to an increase in antibiotic resistance that requires the continual formulation and testing of new chemical compounds to fight infectious diseases. Chemotherapeutic compounds, such asanthracyclines and aziridinomitosenes that are used for the treatment of cancer, also show potential to be a viable treatment for antibiotic resistant bacterial infections. In this study, several anthracyclines (Doxorubicin [DOX],Pyrrolinodoxorubicin [P-DOX], GPX, PGPX, Mitomycin C) and aziridinomitosenes (UMMS, C6, C10) were tested for their antibiotic potential against gram-negative and gram-positive bacteria. Using disk diffusion assays, theanthracyclines P-DOX and DOX showed promising activity toward gram positives, while the GPX derivatives appeared inactive. The aziridinomitosenes also showed activity, with UMMS appearing active against both*Staphylococcus aureus* and *E. coli.* Further exploration of the antimicrobial activity of these compounds appears warranted.

U4. Inhibition of *Streptococcus mutans* biofilms

Geraldine Lerner, Shima Taghikhani, *Irin Girgis, Sandy Gramajo, and Poonam Gulati Department of Natural Sciences, UHD

The human oral cavity contains over 700 diverse bacterial species, some of which are commensal, harmless and found in healthy persons, while others are pathogenic and can lead to dental caries, periodontitis, and other diseases. Most bacteria reside in organic biofilms, communities of microbes attached to moist surfaces, such as teeth and gums, using extrapolymeric substances (EPS.) *Streptococcus mutans* and *Streptococcus salivarius* are two members of oral biofilms*. S. mutans* has been implicated in dental caries, while the role of *S. salivarius* has not been fully elucidated. *S. salivarius* is one of the first colonizers of a biofilm and facilitates the attachment of *S. mutans,* thus assisting in the pathogenicity of *S. mutans.* However, recently it has been suggested that *S. salivarius* is acting as a probiotic in the mouth and may hinder harmful bacteria such as *S. mutans*. In our lab we have been studying ways to inhibit *S. mutans*. Mouthwashes with Cetylpyridinium Chloride (CPC) worked the best among commercial mouthwashes tested. Since then we have tested extracts of natural products, garlic, turmeric and saffron all of which can also inhibit *S. mutans*. We are studying the mechanism of inhibition of saffron inhibition - preliminary studies reveal that biofilms are not disturbed even though the bacteria within are killed. Additionally, supernatants from *S. salivarius* cultures are being tested for inhibitory properties.

U5. Surface proteins of *Leishmania* parasites.

Emmy Hammonds
Lamar University

Leishmaniasis is a disease that causes disfigurement and death of humans throughout the tropical regions of the world. It is caused by the protozoan parasites of the genus, *Leishmania.* The goal of this project is to identify the surface proteins of Leishmania. The surface proteins are hypothesised to help the parasite invade its host. In order to do this, *Leishmania amazonensis* was grown using Schneider's insect medium. A SulfoLink Immobilization Kit and Coupling Resin were used to separate the surface proteins according to the manufacturer's protocol. The surface proteins were then subjected to two-dimensional gel electrophoresis. The process works in two steps; the first steps separates proteins based on isoelectric points and the second separates them based on molecular weights. In the first step the sample is put into the gel and a pH gradient, the electric charge is applied and the proteins move along the gradient until they no longer have an electrical charge, where they stop indicates their isoelectric points. In the next step proteins of the same size but different isoelectric points are resolved. The samples are then sent to UTMB service facility for analysis using MALDI-TOF. Employing the above listed methods, we will be able to gain information on the different surface proteins present on the amastigote and promastigote forms of *Leishmania*. With the help of this knowledge, future work on finding the specific functions of these proteins will be attempted. This work is supported by a McNair's research scholarship.

U6. A Little Bit of Salt and Peppersauce

Ariane Kubena,
Baylor University

A team of researchers from twenty different institutions was organized in the fall of 2014 under the Small World Initiative Program piloted by Yale University. During that time, soil from Peppersauce Cave was tested for the presence of microbes and their potential antibiotic production. This semester's research has involved more thorough testing of the five microbes that previously exhibited unique behavior. The urgency of the antibiotic crisis is best characterized by the antibiotic resistance of ESKAPE pathogens, the leading cause of nosocomial infections (hospital acquired infections). The purpose of this study was to explore the connection between the ESKAPE pathogens and potential antibiotic-producing bacteria from the soil. Following the dilution of the soil sample to determine the number of microbes per gram and an estimate of colony diversity, select isolates were tested by plate inoculation with the six ESKAPE pathogens. Various biochemical tests were completed for those that created zones of inhibition against the ESKAPE pathogens. Current laboratory goals include the identification of the genus and species of these potentially novel microbes. The behavior of the microbial community and the resilience of young scientists give the medical world hope for a better future.

U7. Autoinducer 2 transport in *Salmonella* Typhimurium
Kallie McWhinney
Texas A&M University

Bacteria rely on the concentration of signaling molecules in their environment to recognize population density and express phenotypic traits. This cellular communication is referred to as quorum sensing and has implications for the treatment of food-borne illnesses caused by pathogens like *Salmonella enterica* serotype Typhimurium (*S.* Typhimurium). The chemical signal, autoinducer-2 (AI-2), encoded by the gene *luxS*, plays a central role in *Salmonella* quorum sensing. AI-2 is produced and released into the extracellular environment. When AI-2 is present in high concentrations, the ABC-transporter, encoded by *lsrBCD,* transports the AI-2 into the bacterial cytoplasm. In the absence of this transporter, some of the AI-2 is removed from the external environment possibly through the actions of an alternate transporter. The focus of this study is to determine whether the *rbs* operon encodes an alternate AI-2 transporter. A Δ*rbs* mutant was made by homologous recombination and its ability to transport the AI-2 signal was compared with that of a Δ*lsrD* mutant and a *Δrbs lsrD* double mutant. Negative and positive controls consisted of a Δ*luxS* mutant and wild type *S.* Typhimurium (WT). Measurements of the amount of AI-2 in the *Salmonella* culture supernatant were made using an established *Vibrio harveyi* bioassay. Observation of AI-2 activity in the *V. harveyi* bioassay revealed an increase over time in the amount of AI-2 in the culture media from the Δ*lsrD* mutant relative to WT, however over time, the amount oscillated. The Δ*rbsB* mutant AI-2 activity resembled that of the WT. Lastly, the Δ*rbs lsrD* double mutant produced the same results as the Δ*lsrD* mutant. The negative control, Δ*luxS* mutant, produced low amounts of AI-2 that were likely produced by alternative genes. Together these results suggest that *rbsB* does not participate in the transport of AI-2 in *Salmonella*.

U8. The Road To Discovering New Antibiotics
Danielle Natividad
Baylor University

ESKAPE pathogens are responsible for a wide range of nosocomial infections. This has led to an increased demand for new antibiotics to address the growing threat of multidrug resistant bacteria. In situ bacteria produce antibiotic substances to protect "their turf." Humans have benefitted from these substances since the initial discovery was "harnessed" in the 1940s. Examining soil bacteria for antibiotic production was an endeavor of the next two decades. Since then, pharmaceutical companies have altered the natural products by adding functional groups to the original compound. Usually, these synthetic medications last longer in the body and fewer doses are required. We are going back to the original plan in an effort to identify novel substances that have potential as antibiotics to fight the ESKAPE pathogens.
A soil sample taken from Tacoma, Washington was examined by performing serial dilutions to isolate soil colonies. Isolates averaged 7.625×10^7CFU/g. Twenty-four morphologically different colonies were

chosen from dilution plates for further study. These isolates were plated on separate Mueller-Hinton agar plates and challenged by toothpick inoculations with each of the 6 ESKAPE pathogens. Following 48 hours of incubation, plates were examined for the evidence that the ESKAPE pathogen growth was suppressed. Of these 24 colonies, 7 displayed zones of inhibition against one or more of the ESKAPE pathogens. Organism 2 showed consistent inhibitory effects for all ESKAPE pathogens. These seven isolates were further analyzed by microbial staining and biochemical testing.

U9. Resistance Selection in the Microbiome by Various Antibiotic Treatment Regimens
John B. Pinard, Jeanette M. Carlson, and Todd P. Primm
Sam Houston State University

Antibiotic resistance is an ongoing battle for medicine, and seriously challenges our ability to control infections. Resistance in pathogens is well studied, but how organisms of the normal microbiome relate is unclear. Antibiotic therapy can cause side effects such as diarrhea and enterocolitis by depletion of the microbiome. 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. A major advantage of our fish model is that the skin is mucosal, giving a system that is easy and non-lethal to sample. We utilized the Gram negative *Edwardsiella ictaluri* as a model pathogen to determine antibiotic regimen effectiveness. Previous data has shown that after three days of exposure to the broad-spectrum antibiotic rifampicin, the bacterial skin community composition is strongly altered (determined by 16S profiling) and assumed over ninety-percent resistance (measured by plating). We hypothesize that altering the treatment regimen will affect resistance rates.
Regimes being investigated include pulsing the antibiotic in an on-and-off manner, alternating between two antibiotics, or dual concomitant therapy. Culturable resistance rates are monitored using plating, and genetic community diversity by Ribosomal Intergenic Spacer Analysis. Preliminary data shows lower resistance rates using the pulsed method (alternative two-day periods of antibiotic or resting) with rifampicin, although community composition is still altered. This project seeks to optimize antibiotic treatment by including how it affects the normal microbiome.

