43rd Annual Fall Meeting

of the

Texas Branch
American Society for Microbiology

10-12 November 2011

E.H. Hereford Center
The University of Texas Arlington
Arlington, TX 76019

Hosted by

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Texas Branch ASM – Fall 2011 Meeting Program

Quick view

Thursday Evening
5:00-7:00 Registration
7:15-8:30 Welcome and Plenary given by Dr. James M. Tiedje, Michigan State University

Friday
7:00-8:15 Registration and Breakfast

General Microbiology Session I
8:30 - Trahan on chromosomes
8:45 – McKethan on transcriptional regulation
9:00 – Cullen on *Campylobacter*
9:15 – Radwan on *Sporisorium*
9:30 – Myagmarjav on *Rhodobacter*

General Microbiology Session II
8:30 – Mirza on nitrogen fixation
8:45 – Valenzuela on indole degradation
9:00 – Pound on diatoms
9:15 – Foster on predator-prey interactions
9:30 - Hidalgo-Ramano on quorum sensing

9:45 – 10:15 Coffee Break

General Microbiology Session III
10:30 – Edwards on *Clostridium* liposysis
10:45 – Roy on plant immunity
11:00 – Panchal on coronatine biosynthesis
11:15 – Mann on Caribbean sea fans

Medical Microbiology Session I
10:30 – Darkoh on *Clostridium difficile*
10:45 – Watters on biofilm associated infection
11:00 – Johnson on saponins and viruses
11:15 – Pandhe on cytomegalovirus

11:30 – 12:00 Poster set up
12:00-2:15 Lunch and Posters (50 posters)

2:30 -3:30 Plenary by Dr. James M. Musser, Methodist Hospital Research Institute

Invited General Microbiology Session
3:45 – Bextine on insects and bacteria
4:05 – Melotto on host and pathogen defense
4:25 – Wawrick on nitrogen cycling
4:55 – Sobolev on cyanobacterial mats
5:15 – Allen on sustainable bioproducts

Invited Medical Microbiology Session
3:45 – Jiang on *Clostridium difficile*
4:05 – Trent on *Helicobacter pylori*
4:25 – Wei on SOS inducible biofilms
4:55 – Berg on neutrophils and infections
5:15 – Bowden on the immune system

6:00-7:15 Awards and Dinner

7:15-8:30 ASM Lecturer and Keynote Speaker, Dr. Kathyrn Boor, Cornell University

Saturday
7:00-8:00 Coffee and Breakfast

Mini CUE Session I
8:00 – Reynolds on curriculum guidelines
8:45 – Choudhary research in large lab settings

Mini CUE Session II
9:30 – Bowling on using games in science
10:30 – Panel Discussion led by Bauman

Texas Branch ASM – Fall 2011 Meeting

Full Program

Thursday 10 November 2011

5:00 – 7:00 pm Registration 2nd floor Hereford Center

7:15 Welcome and Plenary at The Planetarium

Dr. Todd Primm, President Texas ASM Branch
Dr. Thomas Chrzanowski, Chair of the Organizing Committee

Plenary
Mining Metagenomes for Meaning

James M. Tiedje
University Distinguished Professor
Director of the Center for Microbial Ecology
AAAS Fellow
Member of the National Academy of Science
Past President of the American Society for Microbiology
Michigan State University

Metagenomics has become a new means to understand microbial communities, in this case from the gene and gene product level. I will discuss the current and developing approaches to extract meaning from this increasingly huge data resource, using soil as the primary example. Soil houses the most diverse microbial communities resulting from its ancient history; complex sets of interrelating gradients; and protective, isolating, stable and relatively resource poor environment. Because of the tremendous advances in DNA sequencing technologies, we now also see incredibly diverse sets of genes in soil communities. These genes and their host microbes catalyze vital functions such as nutrient cycling, organic matter decomposition, greenhouse gas flux, restoring water quality, and plant and animal health. While the new molecular and omic technologies provide the chance to understand and eventually better manage some of these communities, the analysis of the massive amount of sequence data is today's challenge. I will discuss three components that underpin the understanding of soil communities: their compilation of sub communities, the species and pangenome perspectives, and the extraction and analysis of key (eco)functional genes. Because of its diversity, the soil is arguably the most challenging frontier in biology; plus, if we can analyze and interpret soil metagenomes, we can do so for any other microbial habitat.

Friday, 11 November 2011

7:00 – 8:15 Registration, Breakfast and Coffee

2nd floor Hereford Center
Carlisle Suite

8:30 – 9:45
General Microbiology Session I (concurrent)

Red River, 2nd floor Hereford Center
Moderated by: Woo-Suk Chang
Papers in competition for the O.B. Williams Award

8:30 **A Tale of Two Chromosomes in Prokaryotes**
Cheramie Trahan[1*], Hyuk Cho[2], Madhusudan Choudhary[1], [1]Department of Biological Sciences, [2]Department of Computer Science, Sam Houston State University, Huntsville, Texas

8:45 **Characterization of the Transcriptional Regulator NrdR in *Escherichia coli***
Brandon McKethan* and Stephen Spiro, University of Texas at Dallas

9:00 **Characterization of a novel surface modification in *Campylobacter jejuni,* linking the assembly of flagella and lipooligosaccharide**
Thomas W. Cullen[a*], James A. Madsen[b], Petko L. Ivanov[a], Jennifer S. Brodbelt[b], M. Stephen Trent[a,c], [a]Section of Molecular Genetics and Microbiology, [b]Department of Chemistry and Biochemistry, and [c]The Institute of Cellular and Molecular Biology, The University of Texas at Austin, Texas 78712

