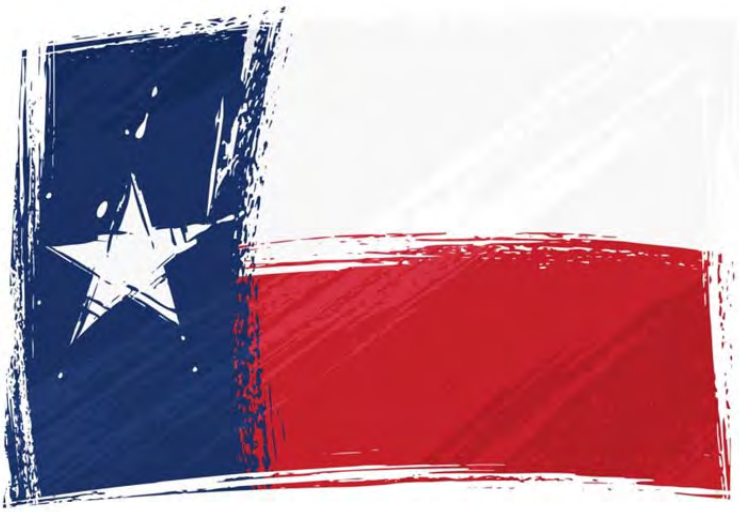
Texas Branch
AMERICAN SOCIETY FOR MICROBIOLOGY

# Final Meeting Program

## 2014 Fall Meeting

## November 6th – 8th, 2014



**Hosted by**

***The University of Texas Health Science Center at Houston***

***UTHealth Medical School***

***Houston, TX 77030***

# Texas Branch Fall Meeting of the American Society for Microbiology
# November 6-8, 2014

## *The University of Texas Health Science Center at Houston*
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## PROGRAM

**THURSDAY, NOVEMBER 6**

| | | |
|---|---|---|
| 12:45 - 3:00 pm | Free guided tour of NASA (limited spaces, tickets required) | |
| 4:00 - 7:00 pm | Registration | 2nd floor lobby |
| 6:00 - 7:00 pm | Reception | 2nd floor lobby |

7:00 - 9:15 pm **WELCOME** MSB 3.001

Heidi Kaplan, Chair, Organizing Committee
Theresa M. Koehler, Chair, UT-Houston Microbiology and Molecular Genetics
Kendra Rumbaugh, President Texas Branch ASM, Texas Tech (Lubbock)

**Cell-Cell Signaling (Danielle Garsin, UT-Houston convener)**

7:15 pm Charles Darkoh, UTHSC School of Public Health (Houston)
*Uncovering the role of quorum signaling in Clostridium difficile pathogenesis*

7:45 pm Marvin Whiteley, University of Texas (Austin)
*Decoding polymicrobial conversations using genomics and micro-3D printing*

8:15 pm Yi Xu, TAMU- HSC (Houston)
*Bacillus anthracis and the host complement system*

8:45 pm Daniel Ebbole, Texas A&M University (College Station)
*Characterization of a virulence factor gene family of the rice blast fungus*

**FRIDAY, NOVEMBER 7**

7:30 - 9:00 am **Continental Breakfast** 2nd Floor Lobby

8:00 - 9:45 **Student Oral Presentations**: MSB 2.103

O.B. Williams Oral Presentation Award in General Microbiology

8:00 am Malintha C. Abeysiri - University of Mary Hardin-Baylor
*Characterization and Weaponization of Imported Red Fire Ant Normal Gut Microbes*

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**Stephen Lory, Harvard Medical School**
***Evolution of Pseudomonas aeruginosa as a Human Pathogen***

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ASM Branches

## Houston, TX
## November 6-8

*Hosted by*

**The Microbiology & Molecular Genetics Department**
**The University of Texas Medical School at Houston**

# TEXAS BRANCH
# AMERICAN SOCIETY FOR MICROBIOLOGY
# 2014-2015


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*University of North Texas*
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Email: Lhughes@unt.edu


## Past Texas Branch ASM Presidents:

- James Stewart 1999-2001
- Karl Klose (University of Texas Health Science Center at San Antonio) 2001-2003
- Robert McLean (Texas State University at San Marcos) 2003-2005
- Heidi Kaplan (University of Texas Medical School at Houston) 2005-2007
- Poonam Gulati (University of Houston Downtown) 2007-2009
- Marvin Whiteley (University of Texas at Austin) 2009-2011
- Todd Primm (Sam Houston State University) 2011-2013

# ACKNOWLEDGEMENTS


**ORGANIZATION COMMITTEE**
**Heidi Kaplan, Chair**

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Daniel Garsin
Barrett Harvey
Nicholas DeLay
Lanny Ling
Nayun Kim
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Yi Dong

**Logistical Support**
Both the Microbiology and Molecular Genetics Department and the Dean's Office of the University of Texas Medical School at Houston have donated their time to making this meeting a success. Their contributions are gratefully acknowledged.

Dr. Lory's presentation is sponsored by the American Society for Microbiology.

**NASA:** We would like to thank NASA for organizing a 'once-in-a-lifetime' tour of the Johnson Space Center facilities. Special thanks go to: Lorraine Wheaton (DB Consulting Group), Jeannie Aquino and Drs. Mark Ott and Duane Pierson.

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SES 1.**Endothelial Cell Response to *Rickettsia massiliae* and *Rickettsia conorii* 'Israeli Spotted Fever'**

Jeremy Bechelli, Claire Smalley, Rong Fang, David H. Walker.
Department of Pathology, University of Texas Medical Branch, Galveston, TX

*Rickettsia massiliae* is a newly documented human pathogen that presents as a mild spotted fever rickettsiosis. The "Israeli spotted fever" strain of *R. conorii* causes a severe disease with a mortality rate up to 30%. Because little is known about the pathogenesis of either of these two bacteria, we hypothesized that *R. conorii* '*ISF'* and *R. massiliae* would induce a differential pathological response in endothelial cells, correlating with disease severity. Using plaque assays and quantitative PCR, we confirm that *R. massiliae* and *R. conorii* 'ISF' grow in similar kinetic curves in the HMEC-1 cell line with the same internalization rate. Size and time to appearance of plaques are larger and faster in *R. conorii* 'ISF' than in *R. massiliae*, 5 and 7 days, respectively. Following 48 hours post infection (HPI), *R. conorii* 'ISF' induced significant IL-8 and IL-6, and *R. massiliae* induced significant amounts of MCP-1 determined by ELISA. Cells infected with *R. conorii* 'ISF' demonstrated a significant increase in cell death and Annexin V staining at 72 HPI compared to *R. massiliae* infected ECs. We show increased LDH release in the cell culture supernatant of cells infected with *R. conorii* compared to *R. massiliae* infected cells and uninfected controls correlating with increased cytotoxicity. Pharmacological inhibitors of caspase-1 significantly reduced the release of LDH, suggesting pyroptosis. Following 72 and 96 HPI, *R. conorii* 'ISF' caused a decrease in electrical resistance, not seen in *R. massiliiae*-infected cells as determined using electric cell-substrate impedance sensing technology. Increased inflammation and pyroptotic cell death caused by *R. conorii* 'ISF' compared to *R. massiliae* suggest potential mechanisms of severe rickettsiosis.

SES 2. ***Ehrlichia* TISS Effector TRP32 Targets Host Genes Related to Cell Differentiation and Proliferation**

Tierra Farris[1], Jere W. McBride[1,2] Departments of Microbiology and Immunology[1] and Pathology[2], University of Texas Medical Branch, Galveston, TX

**Background.** *Ehrlichia chaffeensis* is an obligately intracellular bacterium that causes human monocytotropic ehrlichiosis (HME). A group of tandem repeat protein (TRP) effectors that have multiple functions and interactions with the host cell have been identified and associated with *E. chaffeensis* survival. We previously determined that TRP120 is a nucleomodulin that binds a GC-rich DNA motif via a novel tandem repeat (TR) DNA binding domain, and modulates host gene expression. Notably, another effector, TRP32, is highly upregulated during infection of human monocytes and localizes to the host cell nucleus. The purpose of this project is to show that TRP32 is a nucleomodulin and to identify the genes it regulates. **Methods**. To determine if TRP32 indeed binds host DNA, an electromobility shift assay (EMSA) was performed using full-length TRP32 and the tandem repeat domain. Then, chromatin immunoprecipitation with next generation sequencing (ChIP-seq) was used to identify host genes regulated by TRP32. GREAT was used to annotate terms that were significantly enriched in the dataset. Finally, putative DNA-binding motifs were determined using the MEME Suite. **Results**. TRP32 binds host DNA and the tandem repeat domain contains the DNA-binding domain. TRP32 interacts with host genes involved in negative regulation of megakaryocyte differentiation, regulation of gene silencing and in nucleosome assembly and organization.

SES 3. **HTLV-1's Balancing Act: Hematological Transformation versus Oncogene-Induced Mitophagy**

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The human T-cell leukemia virus type-1 (HTLV-1) is an oncogenic retrovirus that induces transformation and proliferation of virus-infected CD4+ T-lymphocytes. Approximately 3-5% of HTLV-1 infections lead to the aggressive hematological malignancy, Adult T-cell Leukemia/Lymphoma (ATL). Most ATL patient isolates contain a highly conserved nucleotide sequence, known as *pX,* which encodes several regulatory and accessory proteins, including the oncoproteins Tax, $p30^{II}$ and Hbz. The HTLV-1 $p30^{II}$ protein enhances cellular proliferation associated with proviral replication while also promoting latency. Our lab has previously shown that $p30^{II}$ cooperates with the cellular oncoprotein c-MYC through the TIP60 histone acetyltransferase promoting c-MYC-dependent cellular proliferation, S-phase progression and multinucleation. The overexpression of oncogenes, such as *c-MYC*, can induce reactive oxygen species (ROS) leading to DNA-damage and p53-dependent apoptosis. The generation of ROS within the mitochondria can cause the autolytic destruction of this vital organelle through a process called mitophagy. Our lab has discovered the induction of TIGAR (*Tp53-induced glycolysis and apoptosis regulator*) in $p30^{II}$-expressing cells combined with the overexpression of c-MYC prevents ROS accumulation and inhibits oncogene-induced cellular senescence and apoptosis. Additionally, under conditions of oxidative stress, the E3 ubiquitin ligase Parkin is targeted to mitochondria derived vesicles to prevent mitophagy and mitochondrial damage. Similar to TIGAR, $p30^{II}$ can induce Parkin protein expression and target Parkin to the mitochondria in response to oxidative stress. However, the roles of antioxidant-signaling pathway in viral carcinogenesis are not well understood. By targeting Parkin and TIGAR to mitochondria, HTLV-1 $p30^{II}$ could provide a survival mechanism which protects malignant ATL cells against mitochondrial damage induced by ROS. These studies may reveal novel strategies to sensitize chemoresistant ATL patient lymphocytes to anticancer therapies.

SES 4. **Evolution of Virulence and Drug Resistance in *Pseudomonas aeruginosa* LESB58**

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Persistent respiratory infections caused by *Pseudomonas aeruginosa* is the leading cause of fatality in cystic fibrosis patients. Liverpool epidemic strain of *P.aeruginosa,* the first epidemic strain *of P.aeruginosa* to be isolated, is an opportunistic pathogen implicated in cystic fibrosis. This strain, namely, LESB58, is known to have a propensity to acquire virulence associated and antibiotic resistance genes through the process of horizontal gene transfer. Often these genes arrive in large clusters, referred to as “genomic islands”. Horizontal transfers of genomic islands harboring virulence and antibiotic resistance genes have spawned many new virulent and antibiotic resistant strains. In order to decipher the evolution of pathogenicity and resistance in the highly resistant and aggressive bacterial strain LESB58, we implemented a recursive segmentation and agglomerative clustering procedure based on Jensen-Shannon entropic divergence to delineate large regions with atypical composition. Because genomic islands are often mosaic, arriving from different donor strains, we also leveraged the ability of our integrative methodology in deciphering the mosaic compositional structure of islands in LESB58 strain and in understanding the contribution of mosaicism in pathogenicity. We further used gene context, biological functions of genes, presence of motility genes and unusual phyletic pattern as an indicator of horizontal gene transfer to further bolster our predictions. Of 6062 genes in this strain, we identified 605 genes residing on 20 predicted genomic islands, including 12 previously unidentified islands. These newly found islands, harboring many pathogenicity associated genes revealed by sequence comparisons, have likely made LESB58 strain hyper producer of biofilm, hypervirulent, metal resistant and multidrug resistant. We further built phylogenetic trees to identify potential donor strains. Most donor strains were opportunistic virulent pathogens implicated in cystic fibrosis. Horizontal transfers of genes among these virulent and antibiotic resistant microorganisms living within cystic fibrosis patients have led to a more morbid and multi-drug resistant LESB58 strain, specifically evolved to adapt to the conditions in the lungs of cystic fibrosis patients.

SES 5. **The Pilus Tip is Required for *E. faecium* Biofilm Formation and Infection**

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**Background.** *Enterococcus faecium* is now considered one of the leading causes of hospital-associated infections in the United States, accounting for approximately 38% of clinical enterococcal isolates. These infections include infective endocarditis, bacteremia and urinary tract infections (UTIs), among others and represent a therapeutic challenge due to the resistance profile of this species to multiple antibiotics. It is recognized that *E. faecium* expresses factors that participate in its adhesion to host tissues including pili. Our group previously showed that the genome of *E. faecium* TX16 (DO) encodes four predicted pilus-encoding gene clusters, and demonstrated that in the endocarditis-derived *E. faecium* strain TX82, deletion of one of these operons, the *E. faecium* pilus-encoding operon (*empABC*), affected biofilm formation and the resulting mutant was also significantly attenuated in an experimental model of UTI.
**Methods.** We created unmarked, non-polar single deletion mutants of the pilus subunits EmpA, EmpB and EmpC in *E. faecium* TX82, and evaluated the effect of the single deletions on the ability of the strains to form biofilms. In addition, we investigated the contribution of the tip pilin, EmpA, to the ability of *E. faecium* to cause UTIs.
**Results.** We found that deletion of *empA* (*ΔempA*) reduced biofilm formation to the same level as observed with the deletion of the operon (*ΔempABC*) ($P < 0.001$ vs. WT), a phenotype that was restored by reintroducing *in situ* the *empA* gene (*ΔempA::empA*); deletion of *empB* (*ΔempB*) also caused a significant reduction in biofilm ($P < 0.001$), while EmpC (*ΔempC*) was found to be dispensable. Furthermore, we showed that the *ΔempA* deletion strain is significantly attenuated vs, WT TX82 in a mixed infection UTI experiment, in kidneys ($P = 0.028$) and bladders ($P < 0.001$).
**Conclusion.** Our results indicate that the tip subunit, EmpA is the main component of the Emp pili that mediates biofilm formation and is important in the ability of *E. faecium* to cause UTIs.

SES 6. **Pilus Hijacking by a Bacterial Coaggregation Factor Critical for Oral Biofilm Development**

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The formation of dental plaque, a highly complex biofilm that causes gingivitis and periodontitis, requires specific adherence among many oral microbes, including the coaggregation of Actinomyces oris with Streptococcus oralis that helps to seed biofilm development. Here, we report the discovery of a key coaggregation factor for this process. This protein, which we named coaggregation factor A (CafA), is one of 14 cell surface proteins with the LPXTG motif predicted in *A. oris* MG1, whose function was hitherto unknown. By systematic mutagenesis of each of these genes and phenotypic characterization, we found that the Actinomyces/Streptococcus coaggregation is only abolished by deletion of *cafA*. Subsequent biochemical and cytological experiments revealed that CafA constitutes the tip of a unique form of the type 2 fimbria long known for its role in coaggregation. The direct and predominant role of CafA in adherence is evident from the fact that CafA or an antibody against CafA inhibits coaggregation, whereas the shaft protein FimA or a polyclonal antibody against FimA has no effect. Remarkably, FimA polymerization was blocked by deletion of genes for both CafA and FimB, the previously described tip protein of the type 2 fimbria. Together, these results indicate that some surface proteins not linked to a pilus gene cluster in Gram-positive bacteria may hijack the pilus. These unique tip proteins displayed on a common pilus shaft may serve distinct physiological functions. Furthermore, the pilus shaft assembly in Gram-positive bacteria may require a tip, as is true for certain Gram-negative bacterial pili.

SES 7. **Deep Sequencing Reveals Complex Lung Microbiome in Cultivation-Negative Bronchoalveolar Lavage Samples From Mechanically Ventilated Trauma Patients**
Ashley Smith, University of North Texas Health Science Center, Denton, TX

To screen mechanically ventilated patients for ventilator-acquired pneumonia, a bronchoalveolar lavage (BAL) is performed to identify pathogenic microorganisms. Clinical laboratories traditionally use culture-based techniques which detect a limited number of known bacteria; consequently, some samples may be falsely reported as “cultivation-negative” or possessing only “normal respiratory tract flora.” To ascertain the potential for false negative results, we performed next generation sequencing (NGS) on BAL samples from ventilated patients.
Samples were taken from mechanically ventilated trauma patients in the Surgical Intensive Care Unit subjected to BAL as part of the standard of care. DNA was extracted from the samples and the 16S rRNA subunit was amplified and sequenced using either Sanger or the Ion Torrent Personal Genome Machine. Sequences were analyzed using the Mothur data-analysis pipeline to identify the taxonomic designation of each sequence. Sanger sequencing results from colonies grown on culture-positive plates corresponded to the organisms identified by the hospital lab. Seven of nine “culture negative” samples were dominated by a single organism (>50% of sample). One third (3/9) of the raw BAL samples analyzed were dominated by *Neisseria spp*. Other potential pathogens found to be numerically dominant within the BAL samples included *Streptococcus*, *Haemophilus*, *Aeromonas*, and *Rothia spp*. Our study demonstrates the potential benefits of using NGS to supplement the current culture-dependent clinical diagnostic methods. Analyzing the lung microbiome may be an important additional tool for identifying difficult to cultivate species associated with lung infections in mechanically ventilated patients.

## O.B. Williams Oral Presentation Award in General Microbiology

OBW 1. **Characterization and Weaponization of Imported Red Fire Ant Normal Gut Microbes**
Malintha C. Abeysiri [1] and Gregory D. Frederick [2] Univ, of Mary Hardin-Baylor [1], LeTourneau University [2]

Since the inadvertent introduction in the early 1900s, *Solenopsis invicta*, the Red Imported Fire Ant (RIFA), has spread through the lower United States. Due to the lack of natural pathogens and environmental predators in the USA, fire ant territories have increased exponentially. IRFA infestation in the USA has staggering impact on human health, on agricultural and livestock production, and on the environment. A number of approaches to bio-control have been implemented with variable impact. This research study focuses on the development of genetically modified normal IRFA gut flora bacteria expressing the *Bacillus thuringiensis Cry* toxin protein for use in bio-control of IRFA populations. Isolated normal gut flora bacteria from IRFA midguts were plated on Brain Heart Infusion agar (BHI) for further characterization. Using polymerase chain reaction (PCR) from targeted primers, a segment of the small ribosomal subunit 16s RNA gene (~1000bp) was amplified from bacteria chromosomal DNA extracted from each of nine IRFA normal gut bacteria. Restriction enzymes, HaeIII (GG^CC) and MboI (^GATC^), were used to restrict PCR products. The resulting digested PCR products were analyzed on agarose gel electrophoresis for Restricted Fragment Length Polymorphisms (RFLP). DNA sequence analysis of each 16s rDNA amplicon was performed. The determined DNA sequences are compared to the known sequences available in the NCBI database. Isolated IRFA gut flora were used in further characterization by biochemical tests and antibiotic susceptibility tests. Approaches to conjugal transfer of the Cry-toxin bearing plasmid from *Bacillus thuringiensis var karstaki* (BT var Kurstaki) to the characterized IRFA normal gut isolates will also be discussed.

OBW 2. **Identifying the Mechanism and Significance of Substrate Binding by the Molecular Chaperone Hsp110/Sse1**

Veronica M. Garcia[1, 2], Julie G. Heffler[1, 3], and Kevin A. Morano[1, 2]
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Proteopathies result from misfolded or alternatively folded proteins escaping cellular protein quality control systems. The highly conserved heat-shock protein 70 (Hsp70) is a molecular chaperone essential for maintaining protein homeostasis. The related chaperone Hsp110 (Sse1/Sse2 in Saccharomyces cerevisiae) functions as a nucleotide exchange factor (NEF) to regulate the Hsp70 protein folding cycle. Hsp110/Sse1 can prevent protein aggregation in vitro via a substrate binding domain (SBD), but lacks the hallmark folding activity of Hsp70. Previous attempts to study in vivo contributions of the Hsp110 SBD have been unsuccessful as SBD truncations also abolished NEF activity. Thus, the cellular impacts of Hsp110/Sse1 chaperone activity remain unknown. We have generated two distinct Sse1 variants that separate, for the first time, the nucleotide exchange and substrate binding functions. Targeted single-residue substitutions were used to generate Sse1-sbd1, and a second approach, fusing a highly unstable degradation domain to the carboxyl-terminus of Sse1 to occlude the SBD, resulted in a variant we termed Sse1-RFA. We show that Sse1-sbd1 exhibits a greater than 50% reduction in aggregation-preventing activity, while both Sse1 variants are stable when expressed in vivo and heterodimerize with Hsp70 as NEFs. Cells expressing Sse1-sbd1 and Sse1-RFA exhibit severe growth deficiencies, indicating that Sse1 chaperone activity is biologically relevant. These studies in the budding yeast will shed light on human cellular protein quality control systems which can then be pharmacologically targeted to combat protein conformational disorders, including neurodegenerative disorders such as Alzheimer's and Parkinson's diseases and triplet repeat ataxias.

OBW 3. **Salmonellae in the Intestines of *H. plecostomus* in the San Marcos River**

Anna Gates and Dittmar Hahn, Texas State University, San Marcos, TX

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 not 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.

OBW 4. **The Fly Associated Bacteria *Ignatzschineria* Tóth 2007 and *Wohlfahrtiimonas* Tóth 2008 (Gammaproteobacteria; Xanthomonadales; Xanthomonadaceae) on Cadavers Through Time**

Daniel P. Haarmann[1]*, Embriette R. Hyde[2, 3], Joseph F. Petrosino[2], Aaron M. Lynne[1], Sibyl R. Bucheli[1]
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The important role insects have during the decomposition process and in estimating the post-mortem interval in forensic investigations is well established. As some of the first colonizers of a corpse, sometimes within minutes of death, flies may play an important role in the establishment of a cadaver-specific microbiome and affect the succession of a cadaver's microbial community structure as

decomposition progresses. To explore the skin microbiome of decomposing human cadavers, three cadaver pairs (six bodies total) were placed outdoors to decompose under natural conditions. A pair was placed during either winter, spring, or summer months 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. Bacterial swabs of the mouth, right cheek, right bicep, medial torso region (above sternum), and of the feces were collected at various points during decomposition. Sample processing, amplification, and Illumina 16s rRNA sequencing were performed following protocols benchmarked as part of the Human Microbiome Project. 16s rRNA data were processed and analyzed using the open source software QIIME version 1.7.0. Results show that in the warmer months when flies are present in larger numbers, fly associated bacteria such as *Ignatzschineria* and *Wohlfahrtiimonas* are present in greater relative abundance resulting in the decrease of overall bacterial diversity. Bacterial data such as these can help in the better understanding of flies and fly associated bacteria on the decomposition process and in determining the time since death interval.