U10. Determining the Carriage rate of *Staphylococcus areus* Among Healthy Undergraduates Enrolled in a Microbiology laboratory Course
Pungwe Prisca
Baylor University

Methicillin Resistant *Staphylococcus aureus* (MRSA) is a bacterial pathogen commonly associated with skin and soft tissue infections; however, it has also been known to causing osteomyleits, bacteremias, and pneumonia. According to the CDC 25-30% of the population carries *Staphylococccus aureus* in their nasal passages, only 1-3% are MRSA. These statistics prompted our research. The objective of the study is to compare the CDC's statistics with the carrier rate of *Staphylococcus aureus* among undergraduate students enrolled in a microbiology lab course. Testing for the presence of *S. aureus* included assessment of mannitol fermentation, Gram staining characteristics, coagulase and catalase production. Gram positive staphylococci that fermented mannitol, produced catalase, and had a positive tube coagulase test were considered to be *S. aureus*. In addition, the Kirby-Bauer disk diffusion method was used to determine the antibiotic sensitivity of *S. aureus* to penicillin, doxycycline, oxacillin, azithromycin, ciprofloxacin, and trimethoprim/sulfamethoxazole). Students tested included 309 participants from BIO 1402 and BIO 4401. Of the students tested, 18.1% were carriers of *Staphyloccoccus aureus*. Antibiotic susceptibility of MSSA (Methicillin –Susceptible *Staphylococcus aureus*) cultures was determined. None of the students harbored MRSA. All students completed a survey to determine whether factors such as shared bathrooms, contact with young children, travel, or participation in contact sports might impact their carriage rate.

U11. Protein tyrosine phosphatase YwlE as a potential regulator of oxidative stress in *Bacillus anthracis*
Madison R. Rogan, Shauna M. McGillivray
Biology Department, Texas Christian University

Bacillus anthracis, the causative agent of anthrax and a Disease Category A priority pathogen, utilizes an array of chromosomally-encoded virulence factors to subvert the host immune response. After screening a pool of randomly generated *B. anthracis* mutants, we identified a mutation in the gene *ywlE* that rendered the bacteria more susceptible to hydrogen peroxide than the wild-type. In *Bacillus subtilis*, YwlE plays a role in modulating the stress response (Musumeci *et al.,* 2005). Therefore, we hypothesized that a disruption in this gene may decreae resistance to stress such as exposure to reactive oxygen species. Experiments investigating this hypothesis are currently in progress with an independent insertional mutant. Concurrently, we wanted to develop a model for *B. anthracis* infection that would be able to provide an *in vivo* demonstration of decreased virulence with the knock-out of YwlE. The nematode *Caenorhabditis elegans* is a commonly used model, especially when access to a mouse model is limited, but a variety of manipulations that must occur in order to establish infection make it a poor representation of a mammalian host. The larva of the greater wax worm *Galleria mellonella* has shown promising results as an infection model for other human bacterial pathogens, such as *Staphylococcus aureus* (Ramarao *et al.*, 2012), leading us to test its usefulness in *B. anthracis* studies. However, I show that infection with *B. anthracis* strains of varying degrees of virulence does not correlate with *G. mellonella* survival rates, confirming that this is not an effective model organism for studying *B. anthracis* virulence. Ultimately, with further investigation into role of YwlE, I hope to elucidate a key player in the stress response of *B. anthracis*.

U12. Effects of Mouse Cytomegalovirus Infection in Lipid Synthesis
Renet Roy and Dr. Laura Hanson
Texas Woman's University

Cytomegalovirus is a large, host-specific member of the herpes virus family. Human cytomegalovirus (HCMV) has been clinically linked to atherosclerosis but the mechanism is unknown. Although we know the infection increases several pathways of lipid synthesis in human fibroblasts, the role in atherosclerosis has not been evaluated. Since HCMV only infects humans, mouse cytomegalovirus (MCMV) is a common model. Since MCMV also promotes atherosclerosis it the natural host it should be a good model to study the importance of lipogenic factor induction in this pathology. Our hypothesis was that MCMV infection in fibroblasts, like HCMV, up-regulates the genes inducing lipid synthesis and that this plays a role in atherosclerosis. This project focused specifically on HMGCoA reductase in the cholesterol pathway, which was consistently increased upon HCMV infection in fibroblasts. Using reverse transcription and quantitative PCR and by evaluating the results with delta-delta Ct analysis, we found that MCMV does not up-regulate the HMGCoA reductase lipid synthesis transcription in fibroblasts. This means that induction of lipogenesis in MCMV fibroblasts is not contingent on an increase in cholesterol production. We are examining the expression from this gene in other types of cells, such as salivary gland cells, (SGC-1) infected with MCMV. If MCMV does not up-regulate HMGCoAR in important target cells, it will show that not only is this pathway not required for CMV replication, and this pathway at least cannot be required for CMV promotion of atherosclerosis. This would help us look into and target other genes and other pathways of lipid synthesis.

U13. Accessory gene regulator polymorphisms in *S. aureus* may play a role in varied responses to blue light inhibition
Sarah Yuen
Baylor University

Staphylococcus aureus is a Gram-positive pathogen responsible for hospital and community-acquired infections, ranging from minor to serious and even deadly infections. The growing resistance of strains to antibiotic treatment, such as Methicillin Resistant *Staphylococcus aureus* (MRSA) leads to an increased difficulty in treating "staph" infections and the need to explore alternatives to antibiotic treatment. One viable alternative is 470 nm blue light photoinactivation, however the mechanism of blue light's bactericidal effect has yet to be elucidated. In addition, significant differences in the response of strains to blue light treatment reveals concerns regarding the development of resistance to this method of treatment as well. Previous work identified differential gene expression in the *S. aureus* transcriptome after treatment with blue light. This study analyzes the diversity of those genes, in an effort to further determine a genetic basis for the differing responses of strains to blue light inhibition. Each of the identified genes

was compared among 11 different strains, looking for variations among the amino acid sequences using BLAST multiple alignments and Scorecons. One group of genes that we found to have high diversity was the Accessory Gene Regulator (*agr*) operon. The role that *agr* plays as well as the fact that it has also been implicated in the literature to be a mutational hot spot with high variability makes *agr* a gene of interest and will be the subject of further studies in our lab group.

U14. Humanized Gnotobiotic Model of *Gambusia affinis* Via Depletion of Gut and Skin Microbiome, and Inoculation with Human Microbiome in Human-Body-Like Conditions.
Oscar Chavez
Sam Houston State University

The majority of modern biomedical research has been carried out using model organisms. In the areas of microbiology and immunology, gnotobiotic (where the microbiome composition of the host is known and controlled) models have been particularly useful, given the enormous complexity of the natural biota (humans have >1,000 bacteria species in their gut). We have established the fish *Gambusia affinis* as a vertebrate model of mucosal microbiomes. This small inexpensive organism allows simply study of the gut microbiota and host interactions. It was accidentally discovered that the normal gut microbiome of *G. affinis* is severely depleted (>3 log loss of plate counts) after a 4-5 day starvation period. Further, we have developed a system to orally dose fish with defined quantities of bacteria or drugs. This will allow inoculation of starved fish with mixtures to generate gnotobiotic organisms. Current work is focused on applying the gnotobiotic system to generate a humanized animal. Human feces are used as the inoculum, followed by fish incubation at 37 degrees. This system can be used to explore the persistence of donor communities following fecal microbiome transfer, a therapy being currently applied to treat irritable bowel diseases in patients.

U15. Determining the effect of *clp*A in the natural transformation ability of *Aeromonas salmonicida*
Kristen Clemons and Dr. Jennifer Huddleston
Biology Department, Abilene Christian University

The genus *Aeromonas* is ubiquitous in water and can be identified as Gram negative, rod-shaped, facultatively anaerobic bacteria. These bacteria are opportunistic extraintestinal and intraintestinal pathogens that can cause disease year-round with varying severity. *Aeromonas salmonicida* is a naturally transformable species. Natural transformation is a six-part process that results in a bacterium incorporating free DNA into its genome. It is possible that aeromonads can become antibiotic resistant through this mechanism. The proteolytic molecular chaperone ClpA has been indicated as affecting the natural transformation process in the genus *Aeromonas.* In this investigation, the effect of ClpA on the natural transformation ability of *Aeromonas* was studied through the creation of a *clp*A mutant fragment in which *clpA* was replaced with a gentamycin resistance cassette. This fragment was assembled by using PCR and SOE-PCR. Having constructed the mutant fragment, the project is now focusing on the gene replacement in the wild-type *Aeromonas* with the intention of making a comparison between the wild-type and mutant strains which will enable the determination of the effect of ClpA on the natural transformation ability of *Aeromonas salmonicida.*

U16. Bacterial Evolution: Phenotypic and Genotypic Analysis of Strains
Donghyoek Jung and Robert Jonas
Texas Lutheran University, Seguin, TX