9:15 **Molecular genetics studies of a- mating type loci of *Sporisorium reilianum*, the head smut fungus in maize and corn.**
Ghada L Radwan and Clint W Magill, Department of Plant Pathology & Microbiology, Texas A&M University, College Station, TX

9:30 **Isolation and Identification of Chromosomal I-origin of replication in *Rhodobacter sphaeroides***
Bat-Erdene Myagmarjav*, Jonathan Stone, and Madhusudan Choudhary, Department of Biological Sciences, SHSU, Huntsville, TX 77341

9:45 – 10:15 Coffee Break

Carlisle Suite

8:30 – 9:45
General Microbiology Session II (concurrent)
Concho, 2nd floor Hereford Center
Moderated by: Maeli Melotto
Papers in competition for the O.B. Williams Award

8:30 **Evaluation of solid medium based methods for isolation of nitrogen fixing microorganisms**
*Babur Mirza and Jorge Rodrigues, University of Texas at Arlington

8:45 **Degradation of *Escherichia coli* Produced Indole by *Pseudomonas aeruginosa* in Mixed Cultures**
Ernesto Valenzuela Jr[1]*, T.R. Zere[2], J. Gollihar[1], M. Whiteley[3], T.K. Wood[4], and R.J.C. McLean[1]. 1-Texas State University-San Marcos, San Marcos, TX, 2-University of Florida, Gainesville FL, 3-University of Texas, Austin TX, 4-Texas A&M University, College Station, TX *-presenting author

9:00 **Diatom species traits vary in response to inorganic versus organic acidity in Adirondack streams**
Katrina L. Pound* and Sophia I. Passy, Department of Biology, University of Texas Arlington

9:15 **Single cell predator- prey interactions: Simple or complex?**
Briony L. Foster* and Thomas H. Chrzanowski, Department of Biology, University of Texas at Arlington, Arlington TX 76019

9:30 **Indole inhibition of AHL-mediated quorum signaling in *Chromobacterium violaceum* and *Janthinobacterium lividum*.** Benjamin Hidalgo-Romano* and Robert JC McLean Dept. Biology, Texas State University, San Marcos TX *-presenting author

9:45 – 10:15 Coffee Break
Carlisle Suite

10:30 – 11:30
General Microbiology Session III (concurrent)
Red River, 2nd floor Hereford Center
Moderated by: Shawn Christensen
Papers in competition for the O.B. Williams Award

10:30 **Ruminal *Clostridium* species potentially implicated as primary contributors to ruminal lipolysis**
Holly Edwards*[1], Robin Anderson[2], Stephen Smith[1], Rhonda Miller[1], T. Matthew Taylor[1], Nathan Krueger[2], David Nisbet[2]; Texas A&M University, College Station, TX, USA[1], United States Department of Agriculture/Agricultural Research Service, Southern Plains Agricultural Research Center, Food & Feed Safety Research Unit, College Station, TX, USA[2]

10:45 **High Relative Humidity Compromises Stomatal Innate Immunity against *Pseudomonas syringae* and *Salmonella enterica* but not *Escherichia coli***
Debanjana Roy*, Shweta Panchal, Maeli Melotto
Department of Biology, University of Texas, Arlington, Texas, 76019, USA

11:00 **Light-independent coronatine biosynthesis activation on the leaf surface**
S. Panchal[1]*, Z. Breitbach[2], L.Price[1], D. Armstrong[2], M. Melotto[1]
[1]Department of Biology and [2]Department of Chemistry, University of Texas, Arlington, Texas, 76019, USA

11:15 **The effect of temperature on pathogen virulence of the Caribbean sea fan, *Gorgonia ventalina***
*Whitney Mann[1], Joshuah Beach-Letendre[1], Laura Mydlarz[1], The University of Texas at Arlington[1]

10:30 – 11:30
Medical Microbiology Session I (concurrent)
Concho, 2nd floor Hereford Center

Moderated by: Mike Roner
Papers in competition for the Sulkin Award

10:30 **Regulation of Clostridium difficile Toxin Production by a Quorum Signaling system**
*Charles Darkoh[1,3], Heidi B. Kaplan[1-2], Herbert L. DuPont[1-4], [1]The University of Texas Graduate School of Biomedical Sciences, [2]The University of Texas Medical School, [3]The University of Texas School of Public Health-Center For Infectious Diseases, [4]Baylor College of Medicine.

10:45 ***Pseudomonas aeruginosa* biofilm-associated infections contribute to chronicity and increased antimicrobial tolerance in the diabetic wound environment**
Chase Watters[1]*, Urvish Trivedi[1], Katrina DeLeon[1], Trevor Dalton[1], Mark Lyte[2] and Kendra Rumbaugh[1], Department of Surgery, Texas Tech University Health Sciences Center, Lubbock, TX 79430[1] Department of Pharmacy Practice, Texas Tech University Health Sciences Center, Lubbock, TX, 79430[2]

11:00 **The ability of saponins from a variety of natural sources to block virus replication**
Alisa M. Johnson and Michael R. Roner. Department of Biology, University of Texas Arlington

11:15 **Finding appropriate transfection normalization technique in the context of MCMV infection**
*Sonali Pandhe, Nathaniel Mills, Laura K. Hanson, Texas Woman's University