OBW 5. **Elucidating the LPS Modification Repertoire of *Pseudomonas aeruginosa***

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Gram-negative bacteria enhance their survival in harmful environments by outer membrane remodeling, particularly at the lipid A moiety of LPS. We recently identified a functional ortholog of the lipid A kinase, *lpxT*, in *Pseudomonas aeruginosa*. $LpxT_{Pa}$ is unique from previously characterized LpxT enzymes in that it is able to phosphorylate both lipid A phosphate groups as well as generate a novel 1-triphosphate species. Low $Mg^{2+}$ results in modulation of $LpxT_{Pa}$ activity and is influenced by transcription of lipid A aminoarabinose (L-Ara4N) transferase ArnT, which is induced when $Mg^{2+}$ is limiting (Nowicki *et al., Mol Micro,* 2014). We have also revealed the identity of a functional phosphoethanolamine (pEtN) transferase, $EptA_{Pa}$, in *P. aeruginosa*, and the first report of pEtN-modified lipid A in this organism. $EptA_{Pa}$ adds pEtN strictly to the non-canonical position of lipid A. Transcription of $eptA_{Pa}$ is regulated by $Zn^{2+}$ via the CoIRS two-component system, contrasting from EptA regulation in enteric bacteria such as *Salmonella enterica* and *Escherichia coli.* Further, although L-Ara4N modification readily occurs at the same site of pEtN addition under several environmental conditions, $Zn^{2+}$exclusively induces pEtN addition to lipid A. The existence of these modification enzymes suggests that coordinated regulation of *P. aeruginosa* outer membrane remodeling occurs to permit adaptation to a changing environment.

OBW 6. **Secondary Structure Analysis of mRNAs in *Rhodobacter sphaeroides* 2.4.1**

Damilola Omotajo[1], Hyuk Cho[2], and Madhusudan Choudhary[1] [1]Department of Biological Sciences and [2]Department of Computer Science, Sam Houston State University, Huntsville, TX

In bacteria, the Shine-Dalgarno sequence (SDS) is well-known for predicting translation start sites and anchoring mRNA to the 30S ribosomal subunit, via attachment to 16S rRNA. One inhibiting factor of translation is the presence of secondary structures around the start codon of mRNA. This study aims to analyze translation efficiency in terms of the possible influence of SDS on mRNA folding in *Rhodobacter sphaeroides* 2.4.1. Two hypotheses were tested: (I) mRNAs with SDS have less secondary structure formation than mRNAs without SDS, and (II) mRNAs with different SDS motifs still retain similar folding properties. To test these hypotheses, the last 40 nt of 5' UTR and the first 40 nt of ORFs for 3579 protein-coding genes in *R. sphaeroides* 2.4.1 were analyzed. Genes were classified based on the presence or absence of SDS and also the type of SDS motif. The minimum free energy (MFE) from all possible mRNA conformations was predicted using Zuker's *mfold* algorithm. Results revealed that on average, mRNAs without SDS have significantly lower MFEs compared to those with SDS. Also, mRNAs with SDS motifs closer to the start codon and less G nucleotides have significantly higher MFEs. This shows that SDS, just by its inherent nature, influences secondary structure formation at initiation region. Further studies will be performed to predict the hybridization affinity of different SDS motifs to 30S subunit. A mathematical model using Markov chains will then be designed and validated with proteomic data.

OBW 7. **The Effects of Road and House Dust on Growth Rate, Sensitivity to Oxidative Stress, and Biofilm Production in Three Representative Human Gut Flora Opportunistic Pathogens**
[1]Mohammed O. Suraju, [3]Sloan-Lalinde Barnes, [3]Sachindra Sanam, [1,2]Jason Rosenzweig. [1]Department of Biology, [2]Department of Environmental Sciences and Interdisciplinary Sciences, Texas Southern University, [3]DeBakey High for Health Professions

For the past 40 years, catalytic converters in automobiles have been using platinum group elements (PGEs, that consist primarily of platinum, palladium, and rhodium) to minimize the amounts of toxic gases in vehicle exhaust. Recently, there have been reports of increased accumulation of automobile-emitted PGEs in the biosphere. Hence, they could potentially become inhaled and ingested by humans. Yet, no study has examined the physiological impact of consistent road and house dust (likely containing PGEs) exposure on normal gut epithelium flora. In this study, the effects of house dust and road dust, which are known to contain high concentrations of PGEs, were assessed in three opportunistic gut bacteria species (*E. coli*, *E. faecalis*, and *P. aeruginosa*). Specifically, bacterial growth rates, bacterial oxidative stress resistance, and biofilm production were measured following house and road dust exposures. Surprisingly, house dust (200µg/ml), enhanced the growth rate of all three bacteria species in nutrient-poor conditions, but slowed growth rates in nutrient-rich conditions. Most interestingly, both house dust and road dust either in nutrient rich or in nutrient poor conditions significantly increased biofilm production in all three bacteria species. These results indicate that PGEs, likely found in road and/or house dust, could pose a serious health concern as they increase the virulence potential of opportunistic gut bacteria through enhanced biofilm formation.

## Postdoctoral Fellow Speakers

PF 1. **Applying Quantitative Experimental Evolution to an *Acinetobacter baumannii* Hypermutator Strain and Antibiotic Resistance**
Troy G. Hammerstrom, Kathryn Beabout, Seung Hyun Ryu, and Yousif Shamoo
Biosciences Department, Rice University, Houston, TX

We use quantitative experimental evolution of the Gram-negative nosocomial pathogen *Acinetobacter baumannii* and tigecycline (TIG), an antibiotic of last resort, to investigate how bacteria adapt to environmental stress. *A. baumannii* causes serious infections in critically ill patients, particularly those with compromised immune systems, and rapidly evolves antibiotic resistance in the clinic. Many TIG-resistant clinical isolates have point mutations in *adeRS*, a two-component signal transduction system that regulates the genes for the AdeABC efflux pump. Other evidence suggests that undiscovered pathways may affect TIG susceptibility. For our experiments, we selected a hypermutator strain, AB210M, derived from AB210, a TIG-sensitive strain isolated from a patient. After the patient received one week of TIG treatment, AB211 was isolated and identified as a TIG-resistant descendent of AB210. AB210M has a mutation in *mutL*, a key component of the DNA mismatch repair system. We utilized quantitative experimental evolution to identify, rank, and characterize proteins involved in TIG resistance. To identify the mutations that confer TIG resistance, first we used a bioreactor to perform two continuous culture experiments where AB210M was maintained at exponential growth rates with increasing TIG concentrations for 26 days. Then, we chose mixed population and end-point isolates originating from planktonic and biofilm sites for comparative whole-genome sequencing. After analysis of the genomic data from the first bioreactor culture, we identified a wide range of mutations including single nucleotide polymorphisms (SNPs), insertions, deletions, and duplications of mobile elements. Each clonal strain contained an average of 144 mutations (range: 58-423), and there were an average of 207 mutations with a frequency >5% in each mixed population (range: 83-334). All clones had mutations in *adeS*, but at five different positions. One SNP in *adeS* was also found in AB211. Mutations in *adeS* deregulate the expression of the *adeABC* operon which leads to increased efflux of TIG. Our methods reveal new evolutionary trajectories and principles for bacterial adaptation to stress.

PF 2. **GTPase Governing Ribosome Assembly**

Nikhil Jain[1], Megha Gulati[2], Joseph H. Davis[3], James R. Williamson[3] and Robert A. Britton[1]
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[2]Department of Molecular Cell Biology, University of California, Merced, CA [3]Department of Chemistry and The Skaggs Institute for Chemical Biology, The Scripps Research Institute, CA

Ribosome biogenesis in prokaryotes involves coordinated assembly of ~55 protein and 3 RNAs. Many different factors participate in this process, among these are a group of GTPases denoted as ribosome assembly GTPases that are essential for ribosome biogenesis in bacteria and eukaryotes. The GTPases that our laboratory focuses on are RbgA, YphC, and YsxC and have been shown to be involved in large subunit biogenesis. RbgA is widely distributed in bacteria and homologues of RbgA participate in mitochondria and cytoplasmic ribosome assembly from yeast to humans. Thus we expect that results we obtain investigating the function of in *Bacillus subtilis* will have important implications for the assembly of ribosomes in all forms of life. A comprehensive mutagenesis analysis in RbgA revealed the molecular functions of several important domains. Based on a comprehensive mutagenesis analysis, construction of a strain defective in RbgA function leads to the accumulation of a 45S immature ribosome intermediate that is competent for maturation and grows ~6 times slower than the wild-type strain was done. We isolated spontaneous suppressors of the RbgA F6A mutations that partially alleviated the slow growth phenotype. Sequencing of suppressor strains genomes discovered that each suppressor strain harbored a point mutation in *rplF* that indicated its functional link with ribosomal protein L6. All six suppressor mutations caused amino acid substitutions in the N terminal part of L6, which is very close to helix 97 and helix 42 of 50S subunits. We hypothesize that RbgA might be regulating the correct positioning of L6 with respect to h97 and h42. We are currently testing this hypothesis with a variety of structural, biochemical and genetic approaches.

PF 3. **Phosphoenolpyruvate Phosphotransferase System Components Modulate Gene Transcription and Virulence of *Borrelia burgdorferi***

Bijay K. Khajanchi[a], Evelyn Odeh[a], Lihui Gao[a], Mary B. Jacobs[c], Mario T. Philipp[c], Tao Lin[a], and Steven J. Norris[a,b] [a]Department of Pathology and Laboratory Medicine, and [b]Department of Microbiology and Molecular Genetics, Medical School, University of Texas Health Science Center at Houston, Houston, TX
[c]Department of Parasitology, Tulane Regional Primate Research Center, Tulane University Health Sciences Center, Covington, Louisiana

The phosphoenol pyruvate phosphotransferase system (PEP-PTS) and adenylate cyclase (AC) IV (encoded by BB0723, *cyaB*) are well conserved in different species *of Borrelia*. However, the functional roles of PEP-PTS and AC in the infectious cycle of *Borrelia* have not been characterized previously. We examined seven PEP-PTS mutants by needle inoculation of mice to assess their ability to cause mouse infection. Mutants in the EIIBC components (*ptsG*) (BB0645, thought to be involved in glucose specific transport) and *fruA2* (BB0629; putative mannose specific transporter), were unable to cause infection in mice while all other tested PEP-PTS mutants retained infectivity. Infectivity was partially restored in a *in trans* complemented strain of the *ptsG* mutant. While the *ptsG* mutant survived normally in unfed as well as fed ticks, it was unable to cause infection in mice by tick transmission, suggesting that the function of *ptsG* is essential to establish infection by either needle inoculation or by tick transmission. Purified recombinant *B. burgdorferi* CyaB protein had adenylate cyclase activity that was increased at higher temperatures. However, *B. burgdorferi cyaB* was unable to complement the cAMP dependent maltose utilization in *cyaA* deficient *E. coli*. *cyaB* mutants were fully infectious in mice when injected by needle inoculation as well as survived normally in unfed as well as fed ticks. *cyaB* mutants were also able to cause infection to naïve mice after tick transmission. These data indicate that *cyaB* is dispensable in the maintenance of mouse-tick cycle of *Borrelia*. By transcriptome analysis, we demonstrated that *ptsG* and *fruA2* genes may directly or indirectly modulate gene expression of *B. burgdorferi*. Overall, the *ptsG* and *fruA2* components play important roles in the pathogenesis of *B. burgdorferi* that appear to extend beyond their transport functions.

PF 4. **Interaction Between Antibiotics and Iron Chelation Drives Free Radical-Based Killing of Bacteria**

Li Ma[1], Yongjun Gao[2], Anthony W. Maresso[1] [1]Molecular Virology and Microbiology, Baylor College of Medicine, Houston, TX [2]Dept. of Earth and Atmospheric Sciences, University of Houston, Houston, TX

Bacterial resistance to antibiotics is precipitating a medical crisis and new antibacterial strategies are being sought. Hypothesizing that nutritional immunity, a growth-restricting strategy employed by the host, could be used to enhance the efficacy of antibiotics, we determined the effect of iron chelator/antibiotic combinations on two problematic bacterial pathogens (*S. aureus* and pathogenic *E. coli*). Here, we report that antibiotics used at sublethal concentrations display enhanced growth inhibition and/or killing when combined with the iron chelator deferriprone (DFP). ICP-OES reveals abnormally high levels of cell-associated iron under these conditions, a response that correlates with an iron-starvation response and supra-physiologic levels of reactive oxygen species (ROS). The high ROS is reversed upon addition of antioxidants, which restores bacterial growth, suggesting the cells are inhibited or killed by excessive free radicals. A model is proposed whereby certain types of antibiotics facilitate the entry of lethal levels of iron-complexed DFP, a process that drives the generation of ROS. This work suggests we should consider targeting cellular pathways that disrupt normal iron homeostasis as a potential entry point for the development of new antibacterials.

PF 5. **The Role of GABA in Susceptibility to *Clostridium difficile* Infection**

Cana Ross, Alex Peniche-Trujillo, Michael Loeffelholz, Samuel Aitken, Numan Oezguen, Petri Urvil, Jahangir Alam, Mathew Pena, George Bennett, Hoonmo Koo, Robert Britton, Kevin Garey, Sara Dann, Tor Savidge, Texas Children's Microbiome Center & Baylor College of Medicine, Houston, TX

*Clostridium difficile* is a leading cause of nosocomial infection in the U.S. and represents a major health problem for hospitals and long-term care facilities. Despite an established correlation between the disruption of the gut microflora and the development of *C. difficile* infection (CDI), the mechanism of CDI susceptibility and recurrence is not well understood. Here we demonstrate that patients who develop CDI have significant antibiotic-associated shifts in stool microbiome and metabolome signatures that are functionally associated with increased L-arginine conversion to g-aminobutyric acid (GABA). Elevated stool GABA and zolpidem use (a GABAeric drug) were predictive of disease susceptibility in patients and in experimental CDI models, and potentiated *C. difficile* toxin virulence via activation of GABA receptor A signaling. Stool GABA levels correlated with closely related GABA-producing Clostridial spp. Further investigation demonstrated that luminal GABA potentiated microbial dysbiosis during CDI and increased mucosal adherence of *C. difficile*. In summary, elevated microbial GABA production constitutes a significant new risk factor in CDI pathogenesis and represents a novel disease target for therapeutic intervention.

PF 6. ***Bacillus anthracis* Camelysin Secretion and Function**

Michelle C. Swick and Theresa M. Koehler
Department of Microbiology and Molecular Genetics, University of Texas Health at Houston, Houston, TX

Apart from the anthrax toxins and capsule, *Bacillus anthracis* produces other proteins that are known or postulated to affect host-pathogen interactions. Here we focus on camelysin, an apparent homologue of a *B. cereus* protein reported to have proteolytic activity. The *B. anthracis* camelysin gene, *calY*, is controlled by the transition state regulator SinR, and is highly expressed in cultures during stationary phase. Our previous studies showed that culture supernates of a *calY*-null mutant had elevated levels of InhA1, a metalloprotease associated with *B. anthracis* virulence. We sought to characterize the activity of camelysin and its influence on InhA1 and virulence. Using camelysin-specific antibodies and a cell fractionation assay, we found that camelysin is secreted into the culture medium of *B. anthracis* cells. This result is in contrast to reports for *B. cereus*, where camelysin was found to be cell-associated. Recombinant camelysin, purified from *Bacillus anthracis*, did not cleave casein, gelatin, or purified InhA1. Our data suggest that *B. anthracis* camelysin differs from that produced by *B. cereus*. Despite the lack of

activity *in vitro*, camelysin negatively affects both InhA1 and toxin protein abundance in culture supernates, as shown using a *calY*-null mutant. This influence on known virulence factors did not translate to hypervirulence in a mouse model for late-stage disease. *B. anthracis* camelysin may require additional cofactors for proteolytic activity or may control InhA1 levels indirectly. These studies will enhance our understanding of interactions between virulence factors during infection and increase our knowledge of differences between these two pathogens.

PF 7. **Norovirus Gastroenteritis in Latin American Pediatric Diarrheal Disease**

Xunyan Ye[1], Theresa Ochoa[2-3], Nadim Ajami[1, 4], Jose Flores[2, 5], John Van[1], Meina Zhao[1], Zhi-Dong Jiang[2], Herbert DuPont[1-2], Mary Estes[1], Robert Atmar[1], Hoonmo Koo[1-2] [1]Baylor College of Medicine, Houston, TX; [2]Univ. of Texas-Houston, Houston, TX, [3]Univ. Peruana Cayetano Heredia, Lima, Peru; [4]Fundacion Univ. del Norte, Baranquilla, Colombia; [5]Unidad de Diagnostico Clinico, Cuernavaca, Mexico

**Background:** Norovirus (NoV) prevalence remains to be elucidated in Latin America. A few studies show antibodies that block NoV & histo-blood group antigens binding are associated with protection from NoV infection. **Objective:** To evaluate the prevalence of NoV gastroenteritis in children in Latin American countries; to determine the correlate of virus blocking antibody level and protection from NoV infection. **Methods:** Three hundred seventy children with acute gastroenteritis in Latin American countries were enrolled. Stools were tested for NoV antigens by qRT-PCR. Genotyping was done by partial sequencing of the capsid gene. Sera were tested for NoV blocking antibodies by blockade assay. **Results:** NoVs were the most common enteric pathogen identified in Mexico (19%), Peru (43%), and Colombia (41%). The most common genotype was GII.4 (50/71=70 %). Fewer children (10/32=31%) with blocking titer of > =100 than children (89/175=51%) with a titer of <100 were GII-infected (p=.041). In addition, there was no significant difference of viral loads between 32 children with blocking titer of > =100 and 175 children with titer of <100 (p=.092). **Conclusions:** NoVs are an important cause of acute gastroenteritis in Latin American children. Protection against NoV infection may be related to the host production of NoV blocking antibody level.

## Millicent A. Goldschmidt Graduate Student Award Speaker

### The mRNA Quality Control Factors Ski7 and Hbs1 Evolved From an Alternatively Spliced Gene That Produced Ski7-like and Hbs1-like Proteins

Alexandra N. Marshall[1], Maria Camila Montealegre[1,2], Claudia Jiménez-López[1], Michael C. Lorenz[1] and Ambro van Hoof[1]. [1]Microbiology and Molecular Genetics, Univ. of Texas Medical School, Houston, Texas. [2]Division of Infectious Diseases, Internal Medicine, University of Texas Medical School, Houston, TX

One major function of mRNA degradation is to maintain fidelity of gene expression by specifically targeting aberrant mRNAs for rapid degradation. Two *Saccharomyces cerevisiae* paralogs play central roles in this process. The recognition and degradation of mRNAs that lack a stop codon requires Ski7 and the RNA exosome, while the recognition and degradation of mRNAs with stalled ribosomes in the middle of an ORF requires Hbs1 and an unknown endonuclease. In addition to its role in recognizing nonstop mRNAs, Ski7 has a second role as a cytoplasmic exosome cofactor. Ski7 and Hbs1 are paralogs that arose in budding yeast about 100 million years ago, and thus most other eukaryotes only contain one corresponding gene. How other eukaryotes recognize and degrade nonstop and no-go mRNAs, and whether they contain a Ski7-like exosome cofactor was not fully understood.

*Lachancea kluyveri* is closely related to budding yeast, but like other eukaryotes has only one *SKI7/HBS1* gene. rt-PCR, RNA-seq and Western blot analysis show that this one gene encodes two distinct proteins through alternative splicing. Furthermore, the longer splice isoform functions as Ski7, while the shorter splice isoform functions as Hbs1. Thus, the expression of two distinct proteins to recognize nonstop and no-go mRNAs is more widespread than anticipated.

While alternative splicing is much less common in fungi than in mammals, the alternative splicing of *SKI7/HBS1* is conserved in both the ascomycetes and the basidiomycetes as shown by rt-PCR and bioinformatics analysis. Although alternative splicing of *SKI7/HBS1* is conserved, the exact mechanism has changed several times during fungal evolution, such that both alternative 3' splice sites, alternative first exons, and likely other mechanisms are used. Strikingly, other than *S. cerevisiae* and its close relatives, the only other fungi where we failed to detect alternative splicing are in the *Schizosaccharomyces* genus. While this genus is very distantly related to *S. cerevisiae*, it also contains an uncharacterized *SKI7* gene in addition to its canonical *HBS1* gene. This comparison of splicing strategies in diverse fungi provides a model to understand the evolutionary changes in alternative splicing.

The human genome also contains only a single ortholog whose major splicing isoform resembles Hbs1, both in sequence and biochemical activity. However, the human gene is also alternatively spliced, and our results suggest that the alternatively spliced isoform functions as the missing Ski7-like cytoplasmic exosome cofactor.

## Undergraduate Posters - Medical Microbiology

UP 1. **Blood Culture: A Hospital Wide Intervention to Reduce Contamination Rate**

Arif Al-Hamad[1,2], Maha Alibrahim[1], Eman Hajhouj[2], Wasilah Alsheikh[1], Jaffar Altoweilib[1,3], Hassan Alfaraj[2]
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**Background:** Compared to truly negative cultures, false positive blood cultures (BCs) not only increase laboratory work but also prolong lengths of patient stay, increase the use of broad-spectrum antibiotics and radiological investigations, which are likely to increase patient morbidity and health care costs. The present study assessed the degree of BC contamination and evaluated the effect of hospital-wide educational intervention on contamination rates over a period of 16-months. **Methods:** All phlebotomy was performed by nurses; therefore twice-weekly educational workshops were directed at all nurses on in-patient units and emergency department over a period of 3-months. Nurses attending were asked to complete a questionnaire at the beginning of each workshop, followed by PowerPoint presentation, video clip and the demonstration of the different materials used to collect BCs and questions handling. Data from questionnaires and laboratory culture results were analyzed using standard methods, comparing data from pre- and post-intervention. **Results:** A total of 216 out of 503 (42.9%) eligible nurses attended the workshops. The attendance rates from critical areas ranged from 45% to 70%, whereas lower attendance rates were noticed for medical wards and emergency department. The survey identified areas for improvement, which included disinfectant application time, volume of blood to be cultured, and that the top of BC bottle need to be disinfected. A total of 9903 BC sets were drawn from 3649 patients during the study period, of which 676 (6.8%) were contaminated. Overall, the monthly BC contamination rates for the 6-month periods before and after intervention were 8.1% and 5.2%, respectively, with a 36% reduction (P = 0.008). Only 3 wards had an acceptable contamination rate of 3% or less before intervention, compared with 8 wards after intervention. **Conclusion**: Continued educational/training activities are essential for high quality healthcare. While contamination of BCs can never be completely eliminated, there is evidence that adherence to best practice in BC technique can minimize BC contamination, which may be best achievable with a dedicated phlebotomy team.