We have cultured the soil bacterium *Bacillus subtilis* in four separate serial passages for over 200 days in some cases, and now present data regarding changes in the phenotype and genotype of the four separated strains.
To investigate the phenotype of growth rate, the strains were grown in liquid media with various types of carbon sources.
To investigate the genotypic changes that may have occurred, we attempted to clone fragments of chromosomal DNA in order to get the sequence of the DNA and compare it to the wild-type *B. subtilis.*

U17. Is the Amount of Programmed Cell Death Affected by Genetic Diversity in Populations of *Chlamydomonas?*

Allyson R. Keathley, Matthew R. Breuer, and Anne R. Gaillard
Sam Houston State University

Chlamydomonas is a single-celled eukaryotic alga that is commonly used for experimental studies. Here, we use *Chlamydomonas* to study programmed cell death, or PCD. PCD is the systematic, controlled death of cell, which may allow for the death of one cell to benefit the other cells around it. While it is known that *Chlamydomonas* undergoes PCD, and that PCD can provide benefits to neighboring cells, it is not known whether genetic relatedness of a cell culture influences a cell's probability of undergoing PCD. We hypothesize that higher rates of PCD will occur in a genetically identical population compared to a genetically mixed population. A higher rate of PCD in a genetically identical population would mean that *C. reinhardtii* cells could somehow detect the genetic relatedness of the cells around them, and selectively undergo PCD when kin cells are present. To test this, two different species of *Chlamydomonas* were subjected to heat stress to induce PCD. To quantify the amount of cell death in these heat stressed cultures, Evans blue dye was used to detect dead cells. Preliminary data shows that *C. eugametos* exhibits a much lower rate of PCD in a mixed culture with *C. reinhardtii* compared to an axenic culture of *C. eugametos* alone, providing support for our hypothesis. To test whether PCD releases substances that are more beneficial to kin compared to genetically unrelated cells, supernatant from heat stressed cultures was removed and added to new, living cells. The absorbance of each solution was taken daily to measure the amount of cell growth over time, and preliminary data has shown some interesting results.

U18. A preliminary study of shifting skin bacterial communities during human cadaver decomposition in southeast Texas

Zachary T. Lueck, Dalton A. Plummer, Lauren R. Smith[1], Daniel P. Haarmann[1], Joseph F. Petrosino[2], Sibyl R. Bucheli[1], Aaron M. Lynne[1]
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Decomposition is a 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 community structure of internal body sites, 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 oral and fecal regions of six cadavers were sampled by internally swabbing the left inner cheek and externally swabbing the rectum 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 1.7.0. Samples were grouped according to body site, cadaver of origin, and accumulated degree hours. Initial results suggest different microbial communities before and after purge. 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.

U19. Diversity and Geographic Distribution of Truffles in Texas

Glenda Mateos
University of Houston Downtown

Truffles (*Tuber* spp.) are ectomycorrhizal fungi that grow in association with roots of various hardwood trees and shrubs. The underground fruiting bodies of several truffle species are highly sought after in the culinary world and command prices ranging from $200 to more than $2000 per pound. Several edible *Tuber* species are known to occur throughout various regions of the U.S. including the Southeast states. To develop an understanding of the diversity and geographic and ecological distribution of *Tuber* species in Texas we are assessing the presence of this fungus in pecan orchards in the state's different vegetation zones and soil types in the eastern, central and coastal regions. Our initial aim is to assess the presence of *Tuber* species based on species-specific β-tubulin DNA sequences amplified from orchard soils using nested PCR. Of the 7 orchards tested thus far all have been shown to support an abundant and diverse community of *Tuber* spp. including *T. aestivum* which is among the highly regarded group known as the black truffles. No obvious trends in species diversity and distribution based on vegetation zones and soil types have been noted to date.

U20. Isolation and Partial Characterization of Bacteria from Lake Waco Abstract

Ashley Nguyen, Claudia Carvalho and Diane Hartman, DVM
Baylor University

The objective of this project was to isolate and characterize bacteria from three sampling sites near Lake Waco in Waco, Texas. Watery soil samples were collected in sterile containers and 20g of each sample was added to 180 mL of sterile water to form a 10^-2 dilution. Next, each sample was diluted to 10^-3, 10^-4, and 10^-5 by pipetting one mL of the previous dilution into 9 mL of sterile water. 0.1 mL of each dilution was spread plated onto two plates each of tryptic soy agar to obtain CFU/mL of sample, mannitol salt agar to select for gram positive halophiles, and salmonella shigella (SS) medium to select for gram negative organisms. The average viable bacterial count from each set of plates was determined. Speegleville Park had lower overall numbers and no growth on the SS medium compared to the other two sample sites. Next, three colonies from each sample site were selected and streaked for isolation. The isolates were Gram stained to determine their basic morphology. All isolates were gram positive rods, except for Colonies 1 and 7, which were gram negative rods. Colony 2 isolates contained terminal spores. The organisms were tested for the production of catalase and cytochrome oxidase c. Biochemical tests used included triple sugar iron agar, SIM deeps, OF glucose deeps, and nitrate broth. None of the isolates produced H_2S or indole. Colony 6 produced gas in a TSI slant. Only Colony 6 was motile as indicated by the SIM deeps. Colonies 1, 2, 3, 4, 7, and 8 were positive for nitrate reduction. Organisms were tested for their ability to ferment the following sugars: mannitol, lactose, glucose, and sucrose. The partial characterization of organisms from Lake Waco sites estimates the number and relative diversity of organisms that each environment supports.

U21. A preliminary study of shifting skin bacterial communities during human cadaver decomposition in southeast Texas

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Decomposition is a 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 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 six cadavers was sampled by externally swabbing the right cheek, right bicep, and torso 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 1.7.0. Samples were grouped according to body site, cadaver of origin, and accumulated degree hours. Initial results suggest different microbial communities before and after purge. 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.

U22. Isolating *Aeromonas* from Precipitation and the Atmosphere

Kathryn Preston and Dr. Jennifer Huddleston
Biology Department, Abilene Christian University

Aeromonas is a ubiquitous, Gram-negative, rod-shaped, oxidase-positive anaerobe. Aeromonads are emerging human pathogens associated with extra-intestinal infections after coming into contact with or consuming contaminated water or food. Though there are various biological particles that are known to be in the atmosphere, microbial communities are poorly characterized at high altitudes and in air masses. Bacteria have the ability to remain suspended in the air for prolonged periods of time and transmit diseases through both aerosolized “airborne” and “droplet” means. Various precipitation samples were taken in Abilene, TX with a sterile sampling jar and plated onto *Aeromonas* blue medium plates with and without ampicillin. Colonies were subcultured onto plates and then tested for the presence of oxidase. 16S rRNA DNA sequences were amplified from oxidase-positive, Gram-negative rods and then sequenced. After receiving results, sequences were analyzed using two online databases: NCBI BLAST and RDP. No isolates appeared to belong to the genus *Aeromonas*. There were various other bacteria found. There are many reasons why *Aeromonas* was not found in the samples: 1) the lack of a proximal lake or stream as an initial source of Aeromonas prevents detection of these types of cells, 2) *Aeromonas* may not be able to survive in the atmospheric or wind conditions of the study, 3) *Aeromonas* may be present in the precipitation but the methods were not sensitive enough to detect them, and 4) the genus may not be present at all in precipitation. Though Aeromonas was not found, many other genera of rod-shaped bacteria were. Future research for this study include expanding the collection site to sites near aquatic environments and other areas outside of Texas, collecting different forms of precipitation and at different seasons of the year.

U23. Microbial Contamination of Private Water Wells

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There are more than 1,000,000 private water wells in Texas. Due to issues with well design, construction, and/or location, some of these wells may be at elevated risk for exposure to contaminants such as nitrates, salinity, fecal coliforms, and *Escherichia coli from* failing septic systems, agricultural and residential runoff, and other sources. If a contaminant reaches groundwater, it may adversely affect the health of residents who obtain their drinking water from private wells. The Texas A&M AgriLife Extension Service provides water quality education programs and contaminant screening for samples from private water wells across Texas as part of the Texas Well Owners Network program. During recent testing in Round Rock, San Antonio, Seguin, Navasota, and Starr County, 158 water samples were tested for the presence of *Escherichia coli* using Colilert®. Of those 158 samples, eight were positive for total coliforms and *E. coli*. Follow-up samples were available from six locations and were collected and enumerated for

total coliforms and *E. coli* using IDEXX® Quanti-Trays®. Total coliform concentrations ranged from 40 *to 2420* MPN (most-probable-number)/100 mL, and *E. coli* concentrations ranged from <1 to 3 MPN/100 mL. These samples are currently being tested using PCR assays for source-specific *Bacteroidales* markers to determine the source(s) of the microbial contaminants. This information will allow for more precise recommendations to be given to these private well owners regarding methods for protecting their water sources from contamination.