11:30 -12:00 Poster set up in Rio Grande B

12:00 -2:15 Lunch and Poster Rio Grande A&B

Visit with the Exhibitors

Poster Board Number and Presentation

SK: Sam Kaplan Award (Graduate Student)
JA: Joan Abramowitz Award (Undergraduate Student)

SK 1. **Modeling ammonia consumption among cyanobacteria, ammonia-oxidizing *Archaea*, and ammonia oxidizing bacteria in microbial mats collected from Hawaiian coral reefs**

Matthew R. Boyett*, Dmitri Sobolev, University of Houston-Victoria, 3007 Ben Wilson Dr. Victoria, TX 77901

Molecular analysis of cyanobacterial mat communities indicated that cyanobacteria, ammonia-oxidizing *Archaea* (AOA), and ammonia-oxidizing bacteria (AOB) coexist in those systems, competing for ammonia. Competition for the same resource may imply competitive exclusion, yet continued coexistence of those organisms suggests niche partitioning. Our model of a diffusion-consumption limited 1-dimensional representation of the mat, based on reported Km and Vmax values for different organisms indicated that, at realistic ammonia concentrations, AOBs outcompete AOAs near the edges of the mat, whereas AOAs become more competitive towards the center. Cyanobacteria are unable to compete with either group, forcing them into nitrogen fixation mode, of which they are capable. This suggests that AOAs and AOBs may be responsible for making the mat a net nitrogen source by competing for ammonia with cyanobacteria and leading to cyanobacterial nitrogen fixation.

2. **Analysis of coral reef cyanobacterial communities: implications for coral health**.

D. Sobolev* and M. R. Boyett, University of Houston-Victoria, 3007 Ben Wilson Dr. Victoria, TX 77901

Nuisance cyanobacterial mats are found in many coral ecosystems worldwide; their invasion often driven by nutrient pollution. The composition of those mats collected in 3 locations around Hawaii was investigated by use of the 16S rRNA gene PCR/DGGE, cloning, and sequencing. Preliminary results indicated that cyanobacterial component of those mats is dominated by organisms of *Trichodesmium-Leptolyngbya* group, as opposed to *Lyngbya majuscula* found elsewhere. Other organisms detected in the mats were *Alpha*-, *Beta*- and *Gammaproteobacteria*. Importantly, a community was substantially different from sediment, water or healthy corals communities described in the literature. A community similar to the mat community was associated with Black Band Disease of corals, suggesting that nuisance mats can act as pathogen reservoirs.

SK 3. **The Microbiome of the Fish *Gambusia Affinis*: Why so Negative?**

Sonya Ramzanali*, Madeeha Ahmed, Sonia Gonzalez, Annie Leonard, and Todd P. Primm, Dept. of Biological Sciences, Sam Houston State University

How the bacteria in microbiomes on animals function and interact as an ecological system is a question of importance to both basic microbiological and biomedical applied science. We are using a small, freshwater fish (Western mosquitofish) as a model organism to study microbiomes. When we cultured bacteria extracted from the skin of *Gambusia affinis,* a major surprise was to identify exclusively gram negative species. Such selectivity is not seen in humans and mice. Gram positive species are not lacking in the aquatic environment, thus the mechanism of selectivity on the fish skin is of interest. The most straightforward hypotheses are that either the microbial species present on the surface of the fish themselves generate the selectively by some exclusion mechanism, or the fish secrete something selective in the slime layer, or a combination of these two prohibits attachment/survival of gram positive bacteria. *Micrococcus luteus* was used as a candidate invasive species to confirm selectivity of the skin microbiome, because it is a non-pathogenic gram positive organism that can be distinctly identified on selective media. Even at high doses in the water for 48 hours, *M. luteus* could not establish on the fish skin, confirming selectivity. UV-sterilized fish slime layer did not inhibit growth of the *M. luteus.* Current work is focusing on the biome bacteria themselves, utilizing a competition experiment between the fish bacteria and the *M. luteus*.

SK 4. **Bacteriological analysis of bone and raw food diets for pets.**

A. Dallas Arnold* and Aaron M. Lynne, Department of Biological Sciences, Sam Houston State University, Huntsville, TX 77341

In recent years little research concerning the microbial content of the popular bone and raw food diets for domesticated pets has been conducted. These diets are shown to contain potential human pathogens, posing a possible health threat to the pet owner from the preparation of these diets or via shedding from the pet. While there is little to no evidence to support that these diets are healthier for the pet compared to the traditional pet foods, there is a potential health risk associated with these diets for the pet and owner. Ten bone and raw food diets, ranging from chicken to buffalo meat, were randomly selected and tested on various media for contamination by bacterial pathogens including *Salmonella spp., Escherichia coli,* and *Shigella*

spp. It was observed that the majority of the diets showed high microbial content of multiple bacterial species including *Escherichia coli* and *Salmonella spp.* Future research may look at the potential for pathogen transmission from pet to humans.