UP 2. **Microbiome Disruptions by an Antibiotic causes Physiological Repercussions in *Gambusia affinis***

Jeanette R. Carlson[1], Chelcy E. Brumlow[1], Annie B Leonard[1], Embriette Hyde[2], Joseph Petrosino[2], and Todd P. Primm[11]Department of Biological Sciences, Sam Houston State University, Huntsville, TX
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Antibiotics are intended to target bacterial pathogens, but by consequence are responsible for killing the normal microbiota as well. Side-effects from this depleted microbiota can lead to patients developing antibiotic-associated enterocolitis, which results in diarrhea, loss of nutrient uptake, and further opportunistic infections. Detailed understanding of how antibiotic therapy affects the host via the microbiota is lacking, therefore hindering proactive methods in solving this problem.
Our research searches to identify what are the effects of a disrupted microbiome caused by exposure to an antibiotic on the model vertebrate host *Gambusia affinis* via a highly controlled experimental system. Previous lab work shows that fish exposed to the antibiotic rifampicin for three days exhibit dramatic changes in the community composition of the skin and gut microbiome. Results of altered microbiomes showed that fish kept in groups exhibit lowered nutrient uptake (less weight gain over time when fed a standardized diet), nutrient measurements on fish individualized had complex outcomes (4 gained and 5 lost weight, yet the average weight gain in those 4 treated fish was higher than untreated fish weight gains),increased susceptibility to infection using the established pathogen *Edwardsiella ictaluri* (mean time to death following exposure shifted from 98 to 56 hours), and osmotic regulation (88% death in high saline conditions as compared to 42% in control). No significant difference 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 quantifiable data suggesting an antibiotic induced disturbed skin and gut microbiome mediates physiological defects of the host. As well as provides

evidence for host individualistic differences in the return microbiota compositions that could have beneficial, neutral, or negative effects. Work is currently underway to understand the mechanism, with an initial focus on host inflammation and/or altered biochemical pathways in the microbiome community.

UP 3. **Effects of Dual Antibiotic Therapy on the Normal Microbiome**
Oscar Chavez and Todd P. Primm
Department of Biological Sciences, College of Science, Sam Houston State University, Huntsville, TX

Development of antibiotic-resistance is a serious threat to our ability to control bacterial infections. A CDC report from last year estimated that over two million patients had DR (drug-resistant) infections in 2013. The most common method to slow resistance clinically is to apply multiple antibiotics at once (multidrug therapy). While this often effectively slows emergence of resistance in pathogens, it is unclear what the effect is on the normal microbiota. Antibiotic therapy can often have negative side effects related to disruption of the microbiome, and multidrug therapy may exacerbate that. The skin microbiome of *Gambusia affinis* is an established model system in our lab. *Gambusia affinis* was exposed to two antibiotics, rifampicin and tetracycline, which have different cellular targets. The microbiome on the skin of *Gambusia affinis* was extracted before, during, and after the one week treatment. Samples are being compared by total culturable number of bacteria, genetic community profile, susceptibility patterns, and community composition. The number of bacteria dropped rapidly and sharply with treatment, then recovered to initial densities. Surprisingly, double-resistant organisms appeared in the microbiome within three days. Current work is focusing on identifying these organisms. Use of multidrug therapy should balance the benefit of lowered selection of resistance in the pathogen with collateral damage to the normal microbiota.

UP 4. **Developing Targeted Cytotoxic Drugs to Kill Cancerous Cells**
Chelvanambi. M [a], Kishton. R [b], Varadarajan. S [b], Akkaraju. G [a]
[a] Department of Biology, Texas Christian University, Fort Worth, TX [b] Department of Chemistry and Biochemistry, University of North Carolina, Wilmington, NC

Over 20% of human cancers are caused by infectious agents. Today over 14 million patients fight some form of cancer or the other (World Health Organization). In females, breast cancer is the most prevalent cancer with nearly 1.5 million cases diagnosed and 500,000 deaths every year. A characteristic feature of some breast cancer cells is that they over-express the estrogen receptor (ER). The cytoplasmic estrogen receptor upon binding to its ligand, estradiol, translocates into the nucleus, binds to the nuclear DNA and regulates the expression of various genes. We have designed a DNA methylating agent, called Melex, which specifically enters ER expressing breast cancer cells. We used carbon linkers of variable lengths to conjugate Melex to estradiol (Est-Melex). This, based on the model described above, will allow Melex to be specifically taken up by ER+ breast cancer cells and brought in close proximity to the cancer cell's nuclear DNA which the drug can now methylate and cause cell death. We designed three variants of this drug, each differing only in the number of carbons in the backbone of the linker. The goal of our project is to find the variant that is most efficient in killing ER+ breast cancer cells. Additionally, if we do observe cell death, we wish to find the mode of cell death. Identification of such a drug that is efficient and specific in killing ER over expression breast cancer cells will give us a new weapon that can be used in our fight against breast cancer.

UP 5. **Plasticizers and Innate Immune Function**
Kallie Davis and James M. Harper
Department of Biological Sciences, Sam Houston State University, Huntsville, TX

Plasticizers, such as Bisphenol A, have become common environmental pollutants with well-known effects on reproductive and metabolic function in multiple species. However, their effect on other physiological processes, including inflammation and innate immune function, have not been well-studied.

Here we asked whether exposure to industrial pollutants alters the inflammatory response of primary mouse dermal fibroblasts using the production of interleukin-6 (IL-6) as an indicator. In particular, cells were treated with lipopolysaccharide (LPS) after 24 hours of exposure to increasing doses of BPA, and a second plasticizer dibutyl phthalate (DiBP), and the production of IL-6 was monitored. Importantly, we find that the levels of IL-6 are changed in response to LPS exposure.

UP 6. **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 hypothesized 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.

UP 7. **Inhibition of *Streptococcus salivarius* with Saffron Extract**
*Elizabeth Hughes and Irin Girgis, Mentor: Dr. Poonam Gulati
Department of Natural Sciences, University of Houston-Downtown, Houston, TX

Dental plaque is one of the most well known and studied examples of biofilms - a multispecies bacterial community protected in a polymeric extracellular matrix and attached to a moist surface. Dental plaque can lead to varied health problems including dental caries, gum inflammation, and periodontitis, and can influence systemic diseases such as diabetes and cardiovascular disease. Dental biofilms begin with the attachment of early colonizers to the acquired pellicle. These organisms consist of mainly streptococcus species such as, *gordonii, mitis*, and *salivarius.* The later colonizers attach to these bacteria and the biofilms develop. Although *Streptococcus salivarius* is an early colonizer and perhaps a facilitator of further colonization, its role as a probiotic is also being investigated by researchers. Since streptococci play a crucial role in the formation of dental plaque, it is important to understand how to inhibit their growth and attachment. Most of the inhibitory dental products contain harsh chemicals such as, alcohols and cetylpyridinium chloride that can have detrimental side effects. We study the inhibition properties of natural products - garlic, turmeric and saffron. In this project, saffron is being investigated. Saffron is a natural product used in Persia and has been shown to have anti-inflammatory and anti-carcinogenic effects. In our experiments, saffron extract at 50, 100 and 200 µg/ml inhibited *S. salivarius* cells. Biofilm density was also studied using the crystal violet assay and no consistent disruption of the biofilm was seen. Thus, the mechanism of inhibition of *S. salivarius* biofilms is probably at the cellular level, not at the level of biofilm integrity.

UP 8. **Probiotic Partial Restoration of Microbiome Metabolic Function Following Antibiotic Disruption**

Brandon Langle, Jeanette Carlson, John Pinard, and Todd P Primm. Sam Houston State University, Huntsville, TX

Studies have shown that the use of antibiotics in humans disturbs the commensal and symbiotic microbes in the gut, often killing many of these beneficial bacteria. The use of probiotic supplements in the human gastrointestinal tract after antibiotic exposure could restore the displaced bacteria or fill biochemical roles to return normalcy to the microbiome. The mucosal skin layer on the fish *Gambusia affinis* may be used as a model system for the human gut microbiome to study the effects of antibiotic exposure and the application of probiotics. In this system, fish were exposed to rifampicin in artificial pond water for three days, a regimen established to rearrange the microbiome composition. During this time, probiotic culture was grown in nutrient broth inoculated by fish skin extraction from non-antibiotic exposed fish, and this probiotic culture was applied to the tank of the fish under study one day after they had been removed from rifampicin exposure. Fish skin microbiome samples were taken from fish before, during, and after rifampicin and probiotic exposure to plate on rifampicin nutrient agar plates and nutrient agar plates, with biochemical community capabilities were assessed with API-20E test strips. Also RISA (rRNA intergenic spacer analysis) will be performed on samples to compare general microbiome composition differences. The results from this trial will be compared to a control group where fish are not treated with the probiotic culture after exposure to rifampicin. The probiotic will be assessed for its ability to decrease the prevalence and persistence of antibiotic resistance, return original biochemical capabilities to the microbiome, and return normal concentrations of bacteria onto the skin. Preliminary data indicates that while biochemical activities were partially restored, the rate of drug resistant bacteria was not suppressed by the probiotic treatment.

UP 9. **A Preliminary Study of Shifting Oral and Fecal Bacterial Communities During Human Cadaver Decomposition in Southeast Texas**

Zachary T Lueck[1]*, Dalton A Plummer[1]*, Daniel P. Haarmann[1], Joseph F. Petrosino[2], Sibyl R. Bucheli[1], Aaron M. Lynne[1] * Co-presenting [1]Department of Biological Sciences, Sam Houston State University, Huntsville, TX [2]Alkek Center for Metagenomics and Microbiome Research, Department of Molecular Virology and Microbiology, Baylor College of Medicine, Houston, TX

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 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.

UP 10. **A Preliminary Study of Shifting Skin Bacterial Communities During Human Cadaver Decomposition in Southeast Texas**

Laura M Paez[1]*, Jacquelyn K Vasquez[1]*, Daniel P. Haarmann[1], Joseph F. Petrosino[2], Sibyl R. Bucheli[1], Aaron M. Lynne[1] * Co-presenting [1]Department of Biological Sciences, Sam Houston State University, Huntsville, X[2]Alkek Center for Metagenomics and Microbiome Research, Department of Molecular Virology and Microbiology, Baylor College of Medicine, Houston, TX

Decomposition is dynamic and continuous process whereby features of a cadaver change in a relatively predictable pattern over time relative to temperature and specific ecological scenario. As a cadaver decomposes, it passes through several major stages of tissue change leading from wet decomposition (fresh, bloat) to dry decomposition (decay, mummification, and/or skeletonization). Early stages of decomposition are wet and marked by discoloration of the flesh and the onset and cessation of bacterially-induced bloat. Intrinsic bacteria begin to digest the intestines from the inside out, eventually digesting away the surrounding tissues. During putrefaction, bacteria undergo anaerobic respiration and produce gases as by-products, the buildup of which creates pressure, inflating the cadaver, and eventually forcing fluids out (purge). In the trunk, purge is associated with an opening of the abdominal cavity to the environment. While bacteria are credited as a driving force of decomposition; relatively little is known about bacterial succession during decomposition. Understanding the bacterial basis of decomposition is crucial to understanding decomposition as a whole and may help explain the variation of decomposition seen between cadavers. To investigate 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.

UP 11. **Resistance Selection in the Microbiome by Various Antibiotic Treatment Regimens**

John Pinard, Jeanette Carlson, and Todd P. Primm Sam Houston State University, Huntsville, TX

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. We also have an established infection model with *Edwardsiella ictaluri* to determine antibiotic effectiveness. Previous data has shown that after three days of exposure to the broad-spectrum antibiotic rifampicin, the bacterial community composition is strongly altered and assumed over ninety-percent resistance. My project is investigating how different treatment regimens 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, although community composition is still decreased. This project seeks to optimize antibiotic treatment by including how it affects the normal microbiome.

UP 12. **A Preliminary Study of Season Effect on Bacterial Communities During Human Cadaver Decomposition in Southeast Texas**

Christine M. Woelfel-Monsivais[1]*, Matthew J. Greenwood[1]*, Daniel P. Haarmann[1], Joseph F. Petrosino[2], Sibyl R. Bucheli[1], Aaron M. Lynne[1] * Co-presenting[1]Department of Biological Sciences, Sam Houston State University, Huntsville, TX[2]Alkek Center for Metagenomics and Microbiome Research, Department of Molecular Virology and Microbiology, Baylor College of Medicine, Houston, TX

Decomposition is dynamic and continuous process whereby features of a cadaver change in a relatively predictable pattern over time relative to temperature and specific ecological scenario. As a cadaver decomposes, it passes through several major stages of tissue change leading from wet decomposition (fresh, bloat) to dry decomposition (decay, mummification, and/or skeletonization). Early stages of decomposition are wet and marked by discoloration of the flesh and the onset and cessation of bacterially-induced bloat. Intrinsic bacteria begin to digest the intestines from the inside out, eventually digesting away the surrounding tissues. During putrefaction, bacteria undergo anaerobic respiration and produce gases as by-products, the buildup of which creates pressure, inflating the cadaver, and eventually forcing fluids out (purge). In the trunk, purge is associated with an opening of the abdominal cavity to the environment. While bacteria are credited as a driving force of decomposition; relatively little is known about bacterial succession during decomposition. Understanding the bacterial basis of decomposition is crucial to understanding decomposition as a whole and may help explain the variation of decomposition seen between cadavers. To investigate the effect of seasonality of the community structure during decomposition, six human cadavers were placed outdoors to decompose under natural conditions at the Southeast Texas Applied Forensic Science (STAFS) facility (a willed body facility) at the Center for Biological Field Studies (CBSF), Sam Houston State University, Huntsville, Texas during winter, spring and summer months. The 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.

## Undergraduate Posters - Molecular Microbiology

UP 13. **Investigating the Adaptive Value of Programmed Cell Death in a Unicellular Organism**

Matthew R. Breuer, Allyson R. Keathley, and Anne R. Gaillard
Department of Biological Sciences, Sam Houston State University, Huntsville, TX

Programmed Cell Death (PCD) has been documented in a variety of unicellular organisms; however, it is paradoxical as to why a unicellular organism would contain a genetic program for a seemingly altruistic behavior. Here, we use the model organism, *Chlamydomonas*, a unicellular green alga, to investigate the adaptive value of PCD in unicellular organisms. PCD is known to be induced in *Chlamydomonas* by a variety of stresses, including heat stress, UV exposure, and exposure to toxins. We hypothesize that PCD in *Chlamydomonas* is not truly altruistic, but occurs due to kin selection. To test this hypothesis, we induced PCD by heat stress in three different populations of *Chlamydomonas*: a pure culture of *Chlamydomonas reinhardtii*, a pure culture of *Chlamydomonas eugametos*, and a 50/50 culture of *C. reinhardtii*/*C. eugametos*. We predicted that the proportion of cells undergoing PCD in response to heat stress would be less in the mixed culture compared to either pure culture. Preliminary results confirm our prediction; PCD rates in a pure culture of *C. reinhardtii* were significantly higher than PCD rates in the mixed culture. We are also conducting experiments to determine how a cell undergoing PCD might benefit its surviving neighbors. Initial results demonstrate that cells undergoing PCD release substances into the medium that enhance the growth of non-PCD cells.

UP 14. **Identification of Aurora Kinase-Like Protein in *Rhodobacter sphaeroides* 2.4.1**
Zechariah Carroll[1], Damilola Omotajo[1], H. Cho[2], and M. Choudhary[1] Department of Biological Sciences[1], Department of Computer Science[2], Sam Houston State University, Huntsville, TX

The Aurora kinases, a family of serine/threonine kinases, have been shown to perform essential functions in chromosomal segregation during the nuclear division of eukaryotic cells. Several taxonomic lineages within *Animalia* and *Plantae* have retained up to three well-conserved Aurora kinase homologs. Bacterial organisms with multipartite genomes, such as *Rhodobacter sphaeroides*, organize and segregate their chromosomes in an orderly way, suggesting the presence of a protein with regulatory function similar to that of the Aurora kinases. Using a bioinformatics-based approach, this study aims to identify an Aurora kinase-like protein in *R sphaeroides.* COBALT was used to generate a consensus sequence from the 20 least-similar Aurora kinase lineages. Using BLAST-P, the primary sequence of RSP_3475 of *R. sphaeroides* 2.4.1 was identified as most similar to the consensus sequence. Additionally, secondary and tertiary structure comparisons performed via RaptorX provide evidence for regions of high similarity to the Aurora kinases. An in-frame deletion of RSP_3475 will be implemented in order to examine the role of this serine/threonine kinase during chromosomal segregation and cell division.

UP 15. **Abundance of Horizontally Transferred Genes in *Rhodobacter sphaeroides***
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Horizontal gene transfer is prevalent among bacterial species. The varied amount of horizontally transferred genes, which are mediated by transformation, conjugation and transduction, is well documented in prokaryotes. These horizontally transferred genes contribute towards the evolution of novel metabolic traits across different bacterial lineages. *Rhodobacter sphaeroides* belongs to the a-3 subdivision of *Proteobacteria*, and its genome has been fully sequenced and annotated. Previous studies have shown that sequences of the larger chromosome (CI) are highly conserved whereas sequences of the smaller chromosome (CII) from the corresponding species have rapidly diverged. Also, both chromosomes have abundance of duplicated genes within and between the two circular chromosomes. This study aims to understand the contribution of HGTs in the evolution of the complex genome of *R. sphaeroides* and the differential sequence divergence of its two chromosomes. Several methods (Island viewer, Markov clustering, and phylogeny) were employed to assess the prevalence of horizontally transferred genes (HTGs) within each of the two chromosomes. Island Viewer and Markov clustering methods yielded different amounts of HTGs in this genome. Island viewer predicted a significantly less number of HTGs (CI: 2.86%, and CII: 6.07%) in comparison to the Markov Clustering method (CI: 20.81%, CII: 21.02%). Moreover, these alien gene-clusters are likely have been acquired from a diversified group of organisms, possibly augmenting the metabolic capabilities of this organism, however, it does not appear to contribute alone towards the rapid divergence of CII.

UP 16. **Growth and Molecular Characterization of *recA* Mutant in *Rhodobacter sphaeroides* 2.4.1**
Michelle Harrel, Travis Tate, Veronica Rodriguez, Madhusudan Choudhary
Sam Houston State University, Huntsville, TX

RecA is an important enzyme that mediates the regulation of the SOS response, a DNA-damage repair system in prokaryotes. While LexA binds to the promoter of the SOS regulon and represses the transcription of over 40 genes under UV exposure, RecA gets activated by UV exposure and derepresses the transcription of the SOS regulon. RecA plays an important role in the SOS response by inducing autoproteolysis of the LexA repressor, and regulates error-prone DNA synthesis that bypasses DNA lesions. *Rhodobacter sphaeroides* is a model organism to study the role of the RecA protein as it shows an expanded range of metabolic capabilities. *R. sphaeroides* has recently been shown to decrease soil radioactivity in Fukushima, Japan by two-thirds, through anaerobic bioremediation. In addition, its

completely sequenced genome reveals the abundance of gene duplications within and between the two chromosomes that are required for the homologous recombination mediated by RecA. This study employs the construction of a Δ*recA* mutant strain, as well as the comparison of phenotypes, DNA damage and repair extent under UV-induced conditions, and the differential gene expression patterns between the wild-type and the Δ*recA* mutant. Identification of genes, which are differentially expressed under SOS response, will be profiled using RNA sequencing of the total RNAs and also be validated by RT-PCR for a number of previously known genes involved in the UV-induced DNA damage and repair system. We have successfully constructed the Δ*recA* strain of *R. sphaeroides,* which has similar growth characteristics as of the wild type under normal growth conditions. The resulting Δ*recA* strain of the *R. sphaeroides* will serve as an important microbiological tool for heterologous gene cloning in *R. sphaeroides*.

UP 17. **The 'Holdase' Activity of the Molecular Chaperone and Nucleotide Exchange Factor Sse1 is Important for Cell Wall Integrity in *S. cerevisiae***

Julie Heffler[1], Veronica M. Garcia[2], and Kevin A. Morano[2] [1]Department of Biology and Biochemistry, UH, Houston TX [2]Department of Microbiology and Molecular Genetics, UTHSC, Houston, TX

Heat shock protein (Hsp) 70 is a ubiquitous molecular chaperone involved in an ATP-dependent hold-and-release cycle of unfolded polypeptide that is regulated by specific cofactors. One of these is Hsp110, a protein which accelerates exchange of ADP for ATP for Hsp70. This nucleotide exchange factor (NEF) has also been shown to prevent aggregation of multiple substrates *in vitro* via its unique substrate binding domain (SBD). However, it is unclear if Hsp110 acts as a 'holdase' chaperone *in vivo* and if this function is physiologically relevant. To answer this question, we created site-directed point mutations within the SBD of Sse1, the *Saccharomyces cerevisiae* homolog of Hsp110, as well as a chimeric fusion with a DHFR degradation domain (DDD) on the carboxyl terminus. The first mutant was designed to inactivate substrate recognition, the latter to physically occlude the SBD with the unfolded DHFR moiety. We found that both mutants were able to form a stable heterodimeric complex with Hsp70, implying preserved NEF activity. Additionally, both displayed a temperature-sensitive growth phenotype at 37˚C that is rescued upon the addition of osmotic stabilizers, a phenotype associated with defects in cell wall synthesis and remodeling. Lastly, inclusion of a drug that stabilizes the DDD partially rescued the temperature-sensitive phenotype of the DDD-fused mutant. These data provide the first *in vivo* evidence that disrupting substrate binding by Hsp110 leads to biologically relevant consequences, indicating that the 'holdase' activity of Sse1 plays an important role *in vivo* for *S. cerevisiae*.

UP 18. **Isolation and Characterization of Gold (III)-Resistant Mutant of *Rhodobacter sphaeroides***

Hannah Johnson and Madhusudan Choudhary. Sam Houston State University-College of Sciences, Elliot T. Bowers Honors College, Sam Houston State University, Huntsville, TX

Heavy metals (arsenic, cadmium, chromium, lead, mercury, and gold) constitute a major pollution that is contributed to by a variety of sources, such as industrial effluents, leaching out metal ions from the soil, and acid rain; these toxic pollutants cause health risks to humans, and therefore require bioremediation from the streams, lakes, and soils. Previous studies have shown that some bacterial species tolerate varying levels of heavy metals in their environments. *Rhodobacter sphaeroides* belongs to α-3 subdivision of the *Proteobacteria*, and is metabolically versatile including the tolerance of high levels of toxic heavy and rare-earth metals. Strains of *R. sphaeroides* were continually selected on minimal medium with varying concentrations (0.05, 0.10, 0.25, 0.50, 1.0, 10, 20, 50 and 100 μM) of $AuCl_3$. *R. sphaeroides* was found to tolerate the gold salt up to a 50μM concentration, and no phenotypic difference was observed between the wild type and the strain selected on minimal medium plus $AuCl_3$. Growth characteristics reveal that metal tolerance is mediated through spontaneous mutation and selection of that mutant in the continuing bacterial culture under induced environment. Gene homologs of previously identified genes involving metal tolerance in *Pseudomonas putida* were identified in the genome of *R. sphaeroides*. These genes encode for proteins in signal transduction, transport, and carotenoid biosynthesis. Promoter analyses of these genes indicate conservation of promoter elements, such as -10 and -35 sequence

motifs. Also, these genes are not similarly expressed under aerobic, anaerobic and photosynthetic growth conditions. Further molecular analyses of the comparison of the wild-type and the mutant strains include the comparison of the expression pattern for genes involving in metal tolerance using RT-PCR and the comparison of the corresponding promoter sequences by amplifying the promoter sequences and their subsequent sequencing.