U24. Microbial diversity and antibiotic production in Sorcerers Cave

David Sanderson and Dr. Jennifer Huddleston
Biology Department, Abilene Christian University

Access was granted with express permission from the landowner to a deep private cave that previously had limited exploration and no conducted microbial research. Cave microbiology is a relatively new field that possesses many environments previously unknown to contain microbes and has the potential for new drug discovery. The goals of this study where to find new species of bacteria as well as antibiotic-producing bacteria. The samples where aseptically taken from deposits on speleothems (cave formations), pools of water deep in the cave, and from bat guano. The samples were pulverized, diluted in a 0.85% NaCl solution, and plated onto various types of growth media, including 0.01X, 0.1X and 1X tryptic soy agar. The colonies where then transferred by replica-plating onto fresh plates of media and then overlaid with *E. coli* and *S. aureus* in 0.7% soft agar to screen for the presence of antibiotic production from the cave microbes. A preliminary investigation indicated the presence of antibiotic-producing bacteria.

U25. A5 Double Mutant Strain of *Chlamydomonas reinhardtii* Lacks the I1 Dynein Arm and has a Defective A-Kinase Anchoring Protein (AKAP)

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Chlamydomonas reinhardtii is a single-celled phosynthetic green alga that phototaxes toward light by way of two flagella located at its polar end. Both the overall structure and function of these flagella are highly conserved among eukaryotic organisms. We hypothesized that a mutation that inhibits the assembly of the I1 dynein arm found in *ida-1* is epistatic to the mutation found in *388* that causes a mis-regulation of cAMP dependent protein kinase (PKA). In this experiment we compared three known strains of *Chlamydomonas*: *cc-125* (wild type), *388* (deficient in PKA regulation), and *ida-1* (lacks I1 dynein arm), and a newly generated double mutant, *A5* (*388* x *ida-1*), and examined two key proteins of interest: IC-138 which is a phosphorylated protein involved in dynein driven microtubule sliding and RSP3 which is an A-Kinase anchoring protein (AKAP) responsible for regulating PKA. To test our hypothesis, several motility experiments were performed to observe the overall motility of each strain under a microscope, including measuring swimming distance and speed. Results from these analyses demonstrate that disruption of PKA anchoring has no effect on motility when the I1 dynein arm is missing. These results confirm the presence of epistasis between *ida-1* and *388.*

U26. Analysis of Grape Cultivars for Pierce's Disease Resistance with a *Xylella*-specific QRT PCR Probe

Reyna Valdez, Csilla Buday, Amanda Markham and Lisa Morano,
Department of Natural Sciences, UHD

Pierce's Disease (PD) is a plant disease of grapevines caused by the gram negative bacterium *Xylella fastidiosa*. This plant pathogen causes a blockage in xylem vessel preventing water flow and killing the vine. *X. fastidiosa* is transferred by an insect vector and both the vector and the bacteria are found at particularly high levels along the Texas Gulf Coast. One strategy to deal with this deadly disease is to plant PD resistant plants. Texas AgriLife Extension scientists have planted an experimental vineyard in Industry, Texas with grape varieties bred by scientists in Texas, Florida and California that may have PD resistance. PD resistant vines should maintain low levels of *X. fastidiosa*. The objective of this study is to evaluate grape isolates for the levels of *X. fastidiosa* using both ELISA and QRT PCR. The ELISA

method requires a high bacterial concentration for a positive result. QRT PCR can be a more sensitive technique but is difficult to optimize. Here, we show results of a *X. fastidiosa* specific probe that shows promise for analyzing *X. fastidiosa* samples from the vines in the experimental vineyard.

U27. Using SSR Fingerprinting to Evaluate Genetic Diversity of *Xylella fastidiosa* in Texas

Sara Valliani, Saima Valliani and Lisa Morano
Department of Natural Sciences, UHD

Pierce's disease (PD) in grapevines is caused by *Xylella fastidiosa*, a Gram-negative bacillus that grows in the xylem and is typically fatal within one year. PD is a problem in Texas, limiting the expansion of the state's grape industry. One of the critical issues to be understood is how PD moves from vineyard to vineyard and from one region of the state to another. To address the epidemiology of PD spread we plan to analyze 8 distinct small sequence repeats (SSR) from *X. fastidiosa* cultures extracted from vineyards in different counties. SSRs change more rapidly than other areas of the genome and can help us determine how bacteria spread on short time scales (months, years). Our main goal is to compare eight different *X. fastidiosa* isolates from Texas counties and create a cluster analysis to better understand how isolates move between counties. DNA will be extracted from cultures, and a cluster diagram of genetic variability built. Rather than estimating the size of SSRs with gels we are using the melt temperatures from the QRT PCR reactions for each SSR. We plan to use this method with all 8 isolates to address our epidemiological questions.

U28. 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 a male cadaver to a female cadaver placed at the same time. Two 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. Both 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 a single sample cohort such as this, this comparison can serve 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.

U29. Identification of Putative Proteins Interacting with Lipid Droplet-Associated Proteins Using Two-Hybrid Yeast System

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Lipid droplet (LD) homeostasis and compartmentalization has been well documented in developing seeds because these tissues synthesize large amount of triacylglycerols (TAGs) for storage of carbon and energy for the growth after seed germination. However, most leaf cells synthesize and store small amount of TAG also. Increasing TAG in the vegetative biomass will increase the total amount of TAG, an important step in the development of biofuels from plant leaves. Recently, we identified a new class of lipid droplet-associated proteins (LDAPs) that target specifically to LDs in non-seed cell types, including in Arabidopsis where three isoforms of LDAPs present (i.e., AT1G67360, AT2G47780, and AT3G05500). To gain a better understanding of the function of LDAPs, we preformed Arabidopsis library screening using yeast two-hybrid system with AT1G67360 protein as bait. We identified proteins interacting with AT1G67360, such as LPXC1 (UDP-3-O-acyl N-acetylglycosamine deactetylase activity), which is involved in lipid biosynthesis and metabolisms, calcium-dependent lipid binding protein, and proteins with unknown functions but specifically and highly expressed in matured seeds.

U30. A preliminary study of seasonal effect on bacterial communities during human cadaver decomposition in southeast Texas

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Decomposition is a 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 six 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 1.7.0. 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.

U31. The Role of RecA in the Natural Transformation of *Aeromonas salmonicida*

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The genus *Aeromonas* is a gram-negative group of bacteria that are ubiquitous in various fresh water bodies. *Aeromonas* has also been found to be an agent of several gastrointestinal diseases and are often opportunistic pathogens. A characteristic of *Aeromonas* that has recently been discovered is natural transformation, which is the ability to take up environmental DNA and incorporate it into the bacterium's genome. However, natural transformation has only recently been found to occur in the species *Aeromonas salmonicida.* Preliminary studies indicate that the gene *recA*, along with several other genes of interest, might be necessary for natural transformation to occur in this species. The gene *recA* encodes a DNA repair protein that is activated in the presence of DNA fragments and assists in the ligation of the free DNA ends. In order to investigate the role of *recA* in natural transformation of *A. salmonicida*, we will knock out the *recA* gene with a gentamycin-resistance cassette through mutation with a process called splice overlap extension PCR (SOE-PCR). Then, screening of the mutant species will occur to detect a lost ability to transform free DNA, thus indicating *recA*'s role in natural transformation in *Aeromonas salmonicida.* This research is part of a larger investigation of multiple genes that preliminary studies suggest might be involved in natural transformation of this species.

U32. Isolation and Annotation of Mycobacteriophage 'Scorpia' and Investigation of Lysin-Producing Genes

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The study of bacteriophage is beneficial to the medical environment because of the increasing risk of antimicrobial resistance in bacteria. Bacteriophage naturally evolve with their bacterial host, making the evolution of bacterial resistance to phage difficult. *Mycobacterium leprae* and *M. tuberculosis* are pathogenic bacteria that show high resistance to antimicrobials in hospitals. 'Scorpia' is a mycobacteriophage that infects *Mycobacterium smegmatis,* a species similar to *M. leprae* and *M. tuberculosis*. Using host bacteria similar to these two microbes can serve as a model system to study the use of bacteriophage to treat these diseases. In this experiment, 'Scorpia' was isolated, purified, and annotated in order to identify gene sequence and functions. Genes 8 and 10 code for Lysin A and B, respectively. These enzymes are responsible for attaching to and lysing the cell wall of the host bacteria. We hypothesize that Lysin A and B are able to lyse the bacteria and make it difficult for the host to become resistant.

U33. Isolating and Characterizing a *Streptomyces griseus* Bacteriophage: Telomer

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University of North Texas

Streptomyces griseus bacteriophage Telomer was isolated during the fall of 2014 from a soil sample collected at Goolsby Chapel on the campus of The University of North Texas. It presented itself as a unique bacteriophage because of its differentiated plaque morphology, which led us to name it Telomer after the immortality enzyme telomerase. Telomer has a high titer lysate of $1.4x10^7$ pfu/mL from which 14.4µg of DNA was purified and isolated. Telomer has a very long tail; it is approximately 179.162 nanometers, based on the measurement from its electron microscopy picture. Telomer is currently waiting to be sequenced, and once it is sequenced it will be annotated. The possible unique genome could aid in future research.