SK 5. **Construction of In-frame Deletion of *recA* in *Rhodobacter sphaeroides***

Leah Severin*, Cheramie Trahan, Phillip Price, and Madhusudan Choudhary, Department of Biological Sciences, Sam Houston State University, Huntsville, Texas

In several bacteria, *lexA* and *recA* encode LexA and RecA protein, respectively, that control the SOS response system, which is induced as an emergency response to mutational damage. RecA protein is also responsible for homologous recombination. To understand the DNA damage repair system, the *recA* mutant was constructed using an in-frame deletion gene knockout method. The in-frame deletion allele was constructed in vitro by amplifying fragments from both upstream and downstream portions of *recA*. These fragments have complementary sequences at the ends and therefore produced a fusion *recA* deletion allele. The *recA* deletion allele was cloned into a suicidal vector, pLO1, and then mobilized into *R. sphaeroides* cells. Km^r colonies were picked and grown in liquid culture, and then plated out on LB agar plate containing 15% sucrose, which selects for the double crossover event, leading to the replacement of the wild type *recA* gene by the deletion allele. Gene replacement was confirmed by amplifying the fragment containing the *recA* deletion allele by performing PCR and DNA sequencing. Since the *recA* mutant lacks the homologous recombination, the *R. sphaeroides recA* mutant strain would be able to maintain cloned DNA sequences in *R. sphaeroides*.

SK 6. **Promoter analysis of duplicate gene-pairs in *Rhodobacter sphaeroides***

Perry Phillips[1], Laurene Bazunu[1], Jeff Copeland[1], Hyuk Cho[2], and M. Choudhary[1], [1]Department of Biological Sciences, [2]Department of Computer Science, Sam Houston State University, Huntsville, Texas 77341

Rhodobacter sphaeroides, a member of α-3 Proteobacteria, possesses a complex genome structure and exhibits metabolic diversity, which includes photosynthetic, aerobic or anaerobic life styles. Genome Analysis of *Rhodobacter sphaeroides* reveals that ~30% of its genome result from gene duplications, majority of which originated prior to the origin of *R. sphaeroides*. Microarray analysis of 234 duplicate gene-pairs demonstrated that ~11% of these duplicate gene-pairs express differently under varied growth conditions, while their protein structures are maintained under negative selection.
Since mutations in the 5' regulatory sequence can regulate gene expression through their interaction with RNA polymerase and other regulatory proteins, it is hypothesized that the differences in mRNA expression patterns between duplicate copies can be explained by the differences in their corresponding promoters. Bacterial promoters span over 100 nucleotide upstream of the transcription start site and possess -10, -35, and other regulatory conserved sequences. These consensus sequences from previously experimentally examined promoters were used to identify putative promoter elements of 234 duplicate gene copies. This analysis provides a basis for molecular analysis of selected duplicate gene-pairs and could reveal the insight into the metabolic differentiation of gene duplication in *R. sphaeroides*.

SK 7. **Molecular analysis of programmed cell death in the unicellular green alga, *Chlamydomonas reinhardtii*: evidence for a p53-like protein**

Terah L. McClendon*[1], Aurora M. Nedelcu[2], and Anne R. Gaillard[1], [1]Department of Biological Sciences, Sam Houston State University, Huntsville, TX, U.S. , [2]Department of Biology, University of New Brunswick, Fredericton, New Brunswick, Canada

Programmed cell death (PCD) is a fundamental process that is defined by the genetically controlled breakdown of a cell, typically in response to stress. Apoptosis is a well-studied form of PCD and is specific to metazoans. Organisms other than metazoans, including unicellular eukaryotes and prokaryotes, have also been observed to show hallmarks of PCD in response to stress. However, the extent at which these pathways are conserved has yet to be elucidated. Here, we show that the unicellular alga, *Chlamydomonas reinhardtii* exhibits hallmarks of PCD in response to heat stress (42°C, 2 hours) such as production of reactive oxygen species (ROS), morphological changes, and DNA laddering. Our findings also suggest that *Chlamydomonas* possess a protein similar to the mammalian tumor suppressor, p53, a key protein in the apoptosis pathway. Antibodies raised against human p53 cross-reacted with an algal protein approximately 53 kD in size. These antibodies were then used as a probe to screen a *Chlamydomonas* cDNA expression library constructed in the λZAP vector system. Once a positive clone was identified, the pBluescript phagemid was then excised in order to further characterize the cDNA insert by sequence analysis. Once sequenced, the cDNA will be used to test the homology of the algal protein to that of mammalian p53. Lastly, the *Chlamydomonas* genome database will be used to obtain the full-length gene encoding the protein identified.

SK 8. **Characterization of the *blr6977 gntR* gene in *Bradyrhizobium japonicum***

May Taw*, Rawan D. Shishakly, and Woo-Suk Chang, Department of Biology, University of Texas at Arlington

The GntR superfamily is one of the most widely distributed and prolific groups of the helix-turn-helix (HTH) transcription factors. In particular, microorganisms that live in complex, fast-changing environments such as soil tend to have a larger aggregate of the *gntR* regulatory genes. *Bradyrhizobium japonicum* is a Gram-negative soil bacterium that can fix nitrogen into ammonia by developing a symbiotic relationship with the leguminous soybean plant. Although these metabolite-responsive *gntR* genes have been found to be involved in many cellular processes in a range of microorganisms including motility, antibiotic production, and virulence, little is known about their role in the *B. japonicum*-soybean symbiosis. A *gntR* (blr6977) mutant strain was constructed. The colony morphology and growth rates were compared, but they were not significantly different in the wildtype and mutant strains. Motility tests using 0.3% minimal medium agar showed enhanced motility by the *gntR* mutant compared to that of the wild type. Nodulation experiments were conducted in order to determine a nodulation phenotype of the mutant strain. The outcome indicated that not only was the *gntR* mutant deficient in the number of nodules compared to the wild type, it also showed delayed nodulation. Furthermore, the expression of 15 *gntR* genes in wild type *B. japonicum* were measured by quantitative reverse transcription-PCR (qRT-PCR). Currently, we are underway to purify the Blr6977 protein, thereby determining whether Blr6977 is capable of auto-regulation by binding to its own promoter region using an electrophoretic mobility shift assay (EMSA).