UP 19. **Searching for a p53-like Protein in *Chlamydomonas reinhardtii***
Allyson R. Keathley, Matthew R. Breuer, Terah Hardcastle, and Anne R. Gaillard
Department of Biological Sciences, Sam Houston State University, Huntsville, TX

Programmed cell death (PCD) has been documented in a variety of unicellular organisms, including *Chlamydomonas reinhardtii*. PCD in *Chlamydomonas* is known to be induced by an assortment of stresses, including heat stress, UV exposure, and exposure to toxins. PCD in *Chlamydomonas* exhibits characteristic PCD events, such as vacuolization, accumulation of reactive oxygen species, and DNA laddering. However, the signal transduction machinery that carries out PCD in *Chlamydomonas* is virtually unknown. Since apoptosis (a type of PCD specific to animals) requires a key protein known as p53, we hypothesized that *Chlamydomonas* contains a p53-like protein that mediates PCD. While traditional BLAST searches for a *Chlamydomonas* protein with similar amino acid sequence to human p53 have failed to reveal any similar proteins, previously published data documents the presence of a homolog of the human *pig8* gene (p53-induced gene 8) in *Chlamydomonas*, further suggesting the presence of p53 in *Chlamydomonas*. In addition, Western blot analysis of *Chlamydomonas* protein has revealed the presence of a ~53 kD protein that cross-reacts with three different mammalian p53 antibodies. Here, we use a novel bioinformatics approach in an attempt to identify a p53-like protein in *Chlamydomonas*, and focus on structural conservation, rather than amino acid sequence conservation in our search.

UP 20. **Antibiotic Resistance and the Emergence of Immune-Deficient Bacteria**
Ardalan Sharifi, Valerie Price, and Kelli Palmer
Department of Molecular and Cell Biology, University of Texas at Dallas

*Enterococcus faecalis* is a gram-positive bacterium and opportunistic pathogen that colonizes the GI tracts of humans and other animals. Some *E. faecalis* strains are associated with hospital infections and have acquired resistance to multiple antibiotics via mobile genetic elements (MGEs) such as plasmids. CRISPR-Cas systems (Clustered, Regularly Interspaced Short Palindromic Repeats with CRISPR-associated proteins) are defense systems that protect bacterial genomes from infection by MGEs. CRISPR-Cas systems use short nucleic acid sequences, called spacers, to guide Cas nucleases to MGEs of similar sequence. CRISPR-Cas presence varies across the *E. faecalis* species, and most multidrug-resistant *E. faecalis* strains lack CRISPR-Cas. The *E. faecalis* T11RF CRISPR-Cas possesses 21 spacers targeting plasmid and phage, with one spacer targeting the pheromone-responsive plasmid pAD1. Here we show that T11RF CRISPR-Cas is active for defense against pAM714, an erythromycin-resistant pAD1 derivative. Despite activity of this system, some T11RF pAM714 transconjugants arose in conjugation experiments. We examined CRISPR spacer sequences and other CRISPR features in CRISPR-escape transconjugants. We observed deletions of varying sizes in the CRISPR (2 to 8 spacers), all of which included the spacer targeting pAD1. We conclude that *E. faecalis* mutants compromised for CRISPR-Cas defense proliferate under antibiotic selection for a MGE. Further, selection generated population heterogeneity for defense against multiple MGEs, since several different deletions arose in the CRISPR.

UP 21. **Clade Specific Restriction-Modification Systems in *Enterococcus faecium***
Cristian Trejo, Hannah Adams, Wenwen Huo, and Kelli Palmer
Department of Molecular and Cell Biology, University of Texas at Dallas, Richardson, TX

*Enterococcus faecium* diverged into two clades (A and B) about 3,000 years ago, correlating with the urbanization of humans and domestication of animals. Strains from clade A are increasingly associated with hospital-acquired infections, while strains from clade B are more often found as commensal inhabitants of the gastrointestinal tract. Clades A and B lie very close to a genomic-based speciation threshold, which suggests that there are mechanisms moderating transfer of genetic information between the clades. Restriction-modification (R-M) systems modify host DNA at specific recognition sites, and thus prevent restriction by cognate endonucleases that recognize the same sequence. In this way, they function as bacterial immune systems, preventing the transfer of genetic material. In this study, we screened for cytosine methylation among eight *E. faecium* strains, representative of each clade, by restriction enzyme digest. We found evidence of 5-methylcytosine ($5^{m}C$) modification in strain 1,141,733 of clade B, suggesting the presence of a $5^{m}C$-specific DNA methyltransferase. We also used dot blot analysis to screen for the presence of methylated bases in the eight strains. Based on these results, we chose to look at the distribution of predicted R-M system subunits, using representative clade A and clade B strains as queries, among a recently published collection of 73 *E. faecium* strains. Interestingly, some R-M system subunits are enriched in either clade A or clade B, supporting the hypothesis that there are clade-specific R-M system enzymes influencing the transfer of genetic material between the clades.

## Graduate Student Posters - Molecular Microbiology

GP 1. **Mosaic MSCRAMM Family in Coagulase-Negative Staphylococci**
Srishtee Arora[1], Anne-Catrin Uhlemann[2], Franklin D. Lowy[2] and Magnus Höök[1]. [1]Center for Infectious and Inflammatory Diseases, Institute of Biosciences and Technology, Texas A&M Health Science Center, Houston, TX.[2] Division of Infectious Diseases, Columbia University Medical Center, New York

Cell wall-anchored proteins of bacterial pathogens play a key role in establishing adherence to host tissues, a critical step in the pathogenesis of microbial infections. Bacterial surface proteins are described as mosaic proteins composed of interlinked domains. These surface proteins are grouped into families based on structural or functional homology. Many surface proteins or adhesins of Gram-positive bacteria contain two linked IgG-like folded domains, which mediate ligand binding using the dock, lock and latch (DLL) model. These surface proteins are grouped into the Microbial surface components recognizing adhesive matrix molecules (MSCRAMMs) family. Here, we present a novel *Staphylococcus epidermidis* protein in the MSCRAMM family named SesJ. Our bioinformatic analyses show that structural homologs of SesJ are present in other coagulase-negative staphylococcal (CoNS) species. We have concluded that SesJ is a mosaic protein containing a unique N-terminal repeat (NTR) domain, an MSCRAMM domain, two B repeats, an aspartic acid containing repeat region and a cell wall anchoring domain.

GP 2. **Microbiome of Blow Flies (Diptera; Calliphoridae) Associated with Human Cadavers**
Berry, King, Haarmann, Petrosino, Bucheli, Lynne. Sam Houston State University, Huntsville, TX

Flies are important members of the decomposition ecosystem and can be important evidence of death investigations when the time since death is questioned. Because flies arrive during the earliest stages of decomposition and have been recorded colonizing the corpse within minutes of death, they may be significant in establishing a cadaver-specific microbiome. To investigate the biodiversity of the microbiome of blow 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 body site, cadaver of origin, and accumulated degree hours. Special attention is paid to bacteria that have only been recorded in association with blow flies before. 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.

GP 3. **Analysis of the Skin Microbiome of the Western mosquitofish, *Gambusia affinis*, Following Disruption**

Chelcy E. Brumlow[1], Jeanette R. Carlson[1], Embriette Hyde[2], Joseph Petrosino[2], and Todd P. Primm[1]
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Antibiotics can have serious side effects resulting from disruption of the human gut microbiome, including diarrhea and opportunistic infections by pathogens such as *Clostridium difficile*.
We have developed a novel biomedical model system to study the effects of disruptions in a mucosal microbiome using *Gambusia affinis*. *G. affinis* has an easily-accessible skin mucosal layer, a vertebrate acquired immune system, and offers a highly tractable system for study. Skin microbiome samples were taken at various timepoints during and following disruptions such as physical washes, with and without biocides, and antibiotic treatment. Biochemical activity assays, antibiograms, plating for culturable numbers, MPN, Ribosomal Intergenic Spacer Analysis, and 16S profile metagenomics were used to evaluate the effects such disruptions had on the skin microbiome of *G. affinis* and use to compare the recovered microbiome to the initial microbiome. DNA-based results show that there is a strong drop in diversity after a disruption and that the stable composition of the recovered microbiome is significantly different from that of the original microbiome. Metabolic and susceptibility results also show differences between pre- and post-treatment microbiomes. There is also an interesting culturable population recovery trend across multiple disruptions showing that an initial overpopulation occurs which then declines to a typical (compared to pre-disruption) density. Uncovering general principles of microbiome recovery from disruptions may allow development of probiotic and prebiotic interventions to ameliorate side effects for patients.

GP 4. **Impact of Mistranslation on Protein Aggregation in *Escherichia coli***

Christopher Evans, Yongqiang Fan, Jiqiang (Lanny) Ling. Department of Microbiology and Molecular Genetics, The University of Texas Medical School at Houston, Houston, TX

Protein synthesis is a central process of cellular life. The incorporation of amino acids into a newly synthesized peptide by tRNA-codon cognate pairs has many checkpoints designed to ensure the fidelity of the protein sequence. Although the incorporation of the correct amino acids into proteins is important, there is evidence that the increased rates of mistranslation may provide some benefit to cells. Using a genetic ribosome mutant with increased mistranslation rates, rpsD I199N (*rpsD**), we are currently investigating how cells respond to mistranslation and under which conditions this may be advantageous. We have used a ClpB-sfGFP fusion construct and biochemical purifications to investigate the aggregation phenotype of *rpsD** and found that, despite its increased mistranslation rate, it forms fewer aggregates after treatment with streptomycin or heat. We have also observed that the *rpsD** strain nearly completely clears the formed aggregates 2 hours after being removed from the stress, whereas the parental strain, MG1655, does not. As expected, the *rpsD** strain is more resistant to heat killing than MG1655. Together with RNA-Seq and qPCR data, these findings suggest that moderate levels of mistranslation my provide benefit to the cells under severe stress by pre-activating stress responses. We are investigating how pre-activation of stress responses by moderate levels of mistranslation in *rpsD** can lead to aggregate prevention and clearance and what benefits this may provide to the cell.

GP 5. **Employment of *tolC* Insertion Mutants to Identify TolC Assembly Gene(s)**
Kay MK, Fralick JA. Department of Immunology and Molecular Microbiology, Texas Tech University Health Sciences Center, Lubbock, TX

Antibiotic efflux in *Escherichia coli,* a Gram negative bacterium, is achieved via tripartite TolC-based efflux pumps. It is believed that there is a unique chaperone or assembly protein responsible for the maturation of this protein to the outer membrane of *E. coli* but as of yet none have been found. We originally attempted to screen for TolC assembly genes via direct negative selection using TolC-receptor U3 phage and colicin E1, but only found truncated LPS mutants. To continue with our search for a TolC assembly gene we turned our focus to selecting for suppressor mutations that suppress defective TolC structures. The equatorial domain in TolC has been implicated in the stabilization of TolC monomers, where point mutations in positions 360 to 400 lead to destabilization of the monomeric form. We utilized a collection of transposon insertion mutations of TolC. These insertions code for an increase of 27 amino acids in the TolC protein. We found that a 27 amino acid insertion at position 379 resulted in unstable TolC monomers resulting in a TolC null phenotype. We reasoned that a intergenic suppressor mutation might map in a gene whose product is involved in TolC assembly. We have isolated such mutations and are currently attempting to map them by complementation analysis of the *tol*C insertion (379) mutants by genomic libraries constructed from the suppressor mutants.

GP 6. **Electrophysiological Analysis of PapC Mutants Provides Insights into the Mechanism of Plug Displacement**
Thieng Pham, Nadine S. Henderson, Gilles Phan, Sebastian Geibel, Maya Topf, Irene Farabella, Gabriel Waksman, David G. Thanassi, and Anne H. Delcour. University of Houston, Houston, TX

The PapC usher is a twin beta barrel pore of the outer membrane of uropathogenic E. coli used for the assembly of the P pilus, a key virulence factor in bacterial colonization of human kidney cells. The usher catalyzes the translocation and ordered addition of folded pilus subunits delivered by the chaperone PapD to the growing pilus. Each PapC monomer is a 24-stranded beta barrel, flanked by N- and C-terminal globular domains and occluded by a large plug domain (PD). An alpha helix and the beta 5-6 hairpin loop are additional structural components that may play a role in controlling plug dynamics. Indeed, structural studies have revealed that the PD is released to the periplasmic side during pilus biogenesis, but the exact mechanism for PD displacement has not yet been elucidated. Several key residues that may be critical for plug stabilization have been proposed. They cluster in regions at the interface of the plug, the barrel, the alpha helix and the hairpin, and appear to belong to either electrostatic or allosteric networks. In order to assess the roles of these residues in plug displacement, we have used planar lipid bilayer and patch-clamp electrophysiology to compare the activity of the wildtype channel with that of mutant channels with either single or multiple alanine substitutions at these sites. Many mutants showed an increased propensity at plug displacement, as witnessed by openings with a conductance similar to that of the previously characterized plugless mutant (~ 3 nS). Others displayed quieter than WT activity. In addition, evidence of modal gating was observed in WT and some mutants. Together, these mutants provide insight into the molecular mechanism of PD displacement for pilus assembly and translocation through the PapC usher.

GP 7. **Development of Expression Vector and its Application to Knockdown Gene in *Clostridium difficile***
Madhab Sapkota and Dr. Julian G. Hurdle Dept. of Biology, University of Texas at Arlington, Arlington, TX

*Clostridium difficile* is a gram positive, anaerobic, spore forming bacteria that causes diarrhea during antibiotic treatment for other infections. Due to the higher relapse rate and recent outbreaks with emergence of hyper-virulent strains, it warrants effective drugs for its treatment. We are looking for essential genes as potential new targets in *C. difficile* and antisense expression has been employed to verify the essentiality of the genes. Antisense expression has been successfully used in human pathogens where the function of a gene can be studied by controlling its expression using the antisense

RNA. Using anhydrotetracycline (aTC) inducible system, we targeted *murA* (a gene involved in bacterial cell wall synthesis). In the site specific approach of antisense knockdown, the antisense clones containing the ends of *murA* gene (N- and C- terminus) were hypersensitized in the sub inhibitory concentration of antibiotic fosfomycin in *C. difficile* 630. The screening of transconjugants obtained from the library of *murA* fragments under the aTC inducible system did not result in replicative growth defective clones. In order to optimize the growth phenotype of antisense clones, we provided the paired termini to antisense RNA in the site specific approach. Expression of paired termini antisense RNA targeted to ribosomal binding site (RBS) and start codon of *murA* mRNA were lethal to *C. difficile*. We propose that such antisense expression system will facilitate *C. difficile* studies for the exploration of new drug targets and understanding of *C. difficile* biology.

GP 8. **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 on and in the bodies of humans. While this microbiome is important to understand in living humans, 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 a collaborative estimate of 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, 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 special change in microbial 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.

GP 9. **Identification of Microbial Populations in Compost Tea Derived from Spent Mushroom Compost Using in situ Hybridization (FISH)**

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Compost tea is a popular sustainable amendment used to improve soil quality and to control diseases in plants. With proper brewing, compost tea contains many of the beneficial microbes and nutrients in compost, but is more readily available for use by plants. The purpose of this study was to (i) determine microbial populations in spent mushroom substrate (SMS) compost tea using FISH, and (ii) determine if corn growth is influenced by constituents in SMS compost tea, including microbes, nutrients, or a combination of both. Beneficial microbes and nutrients were extracted from the compost by suspending it in sterile water, which was aerated to maintain an aerobic environment. One sample of resulting SMS was subjected to gentle centrifugation to yield two fractions (microbes and supernatant). A second sample of SMS contained both microbes and supernatant. Three treatments (SMS compost tea, microbes in sterile water and supernatant) and a sterile water control were applied to a low nutritive soil with three corn seeds that had been allowed to germinate. Soil and plant samples were obtained at days 0, 1, 2, 5,

10, 20, and 30. The FISH probes used to determine total microbial populations were EUB 338 and EUK 516. Root growth, sprout length as well as root, sprout, and overall biomass were determined from each corn plant at each sampling period. In addition, soil samples were analyzed for microbial activity using FISH at each sampling. Statistical analysis indicated that the compost tea from SMS showed higher root, sprout, and total biomass ($P<0.05$). The data suggest that SMS compost tea can be used as an effective soil amendment to enhance growth of corn.

GP 10. **A multiple gene model framework for prokaryotic gene prediction**
Ravi S. Pandey[1] and Rajeev K. Azad[1, 2*] [1]Department of Biological Sciences, University of North Texas, Denton, Texas [2]Department of Mathematics, University of North Texas, Denton, Texas

In the past decades, the genomes of several prokaryotic organisms have been sequenced, which have necessitated the development of efficient computational methods to decode the biological information underlying these data. Gene identification is one of the primary goals of genome annotation projects. Many computational methods have been developed for predicting protein-coding genes in prokaryotic genomes yet the progress in the field is hindered by the inherent heterogeneity underlying prokaryotic genomes. Most popular algorithms, such as, GeneMarkS and Glimmer, address this by using a typical gene model representing native genes and an atypical gene model representing horizontally acquired genes within a self-training model framework. Yet, in reality, the number of gene models that can adequately account for genic variability within prokaryotic genomes could be higher. We posit that gene prediction accuracy could be significantly raised by using multiple gene models trained on distinct gene clusters that underlie a prokaryotic genome. This is based on the premise that atypical genes may have been acquired from different donor organisms, and therefore, the atypical genes should be segregated into as many gene clusters as the distinct donor sources. To circumvent the limitation of current methods and thus augment the gene prediction accuracy, we implemented an integrated segmentation and clustering algorithm that use higher order Markov models to generate compositionally distinct clusters, which are homogeneous within but heterogeneous with respect to the other clusters. Gene models trained on each of these gene clusters were incorporated within the self-learning GeneMarkS program to predict genes for the genomes of interest. Accuracy was assessed on both genuine and artificial prokaryotic genomes. We will discuss the improvement attained in computational gene prediction by using multiple gene models in an unsupervised framework.

## Graduate Student Posters - Gram Positive Microbiology

GP 11. **Evolutionary Dynamics Leading to Tigecycline Resistance in *Enterococcus faecalis* S613**
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The CDC reports that at least two million infections and 23,000 deaths annually are caused by drug resistant pathogens. One such pathogen, vancomycin-resistant *Enterococcus* (VRE), is a leading cause of hospital-associated infections and can become resistant to multiple antibiotics. Tigecycline (TGC), a tetracycline derivative, is a promising drug for treating VRE infections. However, most VRE have the tetracycline-resistance gene *tetM*, which could pose a threat to the efficacy of TGC. Understanding the evolutionary dynamics leading to TGC-resistance in an adapting population of vancomycin-resistant *Enterococcus faecalis* would be useful for developing strategies to maintain the effectiveness of this antibiotic of last resort. Our lab has developed techniques that use experimental evolution to model adaptation to drug resistance in the lab as it might occur in a patient. The goal of our work is to use these methods to identify the most important genomic and biochemical changes responsible for TGC resistance in *E. faecalis*. Over the course of 24 and 19 days, we adapted two replicate populations of pathogenic *E. faecalis* S613 continuously to increasing concentrations of TGC in a bioreactor that maintains a large population of cells at its fastest growth rate. The populations were adapted from growth in 0.05 µg/mL

TGC to growth in a final concentration of 1 µg/mL TGC. Whole genome sequencing of different strains isolated from the polymorphic end-point populations was used to identify adaptive alleles and the linkages between different alleles. To survey the appearance and frequency of individual alleles within the populations during adaptation, whole genome sequencing was used on samples taken from each day of both adaptation experiments. By the end of both adaptation experiments the same two types of mutations had reached high frequency, suggesting that they play an important role in adaption to resistance. A twelve base pair deletion in the gene that encodes the ribosomal S10 protein reached fixation by the end of both experiments. We hypothesize that this S10 deletion could reduce the binding affinity of TGC for the ribosome. Also, deletions upstream of the *tetM* gene increased *tetM* expression and reached high frequencies within both populations. The average copy number of *tetM* within individual genomes also increased within the populations due to movement and duplication of Tn916, the conjugative transposon that carries the *tetM* gene. We hypothesize that deletions upstream of *tetM* result in increased transposition and conjugation of Tn916, which in turn plays a significant role in the dynamics leading to resistance within the population. This work provides important insights into how *E. faecalis* adapts to TGC resistance, which can subsequently be useful for developing strategies to maintain the effectiveness of TGC against these highly adaptive pathogens.

GP 12. **Analyzing the Interaction Between Bile Acids and the *Clostridium difficile* Germinant Receptor, CspC**

Disha Bhattacharjee and Joseph A. Sorg. Dept. of Biology, Texas A&M University, College Station, TX

*Clostridium difficile* infections (CDI) have increased dramatically over the past decade. The widespread use of broad spectrum antibiotics coupled with the emergence of hypervirulent strains has made CDI one of the most common nosocomial infections in the United States. *C. difficile* is a Gram-positive, spore forming, strict anaerobic bacillus. Only *C. difficile* spores can survive for extended periods of time outside the host, thus spores are considered the infectious form of the organism. Once inside a host, they germinate and resume vegetative growth. The vegetative cell produces toxins that elicit the primary symptoms of the disease. Previous work by our lab, and others, has shown that *C. difficile* spores germinate in response to bile acids and glycine. While the signals that stimulate germination are known, the target of these germinants in the *C. difficile* spore was not. *C. difficile* does not encode orthologues of the canonical germinant receptors (e.g. *gerA*, *gerB* and *gerK*) as encoded by the model spore former *Bacillus subtilis*. Recently, we demonstrated that certain SNPs in *C. difficile cspC* can abrogate spore germination, while another SNP alters how spores respond to an inhibitor of germination, suggesting that CspC is a germinant receptor for *C. difficile* spores. Here, we show by isothermal titration calorimetry (ITC) that CspC is a bile acid binding protein. Our preliminary data suggest that CspC binds to taurocholic acid (a bile acid germinant) and chenodeoxycholic acid (a bile acid anti-germinant). A negative control, bovine serum albumin (BSA), does not bind to taurocholic acid (BSA has not been described as a bile acid binding protein). As described previously, StarD5, a mammalian bile acid binding protein, binds bile acids. We are currently trying to enhance our ITC signal by optimizing our CspC purification protocol.