U34. Investigating Tail Structures of the Novel Bacteriophage 'Scorpia'

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In the realm of microorganisms, bacteriophage play a necessary role in the balance of our environment. Phage do this by infecting bacterial hosts involved in these processes and, thus, indirectly regulating the global carbon and nitrogen cycles in our biosphere. Bacteriophages or phages are viruses that infect and

destroy bacteria. 'Scorpia,' a novel bacteriophage was isolated from a soil sample using the host *Mycobacterium smegmatis*, purified, and characterized. We then annotated the sequenced genome and analyzed the gene functions to gain a better understanding of 'Scorpia' genome, particularly the tail structure. Bacteriophages inject their own DNA into bacteria using their tails. We hypothesize that the structure of the tail, which can vary among mycobacteriophage that infect *M. smegmatis*, may provide insight into the phage mechanisms of infection.

U35. OlympicHelado: UNT-HHMI PHAGES discovery
Isabel O. Delwel, Jazmine E. Rosado, Sonya Layton, Swapan Bhuiyan, Dr. Robert C. Benjamin, Dr. Lee Hughes
University of North Texas, Howard Hughes Medical Institute

Bacteriophages are viruses that infect bacteria. Soil was collected and enriched using Streptomyces *griseus*. The bacteriophage, OlympicHelado, was isolated, purified, and characterized. It grew extremely quickly and was simple to handle. Upon isolation, sequencing, restriction digests, it is clear that OlympicHelado is a highly unique lytic phage.

U36. Isolation and Annotation of the Novel Bacteriophage 'Scorpia' and Investigation of HNH Endonuclease
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Bacteriophages are the most numerous viruses on Earth. These viruses have a unique property: they attach to and infect bacteria. Understanding the basic structure and function of bacteriophages is essential to understanding how these life forms interact with bacteria. By isolating and annotating 'Scorpia,' a cluster A5 novel bacteriophage, students at Del Mar College have identified many significant genes within its genome. Two bioinformatic programs, DNA Master and Phamerator, were used to annotate the 'Scorpia' genome. HHPred was then used to assign structure and function to the identified proteins. Of particular interest is gene 1, which encodes an HNH endonuclease, located at bp502-801. HNH endonucleases are small DNA binding and digestion proteins that function to cleave DNA and have been found to be important in viral genome packaging in some phages. We hypothesize that the HNH endonuclease in 'Scorpia' may be important to the ability of this phage to proliferate and kill its host. The ability of bacteriophage to infect and kill bacteria has promising medical applications, such as treating antimicrobial-resistant strains of bacteria and as a new primary treatment against bacterial infections.

U37. Adapting the Bacteriophage Recombineering with Electroporated DNA (BRED) Procedure for Use with *Streptomyces* Phages
Joshua T. Bernal, Rainna E. Coelho, Thalia A. Kanani-Hendijani, Zane A. Gibbs, Richard Donegan-Quick, Lee E. Hughes

Bacteriophages represent the largest population of genetic entities in the biosphere with an estimated 10^{31} viral particles present on earth. Phages have been used to help understand the fundamental biology of many species of bacteria, including several species of *Streptomyces*. The phages that infect *Streptomyces* have likely undergone host-specific evolutionary changes due to the unique development cycle of streptomycetes. Bacteriophage recombineering with electroporated DNA (BRED) has been used to alter the genomes of mycobacteriophages in order to study them further; however, BRED has yet to be successfully adapted for use with *Streptomyces* phages. We will attempt to perform BRED on the phage Xkcd426, which was isolated using *Streptomyces griseus* subs. *griseus* (ATCC 10137) as host. We will knock out two separate genes, the first by introducing a point mutation that will result in early termination and the second by a 225 base pair deletion. PCR will be used to screen for the deletion and a restriction enzyme digest will be used to screen for the point mutation.

U38. Analysis and characterization of Arthrobacteriophage genomes
Tessa Merritt

Baylor University

Research in bacteriophage genomics has recently become more widespread as researchers realize the insights bacteriophages endow in this area. Arthrobacteriophages, a class of viruses specific to *Arthrobacter species*, include both temperate and virulent types. In the fall of 2014, 24 Baylor University biology students isolated, purified, and characterized 22 Arthrobacteriophages from soil samples. Ten phage genomic DNA samples were submitted for sequencing. Here we compare four Arthrobacteriophages, Link, Courtney3, LeeroyJ, and Steve, that 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 four 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.

U39. Exploration of Genetic Factors Involved in Bacteriophage Temperate Life Cycle by Isolation and Annotation of Novel Mycobacteriophage Chupacabra
Sarah Mullenax, John Ramirez, Jamie Vulgamore, Clayton Speed, John Hatherill, and Daiyuan Zhang

Bacteriophages are viruses that survive by infecting and then replicating using a bacterial host's genetic machinery, which leads to the destruction of the host cell. Bacteriophages' diversity of attributes, while still being specialized to their host, appeals to the need for continuing research into their survival mechanisms. Bacteriophages and their host bacteria are locked in an evolutionary struggle that has led to the development of new defense mechanisms and new methods of infection. Given the rise in antibiotic resistant bacterial species, bacteriophages may offer a new alternative for combating bacterial infections. During this project, the novel bacteriophage, named Chupacabra was isolated and its genome was sequenced. Chupacabra belongs to the cluster A and subcluster A10 of bacteriophages and predates on *Mycobacterium smegmatis*. After culturing, Chupacabra was revealed to have a temperate life cycle. Each plaque was approximately 3mm in diameter. Classification was assisted by images taken from a Transmission Electron Microscope, as well as bioinformatic analysis of Chupacabra's genomic DNA. Chupacabra's capsid and tail were observed to be 60nm in diameter and 140nm long, respectively. The quality and quantity of DNA harvested from Chupacabra was measured through restriction enzyme processes. After DNA sequencing, Chupacabra's genome was determined to be 50,286 base pairs in length. Bioinformatic annotation of Chupacabra's genome revealed select genes were atypical when compared to other lytic cluster A bacteriophages. Hypothesis: It is assumed that key dissimilarities between Chupacabra's genome and that of typical lytic cluster A bacteriophages, contributes to Chupacabra's ability to vacillate between temperate and lytic life cycles.

U40. Annotation of Genes 46 and 48 of Mycobacteriophage 'Scorpia'
Nicole Edwards, Kimberley Kissoon, Clayton Speed, Jamie Vulgamore, and Daisy Zhang

Based on genomic architecture, bacteriophage are some of the most diverse life forms on Earth. Mycobacteriophage genomes are highly mosaic yet contain highly conserved genes that mediate their life cycle. This diversity presents itself once the genomes of phage are compared to each other. In this study, the bioinformatics program 'DNA Master' was used to determine the coding regions of the 50,695 base pair 'Scorpia's genome. 'HHpred' was used to determine the possible function of the 94 Open reading frames (ORFs). 'Phamerator' was used to compare the genomes of 'Scorpia' and its close relatives. Genes 46 and 48 are important for the lytic cycle of 'Scorpia' because they are both nucleotide metabolism related genes. Gene 46 codes for a thymidylate synthase, which is critical for DNA biosynthesis. Research into thymidylate synthase (ThyX) suggests its role in regulation of DNA replication. Gene 48 codes for ribonucleotide reductase (RnR), which regulates the rate of DNA synthesis that essential for cell proliferation. Both of these genes are necessary for the propagation of phage and the proliferation of Mycobacteria, including *M. tuberculosis*. This makes, ThyX and RnR both possible and likely drug targets in the development of treatment for pathogenic bacterial infections.

U41. Analysis of Streptomyces Phage Hydra

Naomi Niyahn
University of North Texas

Bacteriophage are viruses that infect bacterial hosts. The Streptomyces bacteriophage, Hydra, was isolated, purified, and characterized using techniques such as electron microscopy and restriction digest. Hydra was characterized as a lytic phage closely related to the R4 family.

U42. Investigation of the Function of Gene 73 in the Novel Mycobacteriophage 'Scorpia'
Katilyn Parker, Patrick McKinney, M.Clayton Speed, Jaime L. Vulgamore, R. Deborah Overath, and J. Robert Hatherill
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By breaking down detritus and carbon-containing compounds, soil bacteria are a necessary component of the global carbon cycle. Bacteriophage infections play a large role in determining the size of soil bacteria populations and the diversity of soil microbial communities. We isolated a novel mycobacteriophage called 'Scorpia' using the soil bacterium, *Mycobacterium smegmatis,* as a host. Phage isolation and characterization was performed at Delmar College and Illumina sequencing was performed at the Pittsburgh Bacteriophage Institute. We then annotated the phage genome using multiple bioinformatics tools, such as DNA Master and Phamerator. Through this analysis, we identified gene 73 as the gene *kaiC*, which codes for KaiC, a protein known to be involved in circadian rhythms. The function of *kaiC* in 'Scorpia' is currently unknown. Based on the proposed domains on Phamerator, we hypothesize that *kaiC* in 'Scorpia' is involved with the DNA replication process because of its association with the AAA domain and DnaB helicase. Understanding genes involved in phage replication will increase our understanding of how soil-born bacteriophage influence these microbial communities and their ecological functions.