SK 9. **Characterization of *Bradyrhizobium japonicum* Extracytoplasmic Function σ Factor Involved in Oxidative Stress.**

Anchana Thaweethawakorn,[1]* Jeong-Min Jeon,[1,2] Hae-In Lee,[1], Andrew J. Donati,[1] and Woo-Suk Chang[1], Department of Biology, University of Texas-Arlington, Arlington, TX[1]; and Department of Marine Science and Biotechnology BK21 Program, Inha University, Incheon 402-751, Korea[2]

Bradyrhizobium japonicum is a nitrogen-fixing bacterium that can establish a symbiosis relationship with the soybean plant (*Glycine max*). This results in the formation of root nodules where *B. japonicum* fixes atmospheric nitrogen into ammonia. In order to be a successful symbiont, *B. japonicum* must have an effective mechanism to deal with plant defense responses such as an oxidative burst, a rapid proliferation of reactive oxygen species (ROS). We investigated the effect of hydrogen peroxide (H_2O_2)-induced oxidative stress on *B. japonicum* gene expression and physiology. Genome-wide transcriptional profiles revealed that 650 genes were differentially expressed at 2-fold cut-off with $p < 0.05$. Genes encoding sigma factor, stress response, $FixK_2$ transcriptional factor, and its response regulatory genes were up-regulated. Interestingly, *carQ* (bll1028) encoding extracytoplasmic function (ECF) sigma factor showed the highest expression (107.8-fold induction). ECF sigma factors have been known to play a key role in combating oxidative stress. Currently, we are underway to construct a *carQ* knock-out mutant and a complementary strain to identify a role of CarQ in the oxidative response of *B. japonicum*. This information will provide an insight into how the ECF sigma factor is regulated in *B. japonicum* to deal with a plant-derived oxidative burst.

SK 10. **NsrR and the Response to Nitrosative Stress in *Escherichia coli***

Shivani Chhabra* and Stephen Spiro, Department of Molecular & Cell Biology, University of Texas at Dallas, Richardson, TX

Nitric oxide (NO) is a highly reactive, water-soluble free radical that reacts with metal centers, Fe-S clusters and amino acids. NsrR (NO-Sensitive-Repressor) is a transcriptional repressor which contains an [Fe-S] cluster, and regulates gene expression in response to NO. In *E. coli*, NsrR may regulate more than 60 genes, including some encoding NO detoxification enzymes. We hypothesize that the inefficient GTG start codon of the *nsrR* gene keeps the abundance of NsrR low, while allowing high-level expression of downstream genes in the same operon. We further hypothesize that the GTG start codon may limit the membership of the NsrR regulon. We overexpressed *nsrR* by changing the start codon to ATG. Promoter activity assays and microarray data show that known targets for NsrR are more tightly repressed when the start codon is ATG. Gene profiling also revealed some new candidate members of the NsrR regulon. The NsrR-repressed *ytfE* promoter showed a response to NO, even in a *ΔnsrR* background. This result led us to search for other possible regulator(s) of *ytfE* expression. NarXL activates the expression of *ytfE* in response to µM concentrations of NO and nitrate under aerobic conditions but only to nitrate under anaerobic conditions. Since NO can get oxidized to nitrate in aerobic cultures, we concluded that the regulatory pair NarXL activates *ytfE* expression in response to nitrate.

SK 11. **Regulation of the *tynA* and *feaB* genes of *Escherichia coli***

Ji Zeng* and Stephen Spiro, Department of Molecular and Cell Biology, University of Texas at Dallas, Richardson TX

Aromatic compounds are widely distributed in nature, from the rhizosphere of plants to the GI tracts of animals. It is possible that the ability of *Escherichia coli* to use these compounds as carbon, nitrogen and energy sources provides with them advantages over other bacteria in the mammalian intestine. Decarboxylation of phenylalanine and tyrosine by the eukaryotic aromatic amino acid decarboxylase generates phenylethylamine (PEA) and tyramine, respectively. Both compounds can be oxidized by the copper amine oxidase of *E. coli* (TynA) generating the corresponding aldehyde, and liberating ammonia. The aldehyde is then oxidized by the NAD-linked dehydrogenase FeaB, generating the corresponding carboxylic acid. The product of the oxidation of PEA by this pathway, phenylacetic acid, can be further catabolised to TCA cycle intermediates. Thus, PEA can be used as a carbon, nitrogen and energy source, while tyramine can be used only as a nitrogen source. It is known that the transcription of the *tynA* and *feaB* genes is regulated by an AraC family protein, FeaR, and a nitric oxide sensitive repressor NsrR. We will report the results of experiments aimed at understanding the mechanism of *tynA* and *feaB* regulation by FeaR and NsrR.

12. **Gene expression of sigma factors rpoE, rpoH, and small RNA-binding protein hfq in Zymomonas mobilis during stress**

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Zymomonas mobilis, a high-yielding ethanologen with an exceptional tolerance to ethanol and other stresses, is an attractive bacterium for industrial production of biofuel. However, the mechanisms and regulation for tolerance to ethanol, osmotic stress, and potential cellulosic, biomass-related inhibitors are incompletely understood. Regulation by alternative sigma factors is a common stress response pathway in bacteria, especially to such stresses as osmotic, ethanol, and heat shock. Here, changes in gene transcription of two sigma factors (*rpoE* and *rpoH*) as well as the small RNA-binding protein *hfq*, which has been shown to play a role in tolerance to acetate and lignin biodegradation products, were examined by qPCR under defined conditions. Whereas exposure to 10% ethanol had no effect on transcription for any of the genes tested, exposure to 15% glucose caused a significant increase in transcription of both *rpoH* and *hfq*.