GP 13. **The Role of the Leader Peptide in the Biosynthesis of the Lanthipeptide Mutacin 1140**

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Mutacin 1140 belongs to a class of ribosomally synthesized peptide antibiotics called lanthipeptides. Lanthipeptides are composed of an N-terminal leader peptide that is cleaved to yield the antibacterial peptide called the core peptide. It has been proposed that the leader peptide is important for recognition of the post-translational modification (PTM) enzymes and for inhibiting the core peptide activity. Herein, we further our understanding of sequence and structural requirements of the leader peptide for the biosynthesis of the lanthipeptide mutacin 1140. We have determined that the length of the peptide and a novel four amino acid motif are important for the biosynthesis of mutacin 1140. The leader peptide cleavage site was found to be promiscuous in its amino acid composition with the exception of arginine at the -1 position. Furthermore, the leader peptide was found to have an additional cleavage site at the -9

position, which may be required for the release of the lanthipeptide from the PTM enzymes prior to being transported out of the cell. Our study on the leader peptide of mutacin 1140 provides a basis for future studies aimed at producing novel peptide based therapeutics that contain post-translational modifications found in mutacin 1140. Furthermore, our study demonstrates that mutacin 1140, which is produced by *Streptococcus mutans JH1140*, is a useful model system for the study of lanthipeptide biosynthesis.

GP 14. **Identifying the *Clostridium difficile* Germinant Receptor Reveals a Novel Pathway for Spore Germination**

Michael B. Francis and Joseph A. Sorg. Dept. of Biology, Texas A&M University, College Station, TX

*Clostridium difficile* is an anaerobic, spore forming bacterium that infects antibiotic-treated hosts. The vegetative form of *C. difficile* produces toxins that elicit the primary symptoms of disease but it is the spore form that is necessary for host infection. Spores are metabolically dormant forms of bacteria that are resistant to harsh environmental conditions, including antibiotics. Even though they are considered metabolically dormant, spores sense changes in environmental conditions and begin the transition from dormancy to vegetative growth (germination) upon sensing appropriate signals (germinants). Work in our lab has focused on understanding how spore germinants initiate germination by *C. difficile* spores. In *Bacillus subtilis*, one of the first measurable processes in germination is the release of dipicolinic acid (DPA) from the spore core. DPA release then activates enzymes found in the spore cortex, a thick layer of specialized peptidoglycan which surrounds the DNA-containing core. Cortex degradation must be completed so that a vegetative cell can grow from the germinated spore. To date, all other germinant receptors encoded by spore-forming bacteria are localized to the spore's inner membrane; *C. difficile* does not encode the canonical germinant receptors found in other spore-forming bacteria. This implies that there may differences in how *C. difficile* spores germinate, when compared to other spore-forming bacteria (e.g. *B. subtilis*). Recently our lab identified the *C. difficile* germinant receptor, CspC, as the subtlisine-like, protease-like protein. We predict that this receptor is embedded within the spore cortex. We hypothesize that activation of the *C. difficile* germinant receptor initiates cortex hydrolysis and this action then allows DPA release from the *C. difficile* core. This proposed mechanism differs from the *B. subtilis* model and suggests a more simplified mechanism of germination for *C. difficile* spores.

GP 15. ***Lactobacillus reuteri* Exerts Anti-Inflammatory Effects via Conversion of L-Histidine to Histamine and Activation of Histamine Receptor 2**

Chunxu Gao[1,3], Angela Major[3], Vanessa Jackson[2,3], Monica Lugo[2,3], James Versalovic[1,2,3]
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Supplementation with probiotic *Lactobacillus reuteri* strains was shown to suppress intestinal inflammation in patients with inflammatory bowel disease and in rodent colitis models, but the underlying mechanisms are not clear. We demonstrate that *L. reuteri* strains containing a complete *hdc* gene cluster which is responsible for the synthesis and secretion of histamine attenuate acute experimental colitis in mice, as indicated by decreased weight loss, colonic injury and serum amyloid A protein concentrations, as well as reduced uptake of [18F]fluorodeoxyglucose ([18F]FDG) in the colon by positron emission tomography (PET). *L. reuteri* treated mice showed decreased gene expression of IL-6 and IL-1β in the colon. Absence of histamine-producing enzyme HdcA in *L. reuteri* or the substrate L-histidine in the mouse diet or blocking histamine receptor 2 (H2R) with its specific antagonist ranitidine diminished the ability of colitis attenuation. Collectively, luminal conversion of L-histidine to histamine by *hdc*+ *L. reuteri* activates H2R and induces anti-inflammatory immunomodulation in mouse colon.

GP 16. **Production and Isolation of MutB Dehydratase**
Steven Lai Hing, James Smith. Dept. of Biological Sciences, Texas A&M University, College Station, TX

*Streptococcus mutans* JH1140 is a strain of bacteria which produces a lantibiotic product, named mutacin 1140. Mutacin 1140 has been shown to be effective at inhibiting Gram-positive bacterial infections caused by *Staphylococcus aureus* and *Streptococcus pneumoniae*. Mutacin 1140 is a ribosomally synthesized peptide antibiotic that undergoes extensive posttranslational modifications (PTM). Several PTM enzymes are used to produce the functional lantibiotic. Mutacin 1140 biosynthesis operon contains genes for a dehydratase (MutB), cyclase (MutC), decarboxylase (MutD), transporter (MutT), and peptidase (MutP). MutB, and MutC are involved in the formation of lanthionine rings, which are essential for bioactivity. The dehydratase Mut B is ~116 KDa protein. In this study we will describe the production and isolation of the recombinant protein from *E. coli* DE3 expression system. We have already isolated and confirmed the identity of MutB post protein prep/yield and extraction by trypsin digestion and MALDI-PMF (Peptide Mass Fingerprinting analysis). The long term goal of the project is to better understand the function of the structural elements within MutB that promotes the biosynthesis of mutacin 1140. The goal is to design approaches that may promote the production of mutacin 1140 and other lanthipeptide containing peptides. Furthermore, additional understanding of MutB activity may promote its use for modifying additional protein targets enabling subsequent selective chemical modifications.

GP 17. **Small Molecule Inhibition of *Trans*-Translation Impairs *Staphylococcus aureus* Viability**
Yueyang Huang[1], John Alumasa[2], Ken Keiler[2] and Shauna McGillivray[1]
Texas Christian University[1] and Pennsylvania State University[2]

*Staphylococcus aureus* is the leading cause of skin and soft tissue infections in the US. Its ability to rapidly develop antibiotic resistance to antibiotics such as vancomycin, linezolid and daptomycin poses a significant healthcare challenge and new antibiotic targets are urgently needed. The process of *trans*-translation is conserved throughout many bacterial species and is often required for their viability, virulence, development, and response to stresses. We have identified two small molecules, KKL35 and KKL40, that inhibit trans-translation in *S. aureus*. Growth inhibition is seen at concentrations of 4 μm and 2 μm for KKL35 and KKL40 respectively. Cytotoxicity assay show no impact on human Hela cell viability even at 100 fold of the concentration of KKL35 and KKL40 used in minimal inhibitory concentration assays for *S. aureus*. In addition, we find that use of these compounds sensitizes *S. aureus* to antimicrobials such as antimicrobial peptides and some antibiotics resulting in a synergistic interaction when used in combination and much enhanced killing. Therefore, we conclude that *trans*-translation is a good antibiotic target for *S. aureus.* Our findings may inspire novel drug discovery efforts aimed at the development of drugs targeting *trans*-translation.

GP 18. **Comparison of Bile Acid Profiles of Patients with Various Stages of *Clostridium difficile* Infection**
Kathleen N. McAllister and Joseph A. Sorg. Dept. of Biology, Texas A&M University, College Station, TX

*Clostridium difficile* is an anaerobic, spore-forming bacterium that has become the most frequent cause of antibiotic-associated diarrhea. Broad-spectrum antibiotic treatment, often unrelated to *C. difficile* infection, leads to disruption of the normal colonic microbiota. In the spore form, *C. difficile* is able to survive in aerobic environments due to the metabolically dormant state characteristic of a spore. When a host ingests spores, the spores must germinate in order to return to vegetative form that elicits symptoms of disease. Host-derived bile acids belonging to the cholic acid family, in conjunction with glycine, are the most efficient germinants for *C. difficile* spores. Conversely, bile acids of the chenodeoxycholic acid family are anti-germinants that competitively inhibit cholic acid-mediated germination. As the bile acids traffic through the intestines, they become modified by the colonic microflora. Bacteria in the GI tract remove the conjugated amino acid, taurine or glycine, (a process known as deconjugation). Other bacteria remove the 7α-hydroxyl group (a process known as 7α-dehydroxylation). The 7α-dehydroxylation of the primary bile acids produces secondary bile acids which are toxic for *C. difficile* vegetative growth. It is clear that

the existing colonic microflora has the potential to influence the relative concentrations of bile acids within a host, thus affecting *C. difficile* spore germination or vegetative growth. Inhibiting *in vivo* spore germination is an attractive, potential, therapy for CDI. Before such treatment options can be explored, it is important to establish the intestinal bile acid profiles of patients currently infected with *C. difficile* or those who are at risk of becoming infected. Bile acid profiles of patients who have reoccurring *C. difficile* infections are also important. If there is a bile acid profile that favors spore germination in CDI patients, chenodeoxycholic acid derivatives, or analogs, could be given to at risk patients to prevent the onset of CDI. In a double-blinded study, we analyzed the bile acid content of healthy patients with or without antibiotic treatment and no diarrheal disease, with or without non-*C. difficile* diarrhea, and with recurring CDI. Our findings suggest that patients can be divided into groups based on bile acid profiles.

GP 19. **L-Histidine Metabolism by *Lactobacillus reuteri***
Christina Morra and James Versalovic M.D., Ph.D. Baylor College of Medicine, Houston, TX

In *Lactobacillus reuteri* the decarboxylation of L-histidine to histamine by HdcA reduces the production of the pro-inflammatory molecule, TNF. We have begun to elucidate novel histidine metabolism pathways in *L. reuteri* using $^{13}C_6{}^{15}N_3$ L-histidine. Analysis of wild type and *hdcA* mutants revealed no significant changes in growth or viability when cultured in different concentrations of L-histidine or $^{13}C_6{}^{15}N_3$ L-histidine. Therefore, histamine production is not essential for *L. reuteri* growth or viability. While, there were no significant changes to growth or viability in *L. reuteri* grown in media where all histidine was replaced with carnosine (β-alanyl-L-histidine), mass spectroscopy determined that the addition of carnosine to the medium significantly increases histamine production. Together, these data have demonstrated that 1) histidine decarboxylation, although providing benefit to the host, is not required for *L. reuteri* growth; 2) the addition of carnosine improves histamine production; and 3) *L. reuteri* strains can import, process, and use carnosine as its sole source of L-histidine. Future work will involve co-culturing of *L. reuteri* with human ileal enteroids to determine the effects of *L. reuteri* and histamine metabolism on the human ileal epithelium.

GP 20. **Whole-Organism Metabolomic Profiling of Anthrax Reveals a Novel Role for Lipid Mediators in Disease Progression**
Chinh Nguyen and Anthony Maresso. Baylor College of Medicine, Houston, TX

The use of metabolomics, described as the study of dynamic small molecule fluctuations, has been gaining interest in the field of infectious diseases. Here, we describe the first metabolomic analysis of a systemic bacterial infection using *Bacillus anthracis* as the model pathogen. Whole organ and blood analysis identified more than 200 different metabolites that changed in response to infection, with most drastic alterations in lipid metabolites. Bioinformatic analysis revealed two prominent lipid pathways affected during infection. Products of the polyunsaturated fatty acid and lysolipid biosynthesis pathways, potent mediators of the innate immune response that rely on the activity of host phospholipase $A_2$ ($PLA_2$) enzymes, were reduced in infected mice compared to uninfected control. Metabolite changes were detected as early as 1 day post-infection, before onset of disease or the spread of bacteria to organs. These findings suggests a novel mechanism in which *B. anthracis* actively suppresses innate immune response by downregulating pro-inflammatory lipid mediators produced by $PLA_2$ enzymes, thus preventing the initiation of host defense mechanisms. Functional studies showed that drug inhibition of $PLA_2$ activity potentiated disease severity and increased mortality in infected mice compared to animals given bacteria or drug alone. Collectively, this study provides a blueprint for using metabolomics as a diagnostic platform that grades the stage and type of infection, as well as an identifier of novel processes that contribute to bacterial pathogenesis.

GP 21. ***Lactobacillus reuteri* as a Biotherapeutic System for the Delivery of Antimicrobial Peptides**
Laura Ortiz, Robert Britton. Microbiology and Mol. Virology, Baylor College of Medicine, Houston, TX

Bacteria have become an attractive option to supply therapeutic molecules or peptides to the gastrointestinal tract (GIT). Particularly, *Lactococcus lactis* is being explored for the treatment of diseases such as inflammatory bowel disease with encouraging results. Nonetheless, *L. lactis* has some limitations that hamper its use as a therapeutic delivery system (TDS), such as its poor survival in some gastrointestinal compartments. *Lactobacillus reuteri* ATCC PTA 6475 *(LR)* is a human derived strain with probiotic properties that has not been associated with any pathological process. These characteristics, along with the development of genetic tools that allow editing of its genome with high efficiency, make *LR* a promising candidate for the delivery of peptides with therapeutic purposes. Regenerating islet-derived protein 3 alpha (Reg3α) is a peptide, normally secreted to the lumen of the human GIT, which has antimicrobial activity against several pathogens including Vancomycin resistant enterococci (VRE). Our purpose is to engineer a *LR* strain that efficiently delivers Reg3α to the GIT and can be used for the treatment of diseases such as VRE infections. For this purpose, we will generate an *LR* strain that efficiently secretes Reg3α *in vitro* under gastrointestinal conditions, and will be used to determine the therapeutic effect of our TDS *in vitro* e *in vivo* models of VRE. So far, we have generated a construct that allows the inducible secretion of Reg3α. Our data show that Reg3α is being produced by *LR*, with 95% of the total protein produced being soluble; and approximately 70% of the protein, being secreted to the extracellular medium. Currently we are working on strategies to increase Reg3α production and demonstrate its activity *in vitro.*

GP 22. **Effect of Gelatinase Cleavage of AtlA on Surface Localization**
Emily K. Stinemetz,[1,2] Peng Gao,[1] Kenneth L. Pinkston[1] and Barrett R. Harvey[1,2]
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Enterococci are a major cause of nosocomial infections including urinary tract infections, endocarditis and bacteremia. *E. faecalis* adheres to and forms surface communities known as biofilms on clinical devices, which inhibits the efficacy of antibiotic therapy creating healthcare concerns. *E. faecalis* produces cell surface proteins important for colonization and growth. Among them, AtlA, a secreted protein, which associates to the bacterial surface and hydrolyzes cell wall peptidoglycan, is important for cell division and establishment of biofilms. We have discovered that AtlA is cleaved by a metalloprotease, GelE, and this cleavage event is important for proper cell division. We are investigating the interaction between GelE and AtlA and the affect on the ability of *E. faecalis* to divide and establish an infection. Using in-house developed monoclonal antibodies that recognize different epitopes on AtlA, we determined that the GelE-cleaved AtlA localizes to the cell septum allowing for cell division. We are exploring which domain is necessary for AtlA septum localization and identifying potential partners that allow for localization. Future experiments will further our understanding of cell division and the roles of AtlA and GelE in establishment of infection.

GP 23. ***Bacillus anthracis* Employs a Secreted Protease to Utilize Serum Proteins as an Amino Acid Source**
Austen Terwilliger[1], Michelle Swick[2], Kathryn Pflughoeft[2], Julie Lovchik[3], C. Rick Lyons[4], Theresa Koehler[2], & Anthony Maresso.[1] [1]Baylor College of Medicine, Department of Molecular Virology and Microbiology [2]University of Texas Health and Science Center at Houston, Department of Microbiology and Molecular Genetics [3]University of New Mexico Health Sciences Center, Department of Internal Medicine [4]Colorado State University, Infectious Disease Research Complex

Bacteria sustain an infection by acquiring nutrients from the host to support replication. The host sequesters these nutrients as a growth-restricting strategy, a concept termed nutritional immunity. Historically, the study of nutritional immunity has centered on iron uptake because many bacteria target hemoglobin as an iron source. Employing mass spectrometry and a novel medium designed to mimic the

chemical composition of nutrients in human serum, we demonstrate that hemoglobin is proteolyzed by the secreted protease InhA1 to liberate essential amino acids, and that InhA1 affects virulence in a mouse model of inhalational anthrax. In addition, we show this property extends to other serum proteins, including serum albumin. The results suggest we must also consider proteolysis of host proteins as a way for bacterial pathogens to obtain essential amino acids and overcome nutritional immunity.

GP 24. **Role of *Lactobacillus reuteri* in Preventing Osteoclastogenesis**

Darin Quach[1,3], Regina Irwin[2], Laura Schaefer[1,3], Fraser Collins[2], Nara Parameswaran[2], Laura McCabe[2] and Robert A. Britton[1,3] Microbiology and Molecular Genetics[1], Dept. Physiology[2], Michigan State University, East Lansing, MI, Molecular Virology and Microbiol., Baylor College Medicine[3], Houston, TX

Osteoporosis is estimated to affect over 200 million people worldwide. All individuals can be affected, but the largest population impacted is postmenopausal women. Current therapies that prevent bone loss have undesirable side effects so the development of novel treatments is crucial. In human and animal studies, it is observed that increased inflammation creates an imbalance between osteoclastic bone resorption and osteoblastic bone formation leading to the development of osteoporosis. In previous studies we showed that the probiotic *Lactobacillus reuteri* (*L. reuteri*) 6475 prevented bone loss in ovariectomized (OVX) mice, a model used to mimic postmenopausal conditions observed in women, and this correlated with decreased osteoclast formation *in vivo*. Additionally, we also demonstrated that *L. reuteri* suppresses osteoclastogenesis by ~70% using an *in vitro* culture model. In the current study, we show that *L. reuteri* decreases the expression level of DC-STAMP, a regulator of cellular fusion required for osteoclast formation, in a cell culture model. Additionally, genes coding for TRAP, CathepsinK, MMP9, and H+ATPase, which relate to osteoclast bone resorption, are downregulated by *L. reuteri.* This is consistent with results showing that *L. reuteri* suppresses bone resorption by ~60%. Lastly, putative mutants that inhibit osteoclastogenesis by ~30% were identified in a library screen and will be the focus of future *in vivo* experiments to investigate their role on bone health.

GP 25. **Specificity and Protein Interactions of *Bacillus anthracis* PRD-Containing Virulence Regulators**

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*Bacillus anthracis* produces three proteins, AtxA, AcpA, and AcpB, that are members of an emerging class of transcriptional regulators termed PRD-containing Virulence Regulators (PCVRs). PCVRs are global regulators, affecting expression of multiple genes associated with virulence in Gram-positive pathogens. A defining characteristic of PCVRs is the presence of phosphoenolpyruvate-dependent phosphotransferase system regulatory domains (PRDs). The phosphotransferase system (PTS) is a well-established carbohydrate transport system found in Gram-positive and Gram-negative bacteria. Thus, PCVRs may link carbohydrate availability to virulence gene expression. AtxA is a model PCVR and the master virulence regulator in *B. anthracis* controlling expression of the anthrax toxin genes (*lef, pagA,* and *cya*) and the other PCVR genes (*acpA* and *acpB*). AcpA and AcpB function downstream of AtxA to positively affect expression of the capsule biosynthetic operon (*capBCADE*). AtxA, AcpA, and AcpB have amino acid sequence similarity and partially overlapping regulons. To investigate the functional similarity of these PCVRs we quantified regulator activity on target promoters. We constructed strains containing individual regulator genes under the control of an IPTG-inducible promoter and harboring transcriptional fusions of target gene promoters to the β-galactosidase gene *lacZ*. AtxA, AcpA, and AcpB induced lethal factor expression. AcpA and AcpB activated the promoter of the capsule biosynthetic operon P*capBCADE*, whereas AtxA had no effect on this promoter. In previous work, we showed that AtxA activity is linked to homomultimerization. To determine if AcpA and AcpB form homomultimers and to test for heteromultimer formation among the regulators, we performed co-affinity purification experiments using epitope-tagged recombinant proteins. We detected homomultimers of AcpA and AcpB and a heteromeric interaction between AtxA and AcpA. Future experiments will address relationships between PCVR domains and target specificity and the *in vivo* relevance of the AtxA-AcpA interaction.

## Graduate Student Posters - Eukaryotic Microbiology

GP 26. **Prevalence of *Typanosoma cruzi* in Texas rodent populations.**
Adriana Aleman, Troy J. Maikis, Michael R. Forstner, Ivan Castro-Arellano, Dittmar Hahn
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Rodents are one of the reservoir hosts for *Trypanosoma cruzi* (*T. cruzi),* the agent of Chagas disease. Chagas disease is considered a major problem in many rural areas in Latin Americas countries. *T. cruzi* is known to cause severe myocarditis in its hosts. The purpose of this study was to determine the incidence of rodents infected with *T. cruzi* in five geographical regions of Texas. Rodents were captured and heart tissues were collected. Heart tissue was diced and DNA extracted using the DNeasy Blood & Tissue extraction kit from Qiagen. DNA was analyzed using real-time quantitative PCR (qPCR) using Cruzi I and Cruzi II primers. Of approximately three hundred samples analyzed, six were shown to be infected with *T. cruzi*. All infected samples were located within the same geographical region. The data indicate that rodent populations in selected regions of Texas are infected with *T. cruzi*. Further studies should be conducted to determine if other animal populations in the regions of Texas with rodent populations infected with *T. cruzi* may also be positive for the organism.