U43. Annotation of the Novel Mycobacteriophage 'Scorpia' and Investigation of the Conserved Gene RecB-like Exonuclease
Reavelyn Pray, M. Clayton Speed, Jaime Vulgamore, R. Deborah Overath, Daiyuan Zhang, and J. Robert Hatherill

Phage are found virtually anywhere you find bacteria. We isolated a novel phage, 'Scorpia,' from soil in Corpus Christi, TX. 'Scorpia' is classified as a cluster A5 phage and is most closely related to mycobacteriophage 'Benedict'. Using DNA Master, HHpred, and Phamerator, we annotated the genome phage 'Scorpia,' identifying the locations of genes, as well as finding their functions. Cluster A5 phages like 'Scorpia' can have some of the same genes as their bacterial hosts. Certain functions, such as RecB exonuclease, can be found in both phage and host. By elucidating the mechanism of a RecB-like exonuclease in phage, we likely can give insight into the hosts' function. RecB is used to repair damaged DNA. We hypothesize that investigating the highly conserved genes found in mycobacteriophage 'Scorpia' will allow researchers to discover novel methods to fight bacterial diseases. In the future, we may target and knockout these genes, potentially dismantling a bacteria's ability to heal itself and increasing the efficacy of antimicrobial therapy by creating a synergistic effect with traditional antimicrobial therapy.

U44. Isolation and characterization of Mycobacteriophage 'Scorpia'
Stephanie Torres, Chelsea Miller, Clayton Speed, Jamie Vulgamore, and Daisy Zhang

Bacteriophages are the most abundant life form on Earth. Phage research allows for a dynamic insight into lateral gene transfer between viruses and bacteria. As the predators of bacteria, phage can help develop unique therapeutic methods to prevent and treat pathogenic bacterial infections. Although phage therapy has been extensively studied and utilized in other countries for years, the United States has recently renewed interest on this subject. In this study, bacteriophage 'Scorpia' was isolated from local soil sample. Plaque morphology results show that 'Scorpia' is a lysogenic phage. Later, transmission electron microscope (TEM) images 'Scorpia' strongly indicate classification as a 'cluster A5' phage. TEM results also show the capsid to be 60nm in diameter and the tail approximately 140nm long. The genomic DNA was isolated from this phage and later analyzed several times by restriction enzyme digests and was sequenced by ion-torrent technology. Bioinformatics tools were used to determine

protein-coding genes from the genomic sequence. The ‘Scorpia’ genome is ~ 50 KB and contains 94 potential protein coding genes and one tRNA gene. By using DNA Master and Phamerator, it is clear that ‘Scorpia’ shares similarities with several Mycobacteriophages such as ‘ElTiger69’, ‘Benedict’, and ‘Airmid’.

U45. The Annotation and Characterization of Gene 78 from the Bacteriophage ‘Scorpia’
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Phages are a class of viruses that infect bacteria and are arguably the most abundant life form on Earth. A typical phage has a hollow capsid that houses the genetic material and often binds to specific molecules on the surface of their target/host bacteria. In the medical field, there is an increasing research interest in the therapeutic use of phage.‘Scorpia’ is a novel bacteriophage isolated at Del Mar College via enrichment protocol with *Mycobacterium smegmatis*. The DNA from bacteriophage ‘Scorpia’ was purified and sequenced. The annotation performed using programs such as DNA Master, HHpred and Phamerator revealed that gene product 78 (gp 78) is a putative regulatory protein. We hypothesize that gp78 allows ’Scorpia’ to avoid destruction by evading the host machinery that targets the phage for degradation.

U46. Discovery and Analysis of the Streptomyces griseus bacteriophage Allonsy90
Katelyn Williamson
University of North Texas and Howard Hughes Medical Institute

Allonsy90 is a bacteriophage that was isolated from the soil collected on the UNT campus. It infects the bacteria, *Streptomyces griseus*. After being isolated, purified and analyzed using various techniques, Allonsy90 was determined to be a lytic phage that produces well defined plaques.

U47. The isolation and purification of Arthrobacteriophages
Jennifer Wilson
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 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 22 individual Arthrobacteriophages. These bacteriophages were discovered from soil samples collected from a variety of geographic locations by Baylor University students. Each soil sample underwent enrichment and plaque purification on plates containing *Arthrobacter*. The phages were characterized by isolating and purifying the DNA. The viral morphology was determined using transmission electron microscopy. The subsequent phage DNA was analyzed by restriction digestion using five endonucleases and gel electrophoresis. Further research into the genomes 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.

Graduate Student Posters

G1. Evolutionary Increase of Polysaccharide Production May Provide a Mechanical Fitness Benefit to Bacterial Biofilms

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Pseudomonas aeruginosa is a biofilm-forming opportunistic pathogen that chronically infects the lungs of Cystic Fibrosis patients. *P. aeruginosa* biofilms have enhanced resistance to clearance by antibiotics and immune cells. Bacteria within a biofilm are embedded in a matrix of extracellular polymeric substances made up of eDNA, proteins, and self-produced polysaccharides which, for *P. aeruginosa,* are Pel, Psl, and alginate. It has long been known that in the course of chronic infections in the Cystic Fibrosis lung, *P. aeruginosa* tends to increase alginate production, giving rise to a mucoid phenotype. Alginate provides chemical protection by sequestering antibiotics and reactive oxygen species. It was recently found that chronic infections in the Cystic Fibrosis lung also evolve to increase Psl production, indicating that Psl likely confers some adaptive benefit to *P. aeruginosa* in the lung. To determine if Psl might confer a mechanical benefit, we do bulk rheological measurements from pulmonary chronological clinical isolates of *P. aeruginosa.* We find that when production of Psl is increased, the biofilm is stiffer. The increase in Psl helps maintain the yield stress of the biofilm when there is increased alginate production; increased alginate production without increased Psl production results in a softer, weaker biofilm. We suggest that Psl could provide a mechanical fitness benefit that complements the chemical benefits of alginate. From others' estimates of the stresses that phagocytotic cells can apply, we estimate that the stiffening we measure could help biofilms avoid clearance by phagocytotic immune cells. We will further investigate the interaction of biofilms with immune cells by subjecting gels of varying stiffness as well as biofilms with varying polysaccharide expression to phagocytic cells. This approach will evaluate bacterial evasion of the immune system from a mechanical standpoint, which is not commonly studied.

G2. Characterizations of Antimicrobial Resistance Phenotype and Genotypes in *Salmonella enterica* serovar Typhimurium Human Isolates

Dawn M. Fisher, Lauren R. Smith, Daniel P. Haarmann, and Aaron M. Lynne

Department of Biological Sciences, Sam Houston State University, Huntsville, Texas 77341 *Salmonella enterica* is gram negative bacterium that causes 42,000 cases of salmonellosis every year in the United States. Salmonellosis is commonly manifested as diarrhea, fever, abdominal cramps and some cases can end in death. Treatment for severe salmonellosis is with antimicrobial agents, and with the rise of antimicrobial resistance, treatment is becoming problematic. To assess the level of antimicrobial resistance, 90 *Salmonella enterica* serovar Typhimurium human clinical isolates obtained from Texas Department of Health were tested for antimicrobial resistance for 15 antimicrobial agents by disk diffusion method and also analyzed by PCR for the presence of 19 antimicrobial resistance genes. The phenotypic and genotypic profiles were then compared. Of the isolates tested 38.9% were resistant to one or more antimicrobial agents with resistance to streptomycin detected the most. Whenever a resistance phenotype was detected, a corresponding resistance gene was detected 36.7% of the time. To determine if antimicrobial resistance is on the rise, further evaluation is needed to track the trends of *Salmonella enterica* infections.

G3. α-Amylase Degrades the Extracellular Polymeric Substance of 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. In this study, we examined the efficacy of α-amylase, a naturally occurring enzyme that breaks down complex polysaccharides by hydrolyzing α-1,4-glycosidic linkages, to eradicate *Staphylococcus aureus* (Sa), *Pseudomonas aeruginosa* (Pa), and Sa+Pa co-culture biofilms in both in vitro and in vivo models. Treatment of Sa, Pa, and Sa+Pa biofilms grown in vitro with α-amylase in ddH2O resulted in significant reduction in biomass. Additionally, treatment of biofilms grown in wound-like media with α-amylase resulted in the dissolution of the biofilm and an increase in the effectiveness of antibiotic treatments. However, α-amylase applied topically to murine chronic wounds did not enhance the efficacy of gentamicin sulfate to eradicate bacteria. Current studies are focused on determining if the *in vivo* environment inactivates α-amylase, if there is an inhibitory effect of α-amylase against certain antibiotics, or if optimized biodelivery methods are required.