SK 13. **Transcriptome and Proteome Analyses of the *Verrucomicrobium* sp. strain TAV2 Reveal Adaptation under Hypoxic Condition**

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The diverse microbial community present in the termite hindgut is key for plant biomass degradation. Among their members, the microaerophilic bacterium *Verrucomicrobium* strain TAV2 was successfully isolated. In order to gain knowledge about its ecological roles, we obtained transcriptomic and proteomic profiles of TAV2 cells by imitating the O_2 concentration of its natural habitat (2%) and setting a stress condition (20%). Competitive hybridization experiments were carried out with a customized oligonucleotide microarray, while proteins were detected with use of tandem mass spectrometry. Physiological experiments confirmed that growth rate for strain TAV2 under 2% O_2 was faster than that under 20% O_2. Our transcriptomic analysis identified 75 genes that were differentially expressed between the two O_2 concentrations ($p < 0.05$), while proteomics indicated that 96 proteins were differentially detected under the same conditions. There were positive correlations between an expressed gene and its detected protein such as acetyl xylan esterase, an enzyme involving in hemicellulose degradation. Our data indicated that a significant effect of O_2 concentration on cellular functions of the strain TAV2 with 50% of enzymes in glycolysis, 75% of nucleotide metabolism proteins, and all secretion and transport proteins being up regulated under 2% O_2. A metabolic map for strain TAV2 was developed and will be presented.

14. **Towards defining the genome structure of angular leaf spot resistance locus**

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Angular leaf spot (ALS), caused by the fungus *Pseudocercospora griseola (Sacc.) Crous & Braun (sin. Phaeoisariopsis griseola (Sacc.) Ferraris).* is one of the most devastating diseases of common bean (*Phaseolus vulgaris* L.). The fungus enters bean leaves and grows intercellularly leading to cell death and necrotic symptoms typical of the disease. In an attempt

to elucidate the genetic resistance against this fungus, a major quantitative trait locus (QTL) that explains 22% of the resistance was identified. To determine the gene composition of this QTL, a bacterial artificial chromosome (BAC) library was screened with several tightly linked molecular markers using bulk colony PCR. Seven BAC clones were identified and preliminary analysis of BAC end sequence (BES) indicates the presence of two genes (a chitinase and a TIR-NBS-LRR) that could be involved in plant resistance. Sequences analysis also indicates that this region contains a high number of transposon elements; four BES showed similarity to retrotransposons sequences. Furthermore, our results suggest that the base pair to centimorgan (cM) ratio in this region is smaller than the predicted median of 120 kb/cM for common bean and the physical length of this QTL might be shorter than expected.

JA 15. **Identification of Arabidopsis Mutants Compromised in Stomatal Immunity**

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Bacteria cannot directly penetrate the epidermis of the plant, so they rely on stomatal pores to enter the leaves. These surface openings play an important role in limiting bacterial invasion. The guard cells, which form the stomatal pore, can sense bacterial molecules, such as pathogen-associated molecular patterns (PAMPs) and close the stomata by changing the turgor pressure of the guard cells. In this study we are using a genetic approach to identify genes that are involved in stomatal immunity. The bacterium pathogen *Pseudomonas syringae* pv. *tomato* strain DC3118 has been shown to induce strong stomatal closure in the wild type plant Col-0. Using a collection of homozygous T-DNA insertion lines that represent mutations in 10,848 different genes of Arabidopsis, we are screening plants for high leaf surface temperature after inoculation (*i.e*. indicative of open stomata) using an infrared camera (Flir System T300). Plants with mutations in genes involved in bacterium-triggered stomatal closure should have leaves as hot as the *ost1-2* mutant and hotter than the Col-0 wild type plant. To date, we have screened 886 mutant plants and selected 53 lines for further investigation. This screen will further our current understanding of the genetic regulation of stomatal immunity and provide insights for additional and/or complementary control measures to alleviate plant diseases in the field. Furthermore, stomatal immunity may be an important mechanism to prevent fresh produce contamination with human pathogens, thus benefiting human health.

SK 16. **Loss-of-function mutations in JAZ proteins compromise Arabidopsis immunity against *Pseudomonas syringae***

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Recent studies have shown that stomatal pores in the leaf epidermis close as a part of the plant innate immune response against bacterial invasion of plant tissues. Counteracting this response, the plant pathogenic bacteria *Pseudomonas syringae* pv. *tomato* strain DC3000 has evolved the virulence factor coronatine, an important strategy contributing to pathogenesis. The mode of action of coronatine in plant cells is beginning to be elucidated. Two components of the coronatine receptor complex have been identified, namely COI1 (the F-box subunit of E3 ligase) and JAZ (a repressor of jasmonic acid pathway) proteins, suggesting that coronatine acts in the plant by inducing the degradation of proteins and hijacking the jasmonic acid (JA) signaling pathway. In this study we focused on determining the function of the JAZ4 protein in mediating plant immunity at early stages of bacterial infection. Using a combination of approaches, including gene expression analysis, yeast-two-hybrid, ectopic expression of truncated proteins, and gene knockout we have determined that JAZ4 occurs in four natural isoforms in Arabidopsis, allowing for additional levels of regulation of plant innate immunity. Furthermore, we have substantial genetic evidence that the N-terminus of JAZ9 is essential for plant's defense against *Pst* DC3000. This study will contribute to refining the current model of the coronatine action in the plant cell and to discerning the molecular function of JAZ4 during infection.