GP 27. **Ato Proteins, a Family of Putative Ammonia/Acetate Transporters, Promote the *C. albicans A*lkalinization Response.**
Heather Danhof and Michael Lorenz. Department of Microbiology and Molecular Genetics, The University of Texas Medical School at Houston, Houston, TX

The innate immune system limits the niches in the human body in which the opportunistic fungal pathogen, *Candida albicans,* can persist and macrophages are vital components of this process. Transcriptional profiling of phagocytosed *C. albicans* with macrophages revealed an up-regulation of genes involved in alternative carbon metabolism, including amino acid catabolism. We have shown that when this species catabolizes amino acids as a carbon source, it excretes the amine group as ammonia, raising the pH. This alkalinization promotes *C. albicans* escape from the macrophage by neutralizing the phagolysosome and inducing hyphal growth. Comparison of transcript profiles of cells in *in vitro* alkalinization conditions to macrophage phagocytosed cells revealed an overlapping set of up-regulated genes, including several members of the poorly understood *ATO* family. RNA-seq analysis revealed that the expression of *ATO* genes is dependent upon *STP2,* a transcription factor that is critical for the alkalinization phenomenon and we thus hypothesized that the Ato proteins are important effectors of the pH change. Deletion of one of the 10 homologs, *ATO5,* or the over expression of a dominant negative *ATO1G53D* allele*,* results in a delay in environmental alkalinization, a defect in hyphal formation, and a reduction in the amount of ammonia released from the cell. Further, these strains form fewer hyphae after phagocytosis and have a reduced ability to escape macrophages. Together, these results suggest that the Ato protein family is important in the host-pathogen interaction of *C. albicans.*

GP 28. **Redox Imbalance Caused by Thiol Oxidant Stress Induces Aggregation of Cytosolic Cysteine-Containing Proteins in *Saccharomyces cerevisiae***
Amy Ford, Sara Peffer, and Kevin Morano. Department of Microbiology and Molecular Genetics, The University of Texas Medical School at Houston, Houston, TX

A distinguishing feature of eukaryotic cells is the presence of membrane bound organelles that allow for compartmentalization of distinct redox environments, such as the oxidizing ER and mitochondrial inner membrane space and the reducing cytoplasm. Mechanisms to maintain redox balance include thioredoxin and glutathione systems present in the mitochondrial matrix and nucleus/cytoplasm. Reactive oxygen species (ROS) in the cytosol can disrupt this reducing environment, resulting in activation of cellular responses to restore redox balance. Several proteomic studies investigating the redox status of proteins

exposed to ROS *in vivo* identified proteins whose thiol groups can be oxidized. We predict cysteine (cys)-containing proteins synthesized and functional within the cytosol may be especially vulnerable to redox imbalance. In addition, thiol-reactive compounds have been shown to induce the cytosolic unfolded protein response (cUPR) in part by modifying reactive cysteines in the yeast Hsp70 chaperone Ssa1. Here we demonstrate that in the absence of a fully functional cytosolic thioredoxin system, the cUPR is constitutively activated, suggesting a link between redox maintenance and protein quality control. The heavy metal cadmium, a thiol chelator, and the thiol-specific oxidizing agent diamide, have previously been shown to induce the cUPR. Using green fluorescent protein fusions, we find that exposure to either compound causes aggregation of the cys-containing protein Tpi1, but not Eft1 or a control lacking cysteines. Thus, demonstrating that cys-containing proteins are differentially susceptible to thiol oxidant stress.

GP 29. **TRX-1 as a Novel Regulator of the Major *C. elegans* Oxidative Stress Transcription Factor, SKN-1**

Katie C. McCallum[1], Antonio Miranda-Vizuete[2], Bin Lui[3], Swathi Arur[3], Danielle A. Garsin[1]
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The ability of an organism to maintain oxidative homeostasis is critical for its survival. At the cellular level, exposure to oxidative insult can irreversibly damage DNA, proteins, and lipids, all of which can lead to cell apoptosis or necrosis. At the organismal level, unresolved oxidative stress can lead to several life-threatening diseases, including Alzheimer's, Parkinson's disease, and atherosclerosis. Our lab uses the model organism *C. elegans* to study the regulation of the major oxidative stress transcription factor, SKN-1, which is a functional homolog of the major mammalian oxidative stress transcription factor, Nrf2. Recently, we identified a thioredoxin as a novel regulator of SKN-1. Thioredoxins are small redox reactive proteins that have been shown to play a prominent role in redox signaling. In light of this, we hypothesized that thioredoxins are the 'first responders' to oxidative stress and may serve as the link between stress sensing and stress signaling that has yet to be fully elucidated in many organisms. In support of this hypothesis, we have shown that the loss of *trx-1* promotes intestinal nuclear localization of SKN-1, even in the absence of stress, indicating that TRX-1 regulates SKN-1 localization. Interestingly, TRX-1-dependent regulation of intestinal SKN-1 nuclear localization is specific, occurs cell non-autonomously, and is dependent on the p38 MAPK pathway. However, while TRX-1 is able to regulate SKN-1 localization, we do not see increased activation of this transcription factor. Interestingly, loss of *trx-1* elicited a general, organismal down-regulation of several classes of genes, with collagens being the most prevalent.

GP 30. **Identification of Functional tRNA-Derived RNA Fragments (tRFs) in Respiratory Syncytial Virus Infection**

Thien Phan. Texas Southern University, Houston, TX

Respiratory Syncytial Virus (RSV) is the leading cause of lower respiratory tract infection in children from infancy up to early childhood. RSV is a negative sense, single-stranded RNA virus, belonging to the *Paramoxiviridae*. Recently, it has been demonstrated that RSV infections result in changes to host cellular small non-coding RNA (sncRNA) expression. With RSV infections, the predominately sncRNA expressions are tRNA-derived RNA fragments (tRFs), as a results of endonuclease ANG cleavage at a specific site. The tRFs function and role they play in viral and host interaction are still unknown. Herein, we examined the role of tRFs derived from the 5'-end of mature tRNA GlyGCC, tRF GlyCCC, tRNA LysCTT and tRNA CysGCA (named as tRF5-GlyGCC, GlyCCC, LysCTT and CysGCA respectively) in controlling RSV replication and associated chemokine/cytokine induction. We found that cells transfected with an anti-tRF5-GlyGCC or anti-tRF5-CysGCA oligos did not lead to significant changes in RSV replication compared to the cells transfected with control antisense oligo, while RSV replication in anti-tRF5-GlyCCC- or anti-tRF5-LysCTT-transfected cells was remarkably suppressed (more than a log). In addition, the inhibited viral replication led to impaired cytokines/chemokines induction. Vice versa, cells

supplemented with tRF5-GlyCCC and tRF5-LysCTT mimics significantly enhanced RSV replication, confirming these two tRFs are important for RSV replication. Further investigations are needed to unravel the molecular mechanisms underlying the functions of tRF5-GlyCCC and tRF5-LysCTT.

GP 31. ***Dictyostelium discoideum*** **Sociality During Growth with Gram-negative Bacteria**

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*Dictyostelium discoideum* is a eukaryotic amoeba that consumes bacteria during growth. Upon starvation, *Dictyostelium* cells cooperate with one another while developing into multicellular structures where some of the cells sacrifice themselves while supporting the sporulation of the others. Currently, sociality is defined as intercellular interactions during *Dictyostelium* development. We became curious as to whether *D. discoideum* is social during growth as well. Preliminary data led us to the hypothesis that *D. discoideum* is social during growth. To address this hypothesis, we used *D. discoideum* mutants that are unable to grow on Gram-negative bacteria (*Klebsiella pneumoniae*). However when mixed together, these mutants are able to grow, they synergize. We have tested several growth conditions and all have resulted in synergy. To verify that the mutant *D. discoideum* growth defect is bacteria specific, we tested growth in axenic medium. We found that the growth of the mutant *D. discoideum* is the same as wild type, verifying that the growth defect is bacteria specific. These data suggest that *D. discoideum* is social during growth, altering how we understand the growth stage of *D. discoideum* life cycle and amoeba-bacterial interaction.

GP 32. **Effect of 4-hydroxyacetophenone on *Chlamydomonas reinhardtii* Mutants Defective in Motility and Phototaxis**

Katrina L. Thornton, Deborah Sewell, and Anne R. Gaillard
Department of Biological Sciences, Sam Houston State University, Huntsville, TX

*Chlamydomonas reinhardtii* is a unicellular green alga that uses two anterior cilia to swim through the environment in search of light necessary for photosynthesis. Once a light source is detected by the eyespot, signaling pathways are activated that lead to the depolarization of the ciliary membrane, causing an influx of extracellular calcium ions which affect ciliary dominance required for phototaxis. As the cell rotates during phototaxis, the eyespot becomes shaded and the membrane repolarizes, leading to a decrease in the intraciliary calcium concentration. The cilia then return to a synchronous breast-stroke beating pattern. Wild-type *C. reinhardtii* (cc-125) undergoes positive phototaxis, the movement toward light, under low light intensities. However, a previous study has shown that cc-125 will undergo negative phototaxis under these same conditions after incubation with 4-hydroxyacetophenone (4HAP). To better understand the components involved in phototaxis and determine the target of 4HAP, we are studying the affect that 4HAP has on phototaxis of different *C. reinhardtii* strains defective in motility and phototaxis. Our preliminary findings using strains cc-124 (wild-type strain of different mating type), cc-2454 (cell wall-less), *agg1* (strongly undergoes negative phototaxis), *ptx1* (defective in the ciliary dominance shift), *ida1* (inner dynein arm defect) and A5/388 mutants have yielded some unexpected results.

GP 33. **Identification of *Candida albicans* Genes Involved in Extracellular pH Modulation in Glucose-Poor Conditions**

Elisa M. Vesely, Slavena Vylkova, Heather A. Danhof, and Michael C. Lorenz. Department of Microbiology and Molecular Genetics, The University of Texas Medical School at Houston, Houston, TX

*Candida albicans* is the most important fungal pathogen of immunocompromised individuals, causing severe systemic infections with high mortality rates including endocarditis, bloodstream infections and invasive candidiasis. Normally a part of the gastrointestinal microbiota, *C. albicans* is kept a commensal

by the innate immune system, notably neutrophils and macrophages. After phagocytosis by macrophages, pathogens are generally destroyed by rapid acidification of the nutrient-poor phagosome. However, we have shown *C. albicans*-containing phagolysosomes are not acidic. In vitro, in acidic, glucose-poor, amino acid-rich conditions predicted to mimic the phagolysosome*, C. albicans* can dramatically raise the extracellular pH by releasing ammonia derived from amino acid catabolism. We believe that *C. albicans* uses this to actively modulate the phagosome, creating a neutral pH that induces the hyphal growth form, allowing the cell to escape from the macrophage. Indeed, mutation of the Stp2 transcription factor impairs fungal survival after phagocytosis. To further identify genes that are important for the environmental alkalinization response, we performed a screen of ~1000 mutants for those that failed to alkalinize in conditions hypothesized to mimic those found in the maturing phagosome. Initial categorization of these mutants revealed several genes that are predicted to be involved in amino acid import and catabolism, consistent with our model, and transcription factors that regulate morphogenesis. These candidates are currently undergoing secondary screening to verify phenotypes associated with this modulatory event.

## Graduate Student Posters – Gram Negative Microbiology

GP 34. **Selection of Potential Protein Candidates for Vaccine Development Against Avian Colibacillosis**

Cindy Botero. Sam Houston State University, Huntsville, TX

Avian pathogenic *Escherichia coli* (APEC) is the etiologic agent of colibacillosis in birds, and is responsible for at least five percent of the mortality rate found in the poultry industry, leading to multimillion dollar global losses annually. APEC is an opportunistic colonizer of the respiratory system, most often following viral infections, and manifests as organ lesions, including cellulitis, air sacculitis, and septicemia. Recent studies have shown a link between APEC strains in contaminated poultry and the extraintestinal pathogenic *E. coli* causing (ExPEC) urinary tract infections in humans, therefore a vaccine developed against APEC could have a beneficial impact on human health (Lynne, 2012). I hypothesize that there are novel proteins that have not been previously explored in the more common virulent strains of APEC that can be upregulated by exposure to chicken serum and can elicit an immunogenic response in the chicken. This will be accomplished by first detecting novel proteins that are up-regulated by exposure to chicken sera, then detecting proteins that are recognized by the avian immune system, and finally by determining the best candidate proteins for an effective vaccine against avian colibacillosis.

GP 35. ***Pseudomonas aeruginosa* Quorum Sensing is Altered by Serum Albumin**

Allie Clinton and Kendra Rumbaugh
Department of Surgery, Texas Tech University Health Sciences Center, Lubbock, TX

Chronic wound infections cause high rates of morbidity and mortality in a large patient population and are responsible for a large medical cost burden in the US annually. *Pseudomonas aeruginosa* (PA) is one of the most common bacterial pathogens in chronic wounds, and much of the virulence associated with PA is controlled by a system of cell-cell communication termed quorum sensing, or QS, which involves three distinct regulatory systems (Las, Rhl and PQS). QS can alter the expression of over 5% of PA genes in response to the amount of specific chemical signals, or autoinducers, within an environment. Our lab has previously shown that the blood protein albumin inhibits the ability of PA to lyse *Staphylococcus aureus* and we hypothesize this is due to an inhibition of QS by albumin. In this study, we sought to identify how albumin is altering QS. We observed that QS-controlled exoproducts, such as LasA and LasB, were downregulated when PA was grown in the presence of albumin. We also saw that growth in albumin inhibited the production of the acylated homoserine lactones (AHL) 3OC12-HSL and C4-HSL, but promoted the production of quinolone-related autoinducers. Preliminary binding assays suggest that albumin can bind and sequester AHL-based autoinducers, which represses QS. We are now investigating the physiological relevance of these observations in albumin-rich and albumin-deplete *in vivo* environments.

GP 36. **L-Arginine as a Potential Treatment Option to Prevent Sepsis in Burn Wound Infections**
J.A. Everett[1], K.H. Turner[2], M. Whiteley[2], K.P. Rumbaugh[1] [1]Texas Tech University Health Sciences Center, Lubbock, TX [2]University of Texas at Austin, Austin, TX

Burn wounds represent a very unique form of physical trauma due to suppressed immunity and their proclivity to precede sepsis. Myeloid-derived suppressor cells (MDSC) are an emerging subset of immunoregulatory cells that are garnering much attention as a key contributor to immune suppression in burn wounds. A well reported mechanism of suppression utilized by MDSC is the sequestering of available L-arginine (Arg) necessary for efficient T-cell activation and effector cell responses. In this study, we investigated whether introducing excess L-Arg to the burn wound environment could overcome immunosuppression and protect against septic infection with the opportunistic pathogen *Pseudomonas aeruginosa* (Pa), which is a common cause of fatal burn wound infections. Here, we report that MDSC are recruited in large numbers to burn wounds and lead to an increase in arginase and NO levels that result in the depletion of L-Arg. Though bacteria were still able to propagate within the burn wound, the supplementation of L-Arg was beneficial in preventing their progression to sepsis in thermally injured mice. Taken together these results suggest that L-Arg supplementation could be used as a potential therapeutic option to aid in preventing bacterial sepsis in severely burned individuals.

GP 37. **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, TX

*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, 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 by disk diffusion method and also analyzed by PCR for the presence of 19 antimicrobial resistance genes. The resistance genes that were tested showed low percentage of resistance. When looking at the antimicrobials and resistance gene profiles together, ten out of the nineteen resistance genes showed resistance in both phenotypic and genotypic that was over 50%. Nine had showed resistance in both phenotypic and genotypic that was below 50%. Eight out those nine had a 0% resistance in the resistance gene but did have resistance in the phenotypic resistance profile. To determine if antimicrobial resistance is on the rise, further evaluation is needed to track the trends of *Salmonella enterica* infections.

GP 38. **Anti-*Salmonella* effect of thymol and thymol-β-D-glucopyranoside**
G. Levent[1,2*], G. Ciftcioglu[2], R. Anderson[1] [1]USDA/ARS, College Station, TX, [2]Istanbul Univ., Istanbul, Turkey

Thymol is a potent bactericide against zoonotic pathogens but its rapid absorption in the proximal gut prevents its passage to lower sites where these pathogens primarily reside. Thymol-β-D-glucopyranoside (β-thymol) has been shown to be bactericidal against *Campylobacter* during culture with β-gylcoside-hydrolyzing gut microbes but its activity has not been studied against *Salmonella*. Presently, we cultured ($39^{o}$C) porcine feces (0.1% wt/vol) in anaerobic 1/2-strength Mueller Hinton broth with $5.8 \pm 0.09$ $\log_{10}$ CFU/mL *Salmonella* Typhimurium and 0, 3, 6 or 12 mM thymol or β-thymol. Results revealed that *Salmonella* in 6 and 12 mM thymol-treated cultures were reduced 5 and 7 log units after 6 and 24 h incubation, respectively, from those in controls ($7.9 \pm 0.07$ and $8.1 \pm 0.09$ $\log_{10}$ CFU/mL, respectively). A transient anti-*Salmonella* effect occurred in cultures treated with 3 mM thymol, with counts being 5 log

units less than controls after 6 h incubation but only 2.6 log units less after 24 h. *Salmonella* were reduced from controls in cultures treated with 6 and 12 mM β-thymol, but there was a lag in the activity as counts were only 0.7 and 1.3 log units lower, respectively, after 6 h incubation but >6 log units lower after 24 h. Cultures incubated with 3 mM β-thymol were not significantly less than controls (0.2 and 2 log units after 6 and 24 h, respectively). Results reveal the anti-*Salmonella* activity of thymol and β-thymol, implicating acquisition of tolerance to low levels of thymol and a role of β-glycosidase-hydrolyzing microbes for the hydrolysis of β-thymol.

GP 39. **Mechanism of Pseudomonas Elastase- Induced Cytoskeleton Remodeling**

Bidisha Pal and Dr. Ali Azghani. Department of Biology, The University of Texas at Tyler, Tyler, TX

*Pseudomonas aeruginosa* is responsible for severe life-threatening infections.The pathogen causes infections in individuals with pre-existing disorders and recurrent pulmonary infections in cystic fibrosis patients. Pathogenesis of *P. aeruginosa* infections is multifactorial owing to numerous virulence factors including toxins and proteases.

We have reported earlier that *P. aeruginosa* elastase (PE) causes dislocation of tight junction (TJ) proteins that alters epithelial barrier function and that the defect is associated with EGFR – PKC signaling pathway. The aim of this study is to identify the mechanisms for PE induced cytoskeleton reorganization including the involvement of members of Ras Homologue gene family.

In addressing our hypothesis, pulmonary epithelial cell, Calu 3, was used to understand the PE induced Rho GTPase activity in presence and absence of specific inhibitors and activators. Redistribution of TJ proteins and reorganization of cytoskeleton were examined under fluorescence microscopy. SDS PAGE andWestern Blot Analysis were performed using specific antibodies. The loss of epithelial barrier function was analyzed by Trans-epithelial electrical resistance measurement.

We found that PE activates Rho GTPases and induces the formation of stress fibers comparable to EGF and specific Rho Activator. Further analysis of data from molecular and functional studies will reveal the mechanism(s) by which PE injures epithelia in vitro. These findings will aid in the prevention and treatment of pulmonary edema.

GP 40. **RNA Interference of *Ehrlichia chaffeensis* TRP47 Host Target Proteins Promotes Intracellular Survival**

Nancy K. Pohl[1], Tian Luo[2], and Jere McBride[1,2] Departments of Microbiology and Immunology[1], Pathology[2], University of Texas Medical Branch, Galveston, TX

**Background.** *Ehrlichia chaffeensis* is an obligately intracellular bacterium that survives in mononuclear phagocytes by circumventing the host cell immune response. *E.chaffeensis* tandem repeat protein (TRP) effectors have been shown to interact with eukaryotic proteins involved in major biological processes, including apoptosis. We previously determined that knockdown of most (73%) TRP32 target proteins had a significant effect on *E.chaffeensis* survival by either inhibiting or promoting ehrlichial infection. Other *E.chaffeensis* effectors including TRP47, TRP75, and TRP120 interact with various host proteins; however it is unknown whether the knockdown of these proteins enhance or reduce ehrlichial survival. The purpose of this project is to determine the role of the target proteins during ehrlichial infection. This project will provide a point of reference for future work to understand the role of TRPs in pathobiology. **Methods.** To determine the effects of the interacting proteins on ehrlichial survival, siRNA knockdown was performed in THP-1 cells. qPCR was used to quantify ehrlichial load. Finally, immunofluorescence was used to visualize the location of highly interacting protein in infected THP-1 cells. **Results.** SiRNA knockdown of TRP47 host interacting proteins CAP1, Lambda5, FYN, and PTPN1 increased ehrlichial load in human macrophages. These findings suggest that *E. chaffeensis* may sequester or promote degradation of these host proteins to promote intracellular survival.

GP 41. **Identifying the Cellular Target of the Novel Antifungal Compound Occidiofungin**
Akshaya Ravichandran and James Leif Smith Dept. Biology, Texas A&M University, College Station, TX

Due to the rampant occurrence of strains of fungi that are resistant to the common classes of antifungals, such as azoles, echinocandins and polyenes, there is a rising need to identify novel antifungal agents. Occidiofungin is a non-ribosomally synthesized glycolipopeptide, produced by Burkholderia contaminans, which is a soil bacterium. Occidiofungin has a base mass of 1200 Da and consists of eight residues which include standard and non-standard amino acids. The compound is rapidly fungicidal against a wide spectrum of fungal species and has been shown to cause minimal toxicity in murine models. Previously, we demonstrated that occidiofungin triggers apoptosis in target fungal cells. The exact molecular target that the compound interacts with, to cause programmed cell death, is yet unknown. In this study, we attempt to use click chemistry to track the localization of occidiofungin inside a fungal cell. Occidiofungin, functionalized with a reactive alkyne group is used to treat the fungal cells. Subsequently, the compound is probed using an azide functionalized fluorophore to detect areas of localization. By utilizing strains of Saccharomyces cerevisiae that have organelles tagged with a different fluorophore, colocalization is done and it is seen that occidiofungin does not co-localize to either the endoplasmic reticulum or the mitochondria. On performing a time course experiment, it can be seen that occidiofungin localizes in a pattern similar to that of actin patches. Further studies to explore actin and the endocytic pathway will be carried out to determine the molecular target.

GP 42. ***Ehrlichia chaffeensis* TRP47 Binds Host DNA and is SUMOylated**
Sarah L. Schmidt[1,] Tierra Farris[2], and Jere W. McBride[1, 2] Department of Pathology[1], Department of Microbiology and Immunology[2], University of Texas Medical Branch, Galveston, TX

**Background:** *Ehrlichia chaffeensis* is an obligately intracellular bacterium and the causative agent of human monocytic ehrlichiosis, a life-threatening tick-borne illness. Using a type 1 secretion system, it translocates immunoreactive tandem repeat protein (TRP) effectors into the host cell. One of these effectors, TRP120, is a nucleomodulin and binds host DNA via its tandem repeat domain. It was also recently identified as the first known bacterial protein to be directly conjugated with the small ubiquitin-like modifier (SUMO). SUMOylation is a multifunctional post-translational modification linked to nuclear localization. TRP47, another effector protein, is also of interest because it is the most highly expressed ehrlichial protein during infection of mammalian cells and interacts with multiple eukaryotic proteins. In this study, we investigated the localization, function, and potential SUMOylation of TRP47. **Methods:** Localization in infected host cells was determined using immunofluorescence microscopy. DNA binding was assessed with an electrophoretic mobility shift assay. *In silico* SUMOylation site predictions were made using GPS-SUMO, and SUMOylation was detected with an *in vitro* SUMOylation assay. **Results:** TRP47 binds DNA and localizes to the host cell nucleus during infection. *In silico* prediction methods identified two putative SUMOylation sites in the N-terminal domain, and in the *in vitro* SUMOylation assay, TRP47 was found to be modified by SUMO-2.

GP 43. **Improved Understanding of the Mechanism of *vlsE* Antigenic Variation Through High-Throughput DNA Sequencing**
Surabhi Tyagi, Diane G. Edmondson and Steven J. Norris. Department of Pathology and Laboratory Medicine, The University of Texas Medical School at Houston. Houston, TX

Antigenic variation plays a vital role in the pathogenesis of Lyme disease. VlsE, a 35 kDa surface-exposed lipoprotein, undergoes antigenic variation during *B. burgdorferi* infection of mammalian hosts, and appears to be an important mechanism by which these organisms undergo immune evasion. Segmental gene conversion between the expressed *vlsE* gene and adjacent *vls* silent cassettes generates a large number of different VlsE variants in infected animals.