G4. Evolution of drug resistance in Methicillin resistant *Staphylococcus aureus*

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Staphylococcus aureus is a versatile pathogen, capable of infections in both human and animals. It can cause furuncles, septicemia, pneumonia and endocarditis. Although recently cases of community acquired *S. aureus* infections are increasing, most life threatening cases of *S. aureus* are associated with hospital acquired infections. Evolution of *S. aureus* to adapt to modern hospital environment has been through the acquisition of drug resistance genes like *mecA* gene which imparts resistance to methicillin. Drug resistance genes often arrive on large clusters of genes, referred to as “genomic islands”. Horizontal transfer of genomic islands harboring virulence and antibiotic resistance genes have made *S. aureus* resistant to commonly used antibiotics. In order to understand the evolution of antibiotic resistance in *S. aureus,* we used hospital associated, methicillin resistant *S. aureus* strains MRSA252 and N315. We used a gene clustering procedure based on Jensen Shannon entropic divergence to identify large regions with atypical composition, characteristic of genomic islands. Our method was not only able to almost accurately identify boundaries of known genomic islands like *sccmec* which harbors *mecA* gene but also predict previously unreported islands. These newly found islands, may likely contribute to drug resistance in *S. aureus.* Since genomic islands are often compositionally mosaic, we used the ability of our integrative methodology in deciphering the mosaic compositional structures of islands in MRSA252 and N315 strain and understanding the contribution of mosaicism in drug resistance. Further, we also analysed a newly sequenced MRSA strain, H29, isolated from a cow. Our method was able to identify novel islands harboring antibiotic resistance genes. Our method was thus able to decipher the promiscuous nature of *S. aureus* which has spawned several drug resistant strains.

G5. Differential Regulation of p53-Dependent Apoptosis by Cellular Factors and Modulation by the HTLV-1 Transactivator Protein Tax

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The human T cell leukemia virus type-1 (HTLV-1) is a complex oncoretrovirus that infects and immortalizes $CD4^+$ T-cells and causes adult T-cell leukemia/lymphoma (ATL), an aggressive lymphoproliferative malignancy usually associated with poor clinical outcomes. The HTLV-1 provirus encodes several regulatory and accessory factors, including the viral transactivator protein Tax. Tax is generally considered to be the major oncoprotein of HTLV-1 and regulates the expression of viral and cellular genes through interactions with a plethora of host transcriptional and signalling components. In addition to its central role in T-cell immortalization and oncogenic transformation, several studies have demonstrated that Tax also induces cellular senescence and apoptosis. In particular, the p300/CBP-binding domain of Tax, spanning amino acid residues 85-109, induces caspase-dependent apoptosis by titrating a limiting nuclear amount of the transcriptional coactivator, p300. *Importantly, the molecular mechanism(s) by which Tax-p300 interactions cause apoptosis remain to be determined.* The p53 tumor suppressor protein is posttranslationally modified in response to genotoxic or physiological stress, which differentially regulates the expression of p53-dependent pro-apoptotic or survival genes. p300 acetylates p53 on lysine residue K372 associated with the induction of cellular growth-arrest genes. Interestingly, the

methylation of lysine K372 by Set-7/9 methyltransferases recruits the chromodomain of the TIP60 acetyltransferase –which, in turn, acetylates p53 on lysine K120. The p53-K120-acetylation has been shown to differentially regulate the expression of p53-dependent pro-apoptotic genes. *We hypothesize that HTLV-1 Tax-p300 interactions may inhibit p53-K372-acetylation and lead to K372-methylation by Set-7/9 and preferential acetylation of lysine K120 by TIP60, associated with the induction of cellular apoptosis.* My project will test whether Tax-p300 molecular interactions modulate the posttranslational modifications of p53, and attempt to identify the cellular cofactors that differentially regulate the expression of apoptotic genes through p53-K120-acetylation.

G6. Mouse cytomegalovirus affects markers of neurodegeneration

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Cytomegalovirus (CMV) is a ubiquitous, species-specific herpesvirus, infecting 60-100% individuals worldwide. CMV infection has primarily been associated with pathologies in immunocompromised individuals but there is mounting evidence for a role in neurological disorders like Alzheimer's disease & glioblastomas. Due to its species-specificity, appropriate model systems are required to study human CMV (HCMV) pathogenesis. Mouse CMV (MCMV) is a useful model for HCMV as both have similar pathologies & MCMV expresses homologs of many HCMV proteins. A recent study showed HCMV infection induced amyloid-β in human fibroblasts. Expanding on this study, we analyzed markers of neurodegeneration; amyloid precursor protein (APP) and tau phosphorylation. In mouse fibroblasts, MCMV infection induced APP levels by 48 hours post infection (HPI). We saw an increase of tau-pSer396 by 24 HPI & it continued to rise. The total tau levels were also increased by MCMV infection. Similar studies were carried out in rat B35 neuroblastomas to test in more relevant cell type. APP levels were increased in these cells with different kinetics. Tau-pSer396 was higher in these cells to begin with, decreased early after infection & came back to original levels by 24 HPI. To elucidate whether these differences are species-specific, cell-type specific, or related to the transformation status of the neuroblastomas, same markers are being tested in mouse SGC1 cells and rat PC12 cells. Our results suggest that CMV infection is capable of inducing changes in markers of neurodegeneration.

G7. The Elk-1 protein could be involved in activation of the essential gene m142 of mouse cytomegalovirus via cellular factors.

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Worldwide 60 to 100% of adults are infected with human cytomegalovirus (HCMV). HCMV causes serious illness or death in immune-compromised people. In immune-competent individuals, there is clinical correlation between HCMV and atherosclerosis. HCMV can only infect humans, limiting the studies done with this virus. Mouse cytomegalovirus (MCMV) is a common model for HCMV due to similarities in pathology and sequence. However little is known about gene regulation in MCMV compared to HCMV. We are examining regulation of the essential MCMV gene, m142, which is related in sequence and function to HCMV IRS1 and TRS1. Using a series of sequential deletion mutants of the m142 promoter we identified a region -901 to -875 from the transcriptional start site which is required for activated in the absence of virus. This region contained consensus transcription factor binding sites for Elk-1, YY1, and CEBP, all of which have been reported to regulating the HCMV major immediate early promoter. Electrophoretic mobility shift assay using a probe for this region showed two shifted bands from infected or uninfected cell nuclear extracts. Mutation of the Elk-1 site, but not the overlapping YY1/CEBP sites led to failure to compete for binding by one of these complexes. Analysis for supershift with anti-Elk-1 antibody confirmed the presence of Elk-1 in this band. Thus Elk-1 is likely to be a factor in the basal activation of the MCMV m142 promoter, supporting similarities in regulation with HCMV immediate early promoters.

G8. Deciphering Horizontal Gene Transfer Events in *Galdieria sulphuraria* 074W

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Unicellular alga *Galdieria sulphuraria* is an interesting species owing to its characteristics like surviving on more than 50 different types of carbon media and adaptation to hostile environments like high heat, toxicity due to heavy metals, high acidity and hot spring water. These environmental adaptations can be attributed to the genes that were transferred horizontally to *G. sulphuraria* 074W during the course of evolution. Horizontally transferred genes are the genes inherited from the genome of a donor organism other than the parental genome. Genome of *G. sulphuraria* was analyzed by an integrative methodology of recursive segmentation and agglomerative clustering. The clusters thus formed revealed that almost 30% genome of *G. sulphuraria* is composed of alien genes. Protein BLAST of these genes revealed the donor organisms, most of which are proteobacteria and archaea. Most of these donor organisms are known to thrive in various hostile conditions due to their harboring genes that can metabolize various toxic elements.

G9. Late murine cytomegalovirus genes induce host lipogenesis regulators.
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Infection with human cytomegalovirus (HCMV), a common herpes virus, has a clinical correlation with atherosclerosis; however the molecular mechanism is not well understood. Since HCMV only infects humans, animal models are required to evaluate possible mechanisms and interventions. Murine cytomegalovirus (MCMV) is a useful model system since it has sequence similarity and the ability to promote atherosclerosis in its host. Thus, comparison of both the similarities and differences in activity of these viruses can lead to identification of likely mechanisms of viral promotion of atherosclerosis. Recently HCMV was shown to induce increased lipid synthesis in infected fibroblasts. Our hypothesis is that: lipogenesis induction by cytomegalovirus is a factor to promote atherosclerosis and that MCMV will serve as a useful mechanistic model. Using western blot analysis we found that, like HCMV, MCMV induced an increase an increase in an important regulator of lipogenesis, sterol regulatory elements binding protein-2 (SREBP-2) in infected fibroblasts. The kinetics of the increase indicated the effect might require viral products made late in infection, which was confirmed using phosphonoformic acid (PFA, foscarnet), which inhibits viral DNA replication which is required for viral late gene expression. However, we found no increase in SREBP-1, which was increased upon HCMV infection. As both viruses cause similar pathologies, further analysis of the similarities and differences between HCMV and MCMV can shed light on factors likely to be important in pathology.

G10. Community Succession and Stability in Mucosal Microbiomes
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It is known that the human gut microbiome composition shifts in response to patient treatment with antibiotics which can consequently have negative side effects on the host. This shift in the mucosal microbiome of the gut often allows opportunistic pathogens such as *Clostridium difficile* to rapidly increase in number and cause gastrointestinal damage via toxin production.
Our model system for studying the vertebrate mucosal microbiome uses the Western mosquitofish, *Gambusia affinis*. The skin of *G. affinis* contains a microbiome community that is adapted for life within a mucosal system, and is also directly accessible through the water in which *G. affinis* resides. This allows for non-invasive (to the fish) disruptions to the mucosal microbiome and a quick, efficient way to sample and analyze the changes occurring within the community. A major point-disruption, i.e. not prolonged, involved the serial rinsing of *G. affinis* until skin bacterial culturable numbers were greatly reduced. At various timepoints after the serial rinse, the skin microbiome of *G. affinis* was sampled and compared to an undisturbed, pre-treatment control sample. These samples were analyzed via biochemical assays, antibiograms, MPN, plating, and a genetic community profile method (Ribosomal Intergenic Spacer Analysis). A second, preliminary point-disruption included a 30 minute treatment of each fish in 20 µg/mL of chlorhexidine dihydrochloride, a commonly used skin disinfectant. Results show there is a drop in diversity immediately following a disruption, as well as an overgrowth phenomenon during the first 24 – 48 hours of recovery. Also, the stable, recovered mucosal microbiome community on the skin of *G. affinis*

is not identical in taxonomic makeup to the pre-treatment control sample, though the two microbiomes show the same metabolic activities. This suggests that metabolic pathways rather than taxonomic diversity drive recovery and stability of the microbiome composition.