SK 17. **Regulation of m140, an early pathogenesis factor**

Bettye I. Lopez*, Lisa D. Fetters, Laura K. Hanson, Texas Woman's University,

Human cytomegalovirus is a ubiquitous virus that can cause complications in immune compromised people and is the most common infectious cause of birth defects. Many other viruses can infect cross-species whereas human cytomegalovirus is host specific. Mouse cytomegalovirus, due to genetic and pathogenic similarities, is a good model. In looking at host and virus interactions in cytomegalovirus it is of interest to look at the genes conserved among the cytomegaloviruses. We are studying one such group of genes, the US22 gene family. The objective of this project is to get a better idea of how, one member of this gene family, m140, is regulated. The m140 gene product is important for efficient replication of MCMV in macrophages, but is dispensable in other cell types, such as fibroblasts. The m140 gene product is an early protein, meaning that it is dependent upon prior expression of viral transcription factors. Two major viral transcriptional regulators are IE1 and IE3, which are spliced variants. Either one or both of these may induce expression of viral early genes. Preliminary data

indicates that either IE1 or IE3 can turn on expression of GFP m140 with co-transfection. Further quantitative work will look at the regulation by IE1 and IE3 to see if it is additive or synergistic.

SK 18. **The Induction, Purification and Host Range of 4 Lysogenic Phages**

Carlo Manzana and Dr. Tamarah Adair

Bacteriophage therapy has resurfaced as a potential treatment option against *Staphylococcus aureus* due to the recent increase in antibiotic resistance. One source of isolating bacteriophages is through phage induction, a process in which a prophage is excised from its lysogenized state. This experiment induced, purified, and determined the host range against 64 *S. aureus* isolates for 4 bacteriophages. To induce the phages, Mitomycin C was added to 58 *S. aureus* cultures and the product phage solutions were then spotted onto a panel of 10 different isolates to test for lysis. Forty of the fifty eight solutions tested positive for phages. Of these phages, the 15 most lytic, to the panel of 10 isolates, were chosen to be purified but only 4 were purified through 4-5 rounds of successive purification. Phage stocks were produced and a plaque assay was performed to determine the phage concentration. These phages (labeled ϕ2734, ϕ346, ϕ349, and ϕ 2069) were then assayed for their host range. ϕ2734 exhibited the widest host range, lysing 10 of 64 isolates through the spot test. While the phages induced in this experiment manifest lytic ability, their host ranges are not as extensive as phage K, a well-known phage that lysed 55 of 64 isolates in this study. This experiment determined that although lysogenized phages can be a source for phage therapy, most of these phages display low levels of lytic activity.

19. **Evaluation of SGC1 Cells for Use in murine Cytomegalovirus Studies**

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Studies have shown that salivary gland tissue is not only a primary target for murine cytomegalovirus (MCMV) replication following initial infection, but that salivary gland tissue also serves as a unique reservoir for the virus to establish a pattern of prolonged replication. The majority of mutated viruses tested, on the other hand, show distinct sensitivities evidenced by reduced or delayed growth in salivary gland cells. This may be due to specific cell characteristics or failure to reach the site. Viral replication is slower in SGC1 cells than in the standard, NIH-3T3 (fibroblasts) cells. However, experimental results show that this is not due to poor viral envelopment as virus is readily released into the supernatant suggesting a delay in viral DNA replication. We are examining viral entry, gene expression and DNA replication to identify possible reasons for the delay.

To help in the effort to understand the unique interactions between MCMV and salivary gland tissue cells, we have characterized the SGC1 cell line to establish parameters for its practical use in MCMVstudies. Outside of organ harvest, there are few practical salivary gland cell models available that offer a relevant alternative for studying the MCMV-salivary gland relationship. The practical implication of this study is the potential use of the SGC1 cell line in conjunction with MCMV research.

SK 20. **A synthetic medium that closely resembles the stagnant mucus within the lung alveoli of cystic fibrosis patients**

Cecily Haley*, Jane A. Colmer-Hamood, and Abdul N. Hamood, Department of Microbiology & Immunology, School of Medicine, Texas Tech University Health Sciences Center, Lubbock, TX

Cystic fibrosis (CF) is a genetic defect characterized by airway obstruction by accumulation of thick mucus within the lung alveoli. This thick mucus supports the growth of different bacterial pathogens including *Pseudomonas aeruginosa. P. aeruginosa* is a gram-negative opportunistic pathogen that produces numerous extracellular virulence factors including exotoxin A, pyocyanin, and the iron-scavenging molecules pyoverdine and pyochelin, all of which are negatively regulated by iron. The environment within specific infection sites influences the production of different virulence factors by the infecting pathogen. In this study, we describe a unique synthetic medium, Cystic Fibrosis-Lubbock (CF-L) that closely resembles the environment within the lung alveoli of CF patients. CF-L contains different salts and all amino acids at concentrations that parallel those found within the CF sputum. Additionally, CF-L contains lecithin (a surfactant), fragmented DNA (for viscosity), which represents DNA produced by lysed cells, as well as an iron chelator. To assess the effect of mucin (M), the main component of mucus, we added it to CF-L producing CF-L/M medium. We examined the effect of CF-L and CF-L/M on different *P. aeruginosa* genes using plasmids that carry transcriptional fusions of each gene by growing PAO1 at 37°C under static conditions for 16 hours. Growth in CF-L significantly increased the expression of the pyoverdine gene *pvdA*, exotoxin A gene *toxA*, and the *toxA* regulatory gene *toxR*. CF-L also significantly increased the expression of *pvdS*, which is essential for expression of *toxA* and the pyoverdine genes, and *pchR*, the main regulator of the pyochelin genes. In contrast, the growth of PAO1 in CF-L/M reduced the expression of the above described genes significantly. Those genes were repressed despite the presence of an efficient iron chelator in CF-L/M. These results suggest that mucin selectively represses the expression of PAO1 iron-regulated genes and that the repression of these genes by mucin is independent of iron.