The study of the mechanism of *vlsE* variation has been hampered by lack of detectable *vlsE* variation during in vitro growth. To develop an in vitro model system to study *vlsE* recombination, various mouse tissue explants were inoculated with *B. burgdorfer*i and examined the ability of the explants to

promote *vlsE* recombination. The goal of this project is to develop a protocol using 454 Next Generation Sequencing technique to characterize *vlsE* recombination events during infection of mice and to delineate rare *vlsE* recombination events in the presence or absence of tissue explants. A second method known as Quantitative PCR will be used as means to verify sequencing results.

GP 44. **Identification of *Ehrlichia chaffeensis* Effector Proteins Targeted for SUMOylation**
Gabriela Uribe[1], Tierra R. Farris[1], Xiaofeng Zhang[2], and Jere W. McBride[1, 2] Departments of Microbiology and Immunology[1] and Pathology[2], University of Texas Medical Branch, Galveston, TX

In eukaryotes, small ubiquitin-related modifier (SUMO) proteins are conjugated to proteins in order to modulate intracellular processes such as protein interactions and nuclear translocation. Previously, we reported the first bacterial protein modified by the host SUMOylation system, the type 1 secreted (T1S) effector, TRP 120, of *Ehrlichia chaffeensis*. The purpose of this study was to identify additional ehrlichial effectors that are modified by SUMO. We used *in silico* analysis using Secretome P and GPS-SUMO by Biocuckoo to identify 3 hypothetical proteins that appear to be type 1 substrates and are predicted to be SUMOylated. These proteins were cloned into prokaryotic expression vectors and antigenicity was determined by immunoblot and ELISA. To further define protein function and molecularly define post translational modifications, genes encoding these proteins were cloned into mammalian pAC-GFP vectors and ectopically expressed in HeLa cells cotransfected with HA-SUMO(1/2/3) and modifications examined by immunoprecipitation and subsequently analyzed by IFA. Future studies include *in vitro* SUMOylation assays to determine specific lysine residues that are modified by SUMO. The goal of this project is to identify additional *E. chaffeensis* effector proteins and determine the role of SUMOylation in effector function. This project is significant because PTMs such as SUMO are critical for effector function and facilitate pathogen-host interactions.

GP 45. **Elucidating the bactericidal activity of *Burkholderia contaminans* MS14**
Adam L. Guerrero and James L. Smith. Dept. of Biology, Texas A&M University, College Station, TX

**Introduction:** *Burkholderia contaminans* MS14 is a Gram-negative soil bacterium that naturally produces occidiofungin, a broad spectrum antifungal that is currently a subject of research in our lab. However, *Burkholderia contaminans* MS14 has recently been shown to have bactericidal activity against Gram-negative bacteria when grown under different culture conditions. Two transposon mutants were made that resulted in the loss of bactericidal activity. One insertion was in a non-ribosomal peptide synthesis (NRPS) module, while the other insertion was in a LuxR regulator. **Hypothesis:** The NRPS module is involved in the synthesis of the bactericidal product. **Methods:** Chrome azurol S (CAS) plates are used to assess siderophore activity. Deferred antagonism and minimum inhibitory concentration assays (MICs) are used to evaluate the bacterial inhibitory activity of isolated fractions. Products were purified using poly-aromatic absorbance resins, high performance liquid chromatography (HPLC), and dialysis. The products were characterized through the use of matrix-assisted laser desorption/ionization (MALDI-MS) and two-dimensional nuclear magnetic resonance (NMR). **Results:** An NRPS product has been isolated from wild-type MS14 that is not present in the NRPS mutant strain MT577; however the NRPS product was present in the LuxR mutant strain MT357. The isolated product inhibits the rate of growth of the Gram-negative indicator strain, but does not have bactericidal activity. The isolated product has potent siderophore activity. This siderophore appears to be important for the production of the bactericidal compound, as co-cultures of the NRPS and LuxR regulator mutants are capable of restoring the bactericidal phenotype. The siderophore structure is being determined by mass spectrometry and nuclear magnetic resonance. The siderophore has a mass of 737 Da and is predicted to be composed of about six to seven amino acid residues. Additional studies aimed at understanding why the production of the siderophore is necessary for the production of the bactericidal compound are ongoing.

## Postdoctoral Fellow Posters

PP 1. **Structural Analysis of *Corynebacterium diphtheriae* Pilus-Specific Sortase SrtA Reveals a Gating Mechanism for Substrate Entry**

Chungyu Chang,[‡,1] Jerzy Osipiuk, [‡,2] I-Hsiu Huang,[1,3] Bo Chen,[1] Erika Flores,[1] Piotr Gornicki,[2] Andrzej Joachimiak,[2,4,*] and Hung Ton-That[1,*]1Department of Microbiology & Molecular Genetics, University of Texas Health Science Center, Houston, TX [2]Argonne National Laboratory, Biosciences Division, Midwest Center for Structural Genomics and Structural Biology Center, Argonne, IL [3]Department of Microbiology and Immunology, College of Medicine, National Cheng Kung University, Tainan, Taiwan [4]Department of Biochemistry and Molecular Biology, University of Chicago, Chicago, IL.

In Gram-positive bacteria, pilus polymerization is catalyzed by a conserved transpeptidase enzyme known as pilin-specific sortase, while cell wall anchoring of the resulting pilus polymers is mediated by a non-polymerizing sortase or the housekeeping sortase. Although all sortases display similar folding, pilin-specific sortase enzymes harbor a structural lid that is absent from the housekeeping sortases. However, a specific role of the lid in pilus assembly is not well understood. Here, we report the X-ray crystal structure of the *Corynebacterium diphtheriae* pilin-specific sortase SrtA, at 2.1-Å resolution. Consistently, SrtA contains the lid made of the lid motif DPW, which covers the catalytic triad Cys222, His160, and Arg231 of the enzyme. Alanine-substitution of Cys222 and His160 abrogated pilus assembly *in vivo*, whereas pilus polymerization was severely defective with R231A mutation. In contrast, alanine/glycine-substitutions of the lid residues D81, W83, or both did not affect pilus polymerization. Significantly, using 4,4'-dithiodipyridine, a sulfhydryl-reactive reagent, to probe the reactivity of the catalytic Cys222, it was shown that the lid DW mutant exhibited an increase in the kinetic rate constant of more than 80 fold as compared to that of the wild-type enzyme. Consistently, the mutant enzyme was more reactive than the wild-type, as demonstrated by its ability to catalyze pilus polymerization *in vitro*. Structural changes within the catalytic pocket caused by the lid mutations were also evident by the crystal structure of the lid DW mutant, which revealed no electron density for the lid, suggesting that the mutated lid was mobile or had multiple conformations. Altogether, our findings support the model that the lid is involved in a gating mechanism for substrate entry, possibly controlling the switch between pilus polymerization and cell wall anchoring of pilus polymers.

PP 2. **A Heme-Iron Sensor Modulates Surface Assembly of Adhesive Pili in *Corynebacterium diphtheriae***

Bo Chen,[‡,1] Jerzy Osipiuk,[‡,2] Chungyu Chang,[‡,1] I-Hsiu Huang,[1,3] Beth Rogers,[1] Andrzej Joachimiak,[2,4,*] and Hung Ton-That[1,*] [1]Department of Microbiology & Molecular Genetics, University of Texas Health Science Center, Houston, TX [2]Argonne National Laboratory, Biosciences Division, Midwest Center for Structural Genomics and Structural Biology Center, Argonne, IL [3]Department of Microbiology and Immunology, College of Medicine, National Cheng Kung University, Tainan, Taiwan [4]Department of Biochemistry and Molecular Biology, University of Chicago, Chicago, IL

In Gram-positive bacteria, surface display of many virulence determinants including pili requires sortase, a conserved transpeptidase enzyme that catalyzes cell wall anchoring of LPXTG motif-containing surface proteins. In some Gram-positive pathogens, cell wall anchored proteins are also released into the extracellular milieu by cell wall hydrolase enzymes, presumably participating in host immunomodulation. Here we describe a novel mode of surface protein release in *Corynebacterium diphtheriae* that involves previously uncharacterized factors encoded by the *safABC* locus in close proximity with the housekeeping sortase gene *srtF*. Upstream of the *saf* locus are *hrrA* and *hrrS*, coding the two-component system HrrA-HrrS previously shown to be responsive to heme. Remarkably, deletion of *safA* resulted in abundant release of sortase-dependent SpaA pili, a virulence determinant of *C. diphtheriae* required for bacterial pathogenesis; concomitantly, sortase SrtF was cleaved and released into the culture medium. In contrast, these defects were not observed in other *C. diphtheriae* mutant strains. To unveil the function of SafA, a crystal structure of SafA was determined to 1.74 Å, and computational analysis by the DALI server predicted that SafA is structurally homologous to the sensor domain of *Mycobacterium tuberculosis* serine/threonine kinase PknH, suggesting that SafA is a sensor molecule. In addition, *Escherichia coli*

cells expressing recombinant SafA (rSafA) turned red/brown upon addition of hemin, indicative of iron-binding affinity. Consistent with the notion that SafA is a heme-iron binding protein, cell growth of the *C. diphtheriae* wild-type strain was subdued in the presence of elevated levels of hemin, and this defect was rescued with addition of rSafA to the cell culture; however, no significant defects were observed in the *safA* mutant with the same conditions. By RNA sequencing, it was found that in the absence of *safA*, genes encoding factors for sulfur metabolism and the SufRBDCU biosynthetic machine for iron-sulfur cluster assembly were up-regulated, whereas a wider range of genes were down-regulated. Altogether, we propose that SafA is a heme-iron sensor molecule for a signal transduction system that regulates expression of iron-sulfur cluster assembly, which modulates surface pilus assembly via SrtF cleavage in response to environmental signals.

PP 3. **Functional Motifs Responsible for Human Metapneumovirus M2-2-mediated Immune Evasion**

Yu Chen[1], Guangliang Liu[1], Xiaoyong Bao[1] [1]Pediatrics, Univ. of Texas Medical Branch, Galveston, TX

Human metapneumovirus (hMPV) is a leading cause of lower respiratory infection in young children, the elderly and immunocompromised patients. Repeated hMPV infections occur throughout life. However, immune evasion mechanisms of hMPV infection are largely unknown.

hMPV encodes nine viral proteins. Compared to other hMPV proteins, M2-2 is unique in its multiple functions. Our group found M2-2 not only promotes viral gene transcription and replication but subverts host innate immunity through binding to the central antiviral signaling molecule(s), therefore, contributing significantly to immune evasion of hMPV. In airway epithelial cells, M2-2 targets mitochondrial antiviral-signaling protein (MAVS) to fight against host immune responses.

Through the sequence analysis, we found that the M2-2 protein is enriched with PDZ domains, which are a common structural domain for protein-protein interaction. Herein, we mutated the PDZ-binding motifs 29-DEMI-32 and 39-KEALSDGI-46, located in an immune inhibitory region of M2-2, to investigate whether they are the responsible motif(s) for M2-2-mediated immune evasion. We found that recombinant hMPV containing mutation in E30 and M31 (rhMPV-E30M31AA) induced more NF-kB-dependent genes than wild type rhMPV (rhMPV-WT), suggesting the motif 29-DEMI-32 was responsible for the suppression of hMPV-induced NF-kB gene expression. However, the motif 29-DEMI-32 did not affect M2-2-mediated IRF-3-dependent gene induction. The mutagenesis studies on the motif 39-KEALSDGI-46 demonstrated that it was critical for both NF-kB- and IRF3-dependnent chemokine/cytokine inductions with a more dominant role in IRF3-mediated pathway. Currently we are investigating the molecular mechanisms underlying the functions of M2-2 motifs in the activation of NF-kB and IRF-3.

PP 4. **MSCRAMMs of *Staphylococcus aureus* USA300 TCH1516 that Interact with Human Fibrinogen**

Ana Luisa V. Cohen, Brooke H. Russell, Magnus Hook. Center for Infectious and Inflammatory Diseases, Institute of Biosciences and Technology, Texas A&M Univ. Health Science Center, Houston, TX

The gram positive bacterium strain *Staphylococcus aureus* USA300 is the most common clinical strain recovered from human skin infections, endocarditis, osteomyelitis, pneumonia, and sepsis in the USA. This bacterium uses its microbial surface components recognizing adhesive matrix molecules (MSCRAMMs) to initiate and establish colonization of the host. A key player is the clumping factor A (ClfA) that allows the bacterium to interact with fibrinogen. Other MSCRAMMs such as clumping factor B (ClfB), Fibronectin-binding protein A and B (FnbpA, FnbpB) and the bone sialoprotein-binding protein (Bbp) have also been shown to play a role.

We set to determine the contributions of each known MSCRAMM of *S. aureus* USA300 TCH1516 in the adherence to fibrinogen. For that purpose we generated combined mutations of these genes and performed a bacterial adherence assay to fibrinogen.

Results show that adherence to human fibrinogen (hFg) is growth phase dependent. In stationary phase, ClfA is the major contribute to this interaction, whereas in early exponential phase, a novel(s)

MSCRAMMs mediate(s) bacterial adherence. Further characterization of the interaction suggests that the protein(s) bind(s) to the C-terminal of the gamma and alpha chains of hFg. Studies are under way to identify this protein(s).

This study highlight the presence of MSCRAMMs that otherwise would remained unidentified in laboratory strains. The presence of this MSCRAMM(s) could justify the increased ability of USA300 strains to colonize the human host.

PP 5. **Humanized Microbiota Mice as a Model of Recurrent *Clostridium difficile Infection***

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*Clostridium difficile* causes disease following antibiotic induced disruption of the indigenous microbiota. Without disruption *C. difficile* infection is extremely rare, furthermore re-introduction of a 'healthy' microbiota in patients with severe *C. difficile* infection is highly effective. A growing concern, however, is the potential to unintentionally transfer infective pathogens or even to introduce a microbial population with the potential to affect metabolism. Because of this the development of a well-defined cocktail of bacterial strains capable of treating or preventing *C. difficile* infection is an attractive alternative.

To examine *C. difficile* infection and test potential treatments/prophylactics we have developed a humanized microbiota (HMb) mouse model. The benefit of which is that bacteria identified as protective or curative in the HMb model were initially isolated from the human digestive tract and are therefore more likely to have a direct clinical benefit in humans.

Here we show that gnotobiotic C57BL/6 mice gavaged with pooled human fecal slurry from healthy volunteers maintain a stable humanized microbiome over multiple generations. The use of antibiotics to disrupt the HMb results in a disease state ranging from asymptomatic, temporary, colonization to rapid, and in some cases severe, disease in a strain specific manner. Furthermore, mice that have recovered from disease and are not producing *C. difficile* spores at a detectable level can be induced to relapse following a single clindamycin IP dose - even when administered up to 30 days post recovery from initial disease state.

PP 6. **Thermal Springs Sediment Microbial Survey**

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The microbial communities of 16 thermal spring sediments in five US states (Wyoming, California, Nevada, Utah, and New Mexico) were compared based on profiles from partial 16s rDNA sequences. The sediments spanned a wide range of both pH and temperatures. A diverse assortment of bacteria and archaea were found throughout the temperature, pH, and geographic ranges. At the extremes of both low pH (2.1 – 4.1) and high temperature (82.1 – 92.7C) the lowest amounts of bacterial community diversity were observed. These communities were dominated by bacteria from the phylum Proteobacteria (>90% of sequences). Archeal diversity appears to relate more to geographic origin of the samples than to the pH or temperature of the sediment. The Archeal phylum Nanoarchaeota was found to dominate (>50% of sequences) in most of the high temperature Yellowstone National Park samples. Nanoarcheota were not found to comprise more than a trace membership within the Archeal diversity of samples from outside of Yellowstone National Park, but were found in samples from the New Mexico Sulfur Springs in New Mexico (pH 2.6, 58.7C) and the Great Boiling Springs in Nevada (pH 8.2, 81.7C). Comparisons of archeal and bacterial community composition across this diversity of sediments from extreme environments are presented.

PP 7. **Unraveling the Interaction Between the Gut Microbiota and Mucus**

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Gut pathogens initially encounter the host at the intestinal mucus layer making its regulation a crucial aspect of gut health. Intestinal mucus provides bacterial binding sites, fuel source (oligosaccharides), and protection from intestinal expulsion. As a result, commensal and pathogenic bacteria derive significant benefit from an ability to bind and regulate mucus synthesis or secretion. Few studies have addressed bacteria-mucus binding or mucus oligosaccharide utilization by commensal or pathogenic bacteria. To address this gap in knowledge, adhered mucus from human stool (MUC2), pig stomach (MUC 5ac; MUC6) and HT-29-MTX secreted mucus (MUC 5ac) or cultured HT-29-MTX cells were incubated with commensal bacteria (*Bacteroides thetaiotaomicron, Clostridium coccoides, Clostridium butryicum, Blauta producta, Akkermansia muciniphila, Faecalibacterium prausnitzii, Lactobacillus reuteri)* and the pathogen *Clostridium difficile.* Differential binding preferences were observed between bacteria and mucin type. *In vitro* studies using brain-hear-infusion media supplemented with oligosaccharides revealed that *B. producta* utilized all oligosaccharides, while the other bacterial species used specific oligosaccharides for growth. FITC-labeled oligosaccharide specific lectins on adhered mucus after bacterial incubation demonstrated that all commensal bacteria utilized fucose, galactose, N-acteylgalactosamine, and N-acetylglucosamine. Only *B. producta*, *A. muciniphila* and *L. reuteri* utilized mannose and *C. difficile* did not utilize any adhered mucus oligosaccharides. Together this data sheds light on mucus binding and oligosaccharide utilization by the gut microbiota.

PP 8. ***Akkermansia muciniphila* Exacerbates Gut Inflammation for *Salmonella* Typhimurium Induced Acute Enterocolitis in Gnotobiotic Mouse Model**

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This study investigated how the presence of a mucin degrading commensal bacterium *Akkermansia muciniphila (Amuc)* affects the out-come of an intestinal *S.* Typhimurium (*STm*)-induced gut inflammation. Using a gnotobiotic mouse model with a background microbiota of 8 bacterial species (SIHUMI) we investigated the impact of *Amuc* (SIHUMI-A) on inflammation and infectious symptoms caused by *STm*. Presence of *Amuc* in *STm*-infected mice caused significantly increased histopathology scores and elevated mRNA levels of IFN-γ, IP-10, TNF-α, IL-12, IL-17 and IL-6 in cecal and colonic tissue. This increase in pro-inflammatory cytokines was accompanied by increased macrophage recruitment and by 1 $\log_{10}$ higher *STm* cell numbers in mesenteric lymph nodes of SIHUMI mice associated concomitantly with *Amuc* and *STm* (SIHUMI-AS) compared to SIHUMI mice with only *STm* (SIHUMI-S). Mucin filled goblet cell numbers were 2 to 3 fold lower in cecal tissue of SIHUMI-AS mice compared to SIHUMI-S, SIHUMI-A or SIHUMI mice. Reduced goblet cell numbers significantly correlated with increased IFN-γ ($r^2$ = 0.8618, ****P<0.001*) mRNA levels of the mice. Independent of *STm* infection, concentrations of N-acetylneuraminic acid (NANA) of cecal mucosa was significantly increased in the presence of *Amuc*. Concomitant presence of *Amuc* and *STm* resulted in a drastic change in existing microbiota composition. This was not observed in SIHUMI mice with either one of the two organisms being present. The proportion of *B. thetaiotaomicron* decreased from 88% to 0.02% while *STm* increased from 2.2% to 94% in SIHUMI-AS mice compared to other groups. We propose that *Amuc* exacerbates *STm*-induced intestinal inflammation by its ability to disturb host mucus homeostasis thereby turning into a pathobiont.

PP 9. **Long Chain Fatty acyl-coenzyem a synthesases as Novel Drug Targets in *Cryptosporidium parvum***

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*Cryptosporidium parvum* infects both humans and animals, and continues to be a significant opportunistic pathogen among AIDS patients and one of the leading diarrheal pathogens in children. Despite decades of research on cryptosporidiosis including screening hundreds of compounds in vitro and in vivo, fully effective therapeutic agents are still unavailable. The major goal of this study is to explore parasite three long-chain fatty acyl-CoA synthetase (CpACSs) as novel drug targets. The molecular and biological data showed that three CpACSs genes are differently expressed in the parasite different life stages and their proteins localized subcellularly in the parasite, suggesting that the three CpACS genes those genes may play different biological roles in the parasite. Using recombinant proteins, we have determined detailed enzyme kinetics for CpACS1 and CpACS2, and observed that the inhibitor triacsin C could inhibit their enzyme activities with $K_i$ in the nanomolar range. Triacsin C also effectively inhibited the growth of *C. parvum* parasites in vitro ($IC_{50}$ = 136 nM). Most importantly, triacsin C effectively reduced parasite oocyst production up to 88.1% with no apparent toxicity when administered to *Cryptosporidium*-infected IL-12 knock-out adult mice at 15 mg/kg/d for one week. These observations not only validate CpACSs as a pharmacological target, but also indicate that triacsin C and analogs may be explored as potential new therapeutics against cryptosporidiosis.

PP 10. **3-D Structure of an Intact Type III Secretion Machine with Its Cytoplasmic Complex *in situ***

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Bacterial type III secretion systems (T3SS) inject host-altering proteins into target eukaryotic cells. T3SS injectisomes possess an extracellular needle, a basal body and a cytoplasmic complex that regulates needle assembly and effector secretion. We used high throughput cryo-electron tomography and sub-tomogram averaging to determine molecular architectures of native and mutant injectisome *in situ* of the diarrheal pathogen *Shigella flexneri*. Our structures revealed that the Spa47 ATPase is anchored to a novel *spa33*-encoded hexameric complex via linker protein MxiN beneath the base of the injectisome. Disrupting these linkages has a dramatic impact on T3SS function. Despite having evolutionarily related components, the structures of the T3SS cytoplasmic complex are distinct from those of bacterial flagella.

PP 11. **Effects of *L. reuteri* Derived Histamine on IL-8 Expression Using *in vitro* Cell Culture Models**

Coreen Johnson[1,2], Bhanu Priya Ganesh[1,2], Monica Lugo[1,2], Vanessa Jackson[1,2] and James Versalovic[1,2] [1]Pathology & Immunology, Baylor College Medicine, [2]Pathology, Texas Children's Hospital, Houston, TX

Probiotics may affect the disease course of patients with chronic immune-mediated disorders such as inflammatory bowel disease via modulation of the host immune system. One such microorganism, *Lactobacillus reuteri* ATCC PTA 6475, has been shown to convert a dietary component, L-histidine, to the biologically active immunoregulatory signal histamine via a histidine decarboxylase. The produced histamine from *L. reuteri* has been shown to suppress the pro-inflammatory cytokine TNF in human myeloid cells thereby modulating host immunity. However, as the primary contact of the *L. reuteri* derived histamine is the intestinal epithelial cell (IEC) layer, our project sought to identify how histamine affects the expression of cytokines from epithelial cells. Our studies showed that *L. reuteri* derived histamine significantly suppresses the expression of the pro-inflammatory cytokine IL-8 by HT-29 cells stimulated with IL-1β, compared to that of the control groups without histamine. In addition, HT-29 cells treated with

commercially available histamine showed decreased expression of IL-8. Overall, *L.reuteri* derived histamine shows beneficial effects by modulating pro-inflammatory cytokines.