G11. Salmonellae in the Intestines of *H. plecostomus* in the San Marcos River

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Heavy rainfall events have been associated with outbreaks of many waterborne diseases including salmonellosis. Salmonellosis is caused by members of the genus *Salmonella* that can enter water systems through sewage contamination, runoff after heavy rainfalls, or flow-through channels through manure fields after heavy rains or flooding. Currently, salmonellae are not closely monitored in regards to water quality. In this study, *Hypostomus plecostomus*, an invasive, algae consuming fish, was sampled from the San Marcos River (San Marcos, TX), the intestines analyzed for the presence of salmonellae by quantitative real-time polymerase chain reaction (qPCR) after semi-selective enrichment, and results related to precipitation for the river area. Salmonellae were detected in the intestines of *H. plecostomus* in 40-100% of the fish after precipitation events >12.5 mm, but was not consistently detected in environmental samples (i.e. water and sediments). This leads us to believe that *H. plecostomus* is ingesting salmonellae through their food sources and that the amount of salmonellae present in those food sources may be increasing after large rainfall events.

G12. Comparison of the microbiome of non-Calliphoridae flies and accompanying cadaver sites associated with human cadavers

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Bacteria are the primary decomposers of human cadavers but also important are invertebrate scavengers such as flesh flies. Understanding the interaction between bacteria found on cadavers and flesh flies may demonstrate variable bacteria community structure at different stages of decomposition responsible for attracting or repulsing flies and other insects. There are five stages of decomposition that a human cadaver can go through depending on the conditions of the body and the environment: initial decay, putrefaction, black putrefaction, butyric fermentation, and dry decay. Preliminary research suggests that each has a unique bacterial community structure that varies through time. To investigate the biodiversity of the microbiome of flesh flies associated with human decomposition, 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. To assess 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 accumulated degree hours. 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. Combining the disciplines of microbiology and forensic entomology may help form a more precise postmortem interval (PMI).

G13. A Preliminary Study of Shifting Bacterial Communities of the Face during Human Cadaver Decomposition in Southeast Texas

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The Human Microbiome Project brought attention to the community of organisms that live and thrive in and on the bodies of humans. While it is important to understand the microbiome as it relates to human health, it is just as important to understand once human life has ceased and the microbial communities are allowed to proliferate over the course of decomposition. The microbiome of human decomposition is an emerging aspect of forensic research and holds the potential of providing another model to estimate the post mortem interval. Preliminary studies have shown a shift in the communities across the varying stages of decomposition. One aspect left to be studied is whether samples that are taken vary temporally or spatially on a cadaver. In this preliminary study of the influence and shift in microbial composition of human decomposition, two 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. Both cadavers were allowed to decompose in a natural setting while external samples were taken at eighteen locations on the face in six-hour intervals for four days. Face samples were processed using 16s rRNA gene amplification on the Illumina MiSeq platform, following the protocol modeled by the Human Microbiome Project. The QIIME software, version 1.7.0, was used to analyze the data produced. The results show a temporal and spatial change in bacterial community structure. Overall, these results can be used to fine-tune sampling protocols in large-scale studies to more accurately sample for the changing diversity of microbes present on a decomposing cadaver and may strengthen the ability to more accurately determine the postmortem interval.

F1. The extracellular polysaccharide Pel promotes cyclic-di-GMP signaling by enhancing mechanosensing of surface-attached *Pseudomonas aeruginosa*

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Biofilms initiate when planktonic bacteria attach to a surface, sense the presence of the surface, and change their gene expression accordingly. The transition from a planktonic to a biofilm phenotype is associated with changes in the expression of myriad genes and is regulated by cyclic-di-GMP, a second messenger whose intracellular levels increase upon adhesion of *P. aeruginosa* to a surface. What specific cues notify bacteria that they are attached to a surface and cause increased cyclic-di-GMP production are unknown. This is a significant gap in our understanding of a fundamental microbiological process.

Here, we demonstrate that mechanical stress leads to increased cyclic-di-GMP levels. We use a GFP reporter for the *cdrA* gene, which other researchers have previously validated as a measure of intracellular cyclic-di-GMP levels. When we increase the flow rate of liquid media, and thereby increase the shear stress on single bacterial cells, the intracellular cyclic-di-GMP levels increase in a dose-response fashion. Moreover, at low shear stress we find that the presence of Pel enhances the cyclic-di-GMP signaling response upon surface attachment – populations of reporter wild-type (WT) and reporter *pel* have indistinguishable intensity distributions when in liquid suspension and at high shear rates, but *pel*. Motility measurements suggest that Pel may increase frictional interactions between the surface and the bacteria as they move using pili-driven twitching motility. To date, the role of Pel in PAO1 biofilms has seemed minor. We infer that a major role of Pel is helping cells to sense surfaces by increasing the mechanical coupling between the surface and the bacteria.

Addendum

Undergraduate Poster Presentations

U48. Investigating an ESKAPE to Nosocomial Infections

Kyra Curtis; Diane Hartman, DVM; Jacquelyn Duke, PhD.

ESKAPE pathogens are responsible for a wide range of nosocomial infections. This has resulted in an increased demand for new antibiotics to address the growing threat of these multidrug resistant bacteria. Since the 1940s humans have benefitted from substances naturally produced from in situ bacteria. Pharmaceutical companies have altered natural products by adding functional groups to extend the effects and minimize dosage. We are replicating the original protocols to isolate and identify novel substances to combat ESKAPE pathogens.

Organisms were isolated from a soil sample from Tacoma, Washington, and were tested against ESKAPE pathogens for evidence that pathogen growth was suppressed. Out of 24 isolated colonies, 7 displayed zones of inhibition on patch plates, and some showed greater potential for producing effective antibiotic substances based on patch plate results than others. Only one of the seven organisms consistently produced zones of inhibition, so gram staining and various media tests were performed on this organism in effort to identify its genus. A new sample from Waco, Texas was collected and analyzed through the same procedures. Similar tests were performed on the eight isolates to better identify the antibacterial-secreting colonies. The isolates were sent for PCR and DNA analysis. ID of these isolates and their subsequent antibacterial secretions could lead to the discovery of new antibacterial compounds and products against the nosocomial ESKAPE pathogens.

U49. “Aliens on Earth”
Elizabeth Andersen

Hospitals are swarming with bacteria that have the potential to infect unsuspecting workers, patients, and visitors. ESKAPE pathogens are a set of particularly virulent nosocomial bacteria. Although pharmaceutical companies offer cures for these ESKAPE pathogens, their antibiotics are gradually losing effectiveness due to their current inability to accommodate the mutations undergone by the ESKAPE pathogens. As a part of the Small World Initiative, instituted by Yale University, we seek to go back to nature in an effort to find more effective cures for these ESKAPE pathogens. This rationale is due to the fact that there is an array of biochemical compounds available in nature as a result of the great competition amongst bacteria. Samples were taken from several locations around Waco, Texas. Microbiology techniques were then implemented in order to isolate bacterial colonies and test the antibiotic potential of the aforesaid colonies against ESKAPE pathogens. The bacterial colonies that exhibited the greatest antibiotic potential are now undergoing PCR testing, gel electrophoresis and 16sRNA sequencing in an effort to identify the genus and possible species. Aliens are generally referred to as foreign species far off in space, but in reality our planet itself is saturated with a great abundance of unknown species waiting to be identified. With enough exploration these species may be identified and potentially contribute to helping cure many infections and disease that the modern human population faces. Thus our hope lies all around us, our hope lies in these aliens on Earth.

Graduate Poster Presentations

G14.

Berry, King, Haarmann, Bucheli, Lynne

Cadavers experience a shift in bacteria community structure as they progress through stages of decomposition. Bacteria associated with the wet stages of decomposition may be brought to the cadaver by flies, which are important members of the decomposition ecosystem and can be significant evidence in death investigations when the time since death is questioned. To investigate the biodiversity of the microbiome of flies associated with human decomposition, 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 first 40 flies visiting the cadavers were collected and submitted to dissection of the tarsi, labellum, and ovipositor. To assess 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 fly identity (genus and species), fly body site, cadaver of origin, and accumulated degree hours. These data are compared to bacteria samples taken from the mouth and anus of the cadaver at the time of collection. Special attention is paid to bacteria that have only been recorded in association with flies before. Sites show an abundance of Ignatzschineria and Wohlfahrtiimonas on the labellum, tarsi and oocytes. 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.