PP 12. **Four Cases of Methylobacterium radiotolerans Bacteremia: Microbiologic and Clinical Features**

Li Li, Jeffrey J. Tarrand, and Xiang Y. Han, Clinical Microbiology Laboratory, The University of Texas M. D. Anderson Cancer Center, Houston, TX

Methylobacterium species are environmental organisms and are rarely isolated in clinical microbiology laboratories. They are fastidious pink gram-negative rods. Among these organisms, Methylobacterium mesophilicum has been occasionally reported as an opportunistic pathogen that may infect the immunocompromised hosts, such as patients with malignancy, organ transplant, HIV infection, renal failure, or alcoholism [1-4]. Methylobacterium radiotolerans is less known, however, with one report of two cases of infection [5]. In this study, we report four cases of central venous line-related M. radiotolerans bacteremia in patients with leukemia.

PP 13. **Examining the Sequence Requirements for Metallo-β-Lactamase CphA Function by Deep Sequencing**

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CphA is a class B2 metallo-β-lactamase (MBL) identified from the Gram-negative bacterial pathogen *Aeromonas hydrophila*. Similar to other MBLs, CphA requires divalent metal ion cofactors such as $Zn^{2+}$ to hydrolyze β-lactam antibiotics. However, CphA differs from other MBLs in that it is stimulated by low concentrations (nM) but inhibited by high concentrations (μM) of $Zn^{2+}$ and is only active against carbapenem antibiotics. Therefore, to understand the sequence determinants for the function of CphA, randomized single site mutation libraries were constructed for amino acid residues around the active site of CphA. The libraries of CphA random mutants were sorted based on function by selecting for growth of *E. coli* containing the libraries on agar plates containing the carbapenem antibiotic, imipenem. Imipenem resistant mutants were pooled, PCR amplified, and subjected to Illumina sequencing, which produced $1.8x10^7$ reads in total and $1.4\ x10^5$ reads on average for each individual library. Sequence analyses showed that in addition to residues involved in $Zn^{2+}$ binding, many other residues were also conserved in libraries selected by high levels of imipenem. Consistently, mutation in any of these residues attenuated the function of CphA in conferring imipenem resistance. Biochemical analysis confirmed that this reduction in function was attributed to a decrease in protein stability and/or catalytic activity of the mutant CphA. On the other hand, a few positions that tolerate substitutions under stringent selection conditions were also identified and some mutants even displayed higher imipenem-hydrolytic activity than wild type CphA. This study conclusively identified essential and non-essential amino acid residues for the function of CphA and suggests a new approach for studying sequence determinants for the function of enzymes or other bioactive proteins.

PP 14. **Human Microbial Strains Capable of Inducing Glucagon-Like Peptide-1 Production as Type II Diabetes Mellitus and Obesity Therapeutics**

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The World Health Organization (WHO) projects that Type II Diabetes Mellitus (T2DM) will be the 7th leading cause of death by 2030. In 2008, according to the WHO, 35% of adults were overweight and 11% were obese. The gut microbiota plays an important role in cardiovascular disease, T2DM, obesity, hypercholesterolemia and non-alcoholic fatty liver disease. The human gut microbiota has been shown to

alter hormone secretion, including glucagon-like peptide-1 (GLP-1). GLP-1 has numerous functions, including promoting insulin secretion, insulin sensitivity and β-cell mass, while inhibiting glucagon secretion, β-cell apoptosis, gastric emptying and appetite. GLP-1 homologs are currently being used as T2DM therapeutics. Unfortunately, these formulations have important limitations. Microorganisms capable of enhancing GLP-1 production may provide a successful alternative therapeutic to combat T2DM and obesity. The objective of this project is to identify human associated bacteria that have the ability to stimulate GLP-1 secretion from intestinal L cells in culture. Over 1000 microbial strains were isolated from human fecal samples, breast milk, and healthy human colon and ileum biopsies. For screening purposes, bacterial supernatants are incubated with GLP-1 producing L cells. Following incubation, supernatant GLP-1 levels are quantified by ELISA. From the screening of 650 microbial isolates, 45 strains capable of increasing GLP-1 expression have been identified and require further investigations. Further experiments are needed to elucidate the mechanism(s) of action behind the observed increases in GLP-1 levels. Ultimately, a safe and efficient microbial therapeutic formulation for combatting obesity and T2DM is desired.

PP 15. **Lethality of Sortase Depletion in *Actinomyces oris* Caused by Excessive Membrane Accumulation of a Surface Glycoprotein**

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Sortase, a cysteine-transpeptidase conserved in Gram-positive bacteria, anchors on the cell wall surface proteins that facilitate bacterial pathogenesis and fitness. Genetic disruption of the housekeeping sortase in several Gram-positive pathogens reported thus far attenuates virulence, but not bacterial growth. Paradoxically, we discovered that depletion of the housekeeping sortase SrtA was lethal for *Actinomyces oris*; yet, all of its predicted cell wall-anchored protein substrates (AcaA-N) were individually dispensable for cell viability. Using Tn5 transposon mutagenesis to identify factors that upend lethality of *srtA* deletion, we uncovered a set of genetic suppressors harboring transposon insertions within genes of a locus encoding AcaC and a LytR-CpsA-Psr (LCP)-like protein. AcaC was shown to be highly glycosylated and dependent on LCP for its glycosylation. Upon SrtA depletion, the glycosylated form of AcaC, hereby renamed GspA, was accumulated in the membrane. Overexpression of GspA in a mutant lacking *gspA* and *srtA* was lethal; conversely, cells overexpressing a GspA mutant missing a membrane localization domain were viable. The results reveal a unique glycosylation pathway in *A. oris* that is coupled to cell wall anchoring catalyzed by sortase SrtA. Significantly, this novel phenomenon of glyco-stress provides convenient cell-based assays for developing a new class of inhibitors against Gram-positive pathogens.

PP 16. ***Cryptosporidium* Lactate Dehydrogenase is Associated with Parasitophorous Vacuole Membrane and Can Serve as a Drug Target**

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The apicomplexan *Cryptosporidium parvum* possesses a bacterial-type lactate dehydrogenase (CpLDH) that is considered as an essential enzyme, as this parasite lacks the Krebs cycle and cytochrome-based respiration, and mainly (if not solely) rely on glycolysis to produce ATP. Here we provide evidence that CpLDH was localized in the cytosol in the extracellular parasites (e.g., sporozoites and merozoites), but became associated with the parasitophorous vacuole membrane (PVM) during the intracellular developmental stages, suggesting the involvement of PVM in the parasite energy metabolism. We also fully characterized the biochemical features of CpLDH and observed that the LDH inhibitor gossypol could inhibit the parasite growth at low micromolar levels ($IC_{50}$ = 11.8 μM), mainly by acting on the parasite later developmental stages. These observations not only revealed a new function of the poorly understood PVM structure hosting the intracellular development of *C. parvum*, but also confirmed that LDH could be

targeted for developing therapeutics against this opportunistic pathogen for which fully effective treatments were yet unavailable.

## Faculty and Staff Posters

FS 1. **Serial Block Face SEM Visualization of Tuberculosis Infected Macrophages.**
Jonas A. Actor[1,2], Shen-An Hwang[3], William Monroe[4], Dustin Morado[3], Angel Paredes[4], Jun Liu[1,3], Jeffrey K. Actor[1] [1]UT-Medical School Summer Research Program, [2]University of Chicago, [3]Dept. Pathology, Univ. Texas-Houston Medical School, [4]U.S. Food and Drug Admon.*, Jefferson, AR

Tuberculosis is the most common infectious disease worldwide; its etiological agent *Mycobacterium tuberculosis* (*Mtb)* is present in more than a third of the global population. While much is known about the overall immune response, it is still unknown as to why granulomatous *Mtb* will cause necrotic tissue damage, develop into latency, or will heal to form a Ghon complex. Current efforts focus on whether these outcomes are determined by the initial innate immune response elicited during *Mtb* infection. We aim to examine the trafficking within an infected macrophage host during the initial stages of infection by three-dimensional (3D) modeling of the *Mtb* bacteria within macrophage host cells during the innate immune response, using the groundbreaking serial block-face scanning electron microscopy (SEM). Such an approach has previously been impractical because of the low noise-to-contrast ratios in other microscopy techniques at this scale. J774A.1 macrophages were exposed to *Mtb* or non-virulent BCG and then prepared with an osmium tetroxide stain. A macrophage monolayer was then embedded in Epoxy resin (LX112). Scanning electron microscopy using Gatan 3View SEM took ~600 cross sections of 50nm thickness at unprecedented resolution. Together with advantaged image processing and segmentation, we revealed novel cellular models of macrophages infected with mycobacteria. This also provided structural evidence of an increase in vesicle formation in *Mtb* infected macrophages compared to the avirulent BCG organisms. These results highlight the usefulness of 3D models generated by this novel application of SEM. Future research will expand this model to quantify the number and the volumes of vesicles that appear post-infection as a measure of the cellular immune response induced by *Mtb*. *The views presented in this article do not necessarily reflect those of the Food and Drug Administration.

FS 2. **Recombinant Lactoferrin Enhancement of BCG Presentation in Mouse Dendritic Cells and Macrophages.**
Kelly O'Shea[4], Shen-An Hwang[1,4], Marian L. Kruzel[2,4], Jeffrey K. Actor[1,3,4]
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Lactoferrin (LF), a natural iron-binding protein, has previously demonstrated effectiveness to enhance efficacy of the *Mycobacterium bovis* Calmette Guerin (BCG) TB vaccine. This report primarily examines targeted immune modulatory effects of Chinese hamster ovary (CHO) cell line expressed recombinant mouse and human LFs on mouse bone marrow derived dendritic cells (BMDC). BCG infected BMDCs were cultured with either mouse LF or human LF (100mg/mL) and examined for class II presentation molecule expression. Culturing of BCG infected BMDCs with both LFs decreased the class II presentation molecule expressing population. At the same time, the mouse LF significantly increased production of IL-12p40, IL-1b and IL-10, while the human LF treated BMDCs increased only IL-1b and IL-10. Overlaying naïve CD4 T-cells onto BCG infected BMDCs that were cultured with mouse LF increased IFN-$\gamma$, whereas the human LF exposed group demonstrated increased IFN-g as well as IL-17 from CD4 T-cells. Overlay of naïve CD8 T-cells onto the BCG infected BMDCs treated with mouse LF increased IFN-g and IL-17, while similar experiments using human LF showed only increase in IL-17. Comparative studies were also accomplished using bone marrow derived macrophages (BMMs); data obtained show a more equivalent response than that seen in the BMDCs. This report is the first to examine both recombinant LFs in parallel experiments, and assess their unique properties to target murine dendritic cell function related to BCG vaccine efficacy. These results help delineate LF's mechanisms as a unique adjuvant to effect monocyte

activity, and detail efficacy of the human counterpart when used in a heterologous system to understand LF-mediated events that confer BCG efficacy to protect against MTB challenge.

FS 3. **Prevalence of *Clostridium difficile* and Their Characteristics In and Around Various Public Environs**

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*Clostridium difficile* is the leading cause of infectious diarrhea in hospitalized adults, with increasing rates reported in people in the community not exposed to a hospital environment. Due to increasing incidence and severity of *C. difficile* infection, interventions to prevent community-associated *C. difficile* infection (CDI) are essential. The objectives of the study were to investigate the prevalence of *C. difficile* in non-clinical public environs and characterize the isolates using bio-molecular methods. Isolate characterization included PCR ribotyping, detection of toxin A, toxin B (*tcd*A and *tcd*B), and binary toxin genes (*cdt*A and *cdt*B). Total 938 environmental samples (surface swab samples from toilet, bench, sink, trash can, door handle, etc.) from park-areas, common public areas, fast-food restaurants, and chain stores and shoe bottom samples (from houses) were collected in Houston, Texas. Samples were processed following our published procedures (Anaerobes 2014; 27:31-33). 22.7% (213/938) samples were *C. difficile* culture positive. Shoe bottom samples had the highest percent of positive samples (71/153; 46.4%) followed by park areas (94/235; 40.0%), common public areas (18/195; 9.2%), chain stores (20/230; 8.7%), and fast-food restaurants (10/125; 8.0%). 107 isolates (107/213; 50.2%) were positive for toxin A/B or both the genes and 16 (16/213; 7.5%) binary toxin genes. A total of 25 different ribotypes have been detected from 190 isolates. Predominant ribotypes were 014-020 (14.7%), UM10 (12.1%), UM11 (11.6%), 078-126 (6.8%), 002(6.3%). Toxigenic *C. difficile* ribotype 027 was isolated from 5 samples (0.53%). Isolation of *C. difficile* from shoe bottom samples was correlated with fecal contamination level (*Enterococcus* counts). In conclusion, the study has illustrated that potentially pathogenic *C. difficile* is highly prevalent in various environmental surfaces in common public environs and may have implications with community acquired *C. difficile* infections.

FS 4. **Investigating Microbial Community Dynamics in Fecal Minibioreactor Arrays**

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Although it is clear that the microbiome plays an important role in human health, determining factors that contribute to disease can be challenging due to the complexity of host-microbiome interactions. Cultivation of fecal communities in continuous-flow bioreactors is one method that has shown some success in elucidating functions of the gastrointestinal microbiome. However, these experimental setups are often cumbersome and expensive, limiting replicate experiments. We developed small, simple fecal mini-bioreactor arrays that allow for anaerobic, continuous-flow cultivation of up to 48 communities simultaneously. We used these reactors to study *Clostridium difficile*, an important nosocomial pathogen whose persistence is inhibited by a healthy microbiota. We established reproducible conditions where unperturbed communities were resistant to *C. difficile* and disruption promoted invasion. Further, we identified members of the microbiota with potential to inhibit *C. difficile* and are currently testing these microbes in reactor and mouse models of disease. We are also investigating how cultivation conditions can differentially impact microbial communities and whether these differences can provide insights into microbiome function.

FS 5. **Clarification and Improved Health of Ponds Using *Bacillus spp.* and Calcified Seaweed**

Kelly Gillespie, MPH, Scott Boyette, PhD, Spencer Naquin, Charles Greenwald, PhD, Jordan Church, Isaac Johnson, Katie O'Neil. NCH Corporation

**Background:** Routine chemical application of and dredging of sludge cost pond owners thousands of dollars annually; however, a new non-chemical treatment has been trialed and presents a more attractive,

less costly opportunity. Reductions in phosphate and nitrate levels are associated with sludge layer formation and creating a clear pond can eliminate commonly encountered problems. This study examined the use of one non-chemical method to reduce sludge levels and enhance pond clarity. **Results:** Sludge depth was significantly reduced by an average of 13% (p-value 0.0486) using a two-sample one-tailed t-test α=0.1. We also observed, using one-sided Mann-Whitney test that phosphate and nitrate levels significantly decreased an average of 57% and 69% respectively after treatment (p-value 0.00 for both tests). Clarity changes could not be determined subjectively due to the nature of using a Secchi disk and inherent variances from measuring near rain events. During an enhanced treatment phase, we observed a significant 93% decrease in phosphate and 100% decrease in nitrate levels from baseline (p-value = 0.00 for both). In one month there was a significant decrease of 85% and 75% in phosphate and nitrate levels (p-value = 0.00, 0.02 respectively). However, sludge depth did not significantly change during the enhanced phase. **Conclusions:** Despite the small sample size (n=11) an observed significant decrease in sludge depth, phosphate, and nitrate levels. The changes in phosphate and nitrate concentrations were also striking. Baseline phosphate levels ranged from 0.2 – 2 ppm; however, after treatment, the range was 0.1 – 0.3 ppm. The enhanced treatment had an even smaller sample size (n=4), yet, significant changes were observed. These results are still being evaluated. Further investigations into this new program will be initiated to confirm these results and establish if additional environmental factors play a significant role in the observations.

FS 6. **A Model of Bacterial Translocation Reveal a Major Role for SSIE in Systemic Disease**
Sabrina I. Green[1], Nadim J. Ajami[1] Nina Poole[1], Joseph F. Petrosino[1], and Anthony Maresso[1]
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*E. coli* is a major cause of life-threatening infections in patients with neutropenia, particularly those receiving chemotherapy for the treatment of cancer. In most cases, these infections originate from opportunistic strains living within the patient's gastrointestinal tract which then translocate to major organ systems. There are no animal models that faithfully recapitulate these infections, and, as such, the host or bacterial factors that govern this process remain unidentified. We present here a novel model of chemotherapy-induced bacterial translocation of *E. coli*. Oral gavage of BALB/c mice with a clinical isolate of extraintestinal pathogenic *E. coli* (ExPEC) leads to stable and long-term colonization of the murine intestine. Following the induction of neutropenia with the chemotherapeutic drug cyclophosphamide, ExPEC translocate from the intestine to the lungs, liver, spleen, and kidneys with concomitant morbidity in infected animals. Translocation can also occur in mice bearing mammary tumors, even in the absence of chemotherapy. Translocation of ExPEC is also associated with an increase in the diversity of bacterial DNA detected in the blood. Finally, the use of the model reveals a putative mucinase, SslE, is a virulence factor for this process. This is the first report of a chemotherapy-based animal model of ExPEC translocation in cancerous mice, a system that can be readily used to identify important mechanisms and factors involved in these infections.

FS 7. **Pathogenesis of Post Primary Tuberculosis**
Robert L Hunter and ShenAn Hwang. Department of Pathology and Laboratory Medicine, The University of Texas-Houston Medical School, Houston TX

Post primary tuberculosis (TB) is a disease that occurs only in human lungs. Unfortunately, lung tissue suitable for study has not been available to researchers since the dawn of the antibiotic age in the 1950's before the development of cellular immunology, molecular microbiology or genetics. Researchers have been forced to use animal models and human tissues that do not have post primary TB. As a consequence, scientists have been led to adopt an erroneous conception of the pathogenesis of post primary TB. For generations, the metaphor of host resistance to TB has been a war of attrition. Can the host recruit and activate macrophages fast enough to contain and kill Mycobacterium tuberculosis (MTB) or can the MTB divide and kill the macrophages to produce an enlarging lesion. Evidence presented herein demonstrates that this metaphor is inappropriate. A better one is the explosive reaction produced by dropping pure sodium into water. Two reactive components, host T cells and MTB antigens, are

produced and stored separately in developing post primary TB. When brought together, they react violently to produce caseous pneumonia that evolves to cavities and/or fibrocaseous TB. Live MTB are not necessary for this process, but are standing by to move in and populate the cavity as the violence subsides.

FS 8. **Lung Histopathology of HIV/MTB and MTB Infected Individuals**
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Co-infection of HIV and MTB (*Mycobacterium tuberculosis)* enhances disease progression. However, limited information is available on the host-pathogen and/or pathogen-pathogen interactions, due to absence of clinical relevant samples. We have an ongoing collaboration with the pathologists in St. Petersburg, Russia to analyze autopsy samples from HIV/MTB or MTB only infected individuals. Both HIV and MTB infection are found in lung tissue. We hypothesize that HIV co-infection disrupts lung pathology that are important for control of MTB infection. MTB induced granuloma development, maintenance, resolution, and/or breakdown differ in HIV co-infected individuals. Using immunohistochemistry techniques, we begin to investigate differences in localized infection sites of the lung. There is no co-localization of HIV and MTB infection in the HIV/MTB co-infected lung. Additionally, MTB infection sites closest (~4mm) to HIV infection sites demonstrated a distinct increase in MTB bacterial load compared to MTB infection sites at a distance (~10mm). At MTB infection sites with high MTB bacterial numbers, there is a lack of $CD4^+$ cell presence, with high presence of $CD8^+$ cells and macrophages. In contrast, in MTB only infected lung samples, $CD4^+$ cells make up 30% of the immune cell population. Sites with low MTB numbers exhibit more organized structures for pathogen containment, with similar levels of $CD4^+$ and $CD8^+$ cells. These preliminary studies offer unique clinically relevant insights into immune changes of HIV/MTB co-infections.

FS 9. **3D Visualization of Bacteriophage λ Infection by Cryo-Electron Tomography**
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λ is a bacteriophage that infects gram-negative Escherichia coli. A member of the family Siphoviridae it is characterized by a non-enveloped isocahedral capsid containing a dsDNA genome and a long, flexible, noncontractile tail. Here, we utilized high-throughput cryo-electron tomography (cryo-ET) in conjunction with a new generation, direct detection device camera to study the initiation of λ infection of *E. coli*. We have visualized several key intermediates during infection that provide new insights into the mechanisms of adsorption and penetration of the cell envelope. We also determine the helical nature of the tail-tube; structural modeling provides visual evidences that the core of the λ tail-tube relates to the tube structure formed by hexameric rings of *Pseudomonas aeruginosa* Hcp1 protein essential to the type VI secretion system. This parallel suggests that the mechanism of phage infection extends more generally to the transport of macromolecules across lipid membranes into the cytoplasm.

FS 10. **Combinatorial Engineering of Intergenic Regions in Operons to Improve the Succinic Acid Production in *Escherichia coli***
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The parameters in a complex synthetic gene network must be extensively tuned before the network functions as designed [1]. Here, we introduce a simple and general approach to rapidly tune gene networks in *Escherichia coli* using hypermutable simple sequence repeats embedded in the spacer region between ribosome binding site (RBS) and initiation code, which we call BRI region. By varying repeat length and different bases in this BRI region, we generated expression libraries that predictably sample gene expression levels from 1-7 fold range. We demonstrate the utility of the approach by regulating

*ecaA* gene and *pepC* gene in the engineered *E. coli*for improving succinic acid yield and production [2, 3]. In engineered *E. coli*libraries LKCA111 (DC1515 containing the *ecaA* gene), the final succinic acid yield and productivity were 0.82 $g \cdot g^{-1}$ and 1.84 $g \cdot L^{-1} \cdot h^{-1}$ respectively by regulating *ecaA* gene expressed 3.53-fold than the unmodified *ecaA* gene; in engineered *E. coli* libraries LKPC111 (DC1515 containing the *pepC* gene), the final succinic acid yield and productivity were 0.86 $g \cdot g^{-1}$ and 1.89 $g \cdot L^{-1} \cdot h^{-1}$ respectively by regulating *pepC* gene expressed 2.89-fold than the unmodified *pepC* gene. Finally, the two genes were co-expressed in engineered *E. coli*libraries LKCO111, the expression level of the two genes were regulated by BRI libraries, when the expression level of *ecaA* gene was 3.53 -fold than the unmodified *ecaA* gene, the expression level of *pepC* gene was 1.06-fold than the unmodified *pepC* gene, the yield of succinic acid and productivity both were highest, they were 0.87 g $l^{-1}$ $g \cdot g^{-1}$ and 2.01 $g \cdot L^{-1} \cdot h^{-1}$, respectively.
This research was supported in part by grants from the National Natural Science Foundation of China (Grant NO.21106191, 21206175). We thank Professor P. Clark for strain DC1515 and kindly guidance.