SK 21. **Serum albumin significantly enhances the expression of *Pseudomonas aeruginosa* iron controlled genes**

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Pseudomonas aeruginosa, which causes serious infections in immune compromised hosts, produces several virulence factors. The production of these virulence factors is influenced by the environment at the infection site. In response to the limitation of iron within the host, *P. aeruginosa* produces exotoxin A and siderophores (iron scavenging molecules). We recently showed that at early stages of growth of *P. aeruginosa*, adult bovine serum (ABS) and human serum significantly enhanced the production of these iron controlled factors. In this study, we describe experiments designed to identify the potential serum factor that produces the observed effect. Fractionation experiments, using different molecular weight cutoff membranes indicated a potential high molecular weight factor which is greater than 50kD but less than 100kD. The observed effect was eliminated when we subjected ABS to heat or trypsin treatment. However, complement inactivation experiments failed to eliminate this effect. Since albumin is a major serum component we tried to eliminate it from serum using albumin absorption experiments. Albumin absorption significantly reduced the effect of serum on the expression of *P. aeruginosa* genes. Similarly, the effect of albumin deficient human serum was significantly reduced. Albumin may accomplish its effect as either a calcium binding protein (CaBP) or an iron binding protein (FeBP). However, neither casein (a CaBP) nor holotransferrin (a FeBP) affected the expression of PAO1 genes. These results suggest that 1) Serum albumin plays a critical role in *P. aeruginosa* virulence in early stages of infection by enhancing the expression of iron controlled genes; 2) such an effect is not related to albumin associated calcium or iron but possibly albumin associated peptides; 3) other serum factors may complement the effect of albumin.

SK 22. ***P. aeruginosa* quorum sensing signals inhibit the development of *S. aureus* biofilms: analysis using a three-dimensional wound biofilm model**

Anastasia Warden[1,2] , *Kyle G. Miller[2], Gunnar F. Kaufmann [3] , Kim D. Janda[3], Colin A. Lowery[3], Janet Dertien[4], Jane A. Colmer-Hamood[2], and Abdul N. Hamood[2], [1]Department of Chemistry & Biochemistry, Texas Tech University, Lubbock, TX; [2]Department of Microbiology & Immunology, Texas Tech University Health Sciences Center, Lubbock, TX; [3]The Scripps Research Institute, La Jolla, CA; and [4]Department of Pharmacology & Neurosciences, Texas Tech University Health Science Center, Lubbock, TX

One of the serious challenges that the health care system faces is the management of infected chronic wounds including diabetic foot ulcers, non-healing surgical wounds, and pressure ulcers. These wounds are usually colonized by different pathogens including the opportunistic pathogens *Pseudomonas aeruginosa* and *Staphylococcus aureus*. At different infection sites, bacteria exist within specific protective structures termed biofilms. Biofilms are difficult to eliminate and are resistant to different antibiotics. Within the infected wound, bacteria utilize different strategies to compete for the limited space and nutrients. Recent research has shown that *P. aeruginosa's* quorum sensing signals, such as acylated homoserine lactones 3-oxo-C_{12}-HSL and 3-oxo-C_{14}-HSL, as well as their tetramic acid degradation products C_{12}-TA and C_{14}-TA, have innate antimicrobial activity. Thus, we hypothesize that *P. aeruginosa* might use these molecules to eliminate other competing bacteria. In this study, we utilized the newly developed three-dimensional wound biofilm model (3-DWBM) to compare the effectiveness of C_{12}-TA and C_{14}-TA in eliminating *S. aureus* biofilms. The main constituents of the 3-DWBM, which mimics the conditions within the infected wound, are collagen and serum. The biofilms were visualized by confocal laser scanning microscopy. *S. aureus* expressing the green fluorescent protein produced a well-developed biofilm that extended within the collagen layers of the 3-DWBM. When applied at the initiation of the biofilm, C_{14}-TA, but not C_{12}-TA prevented the development of *S. aureus* biofilm. However, C_{14}-TA failed to interfere with biofilm development when applied 4, 8, or 24 hours after biofilm initiation. Daily application of C_{14}-TA for 7 days reduced the thickness and depth of the *S. aureus* biofilm. Additionally, quantitative analysis of the biofilms using the MetaMorph program showed that the observed reduction is significant. Neither C_{12}-TA nor C_{14}-TA affected the development of *P. aeruginosa* biofilms. These results suggest that: (1) the 3-DWBM is a suitable model to examine biofilm development by different bacterial pathogens; (2) C_{14}-TA interferes with the development of *S. aureus* biofilms but is ineffective in eliminating already established ones, and (3) C_{14}-TA is a potential therapy against *S. aureus* biofilms within chronically infected wounds.

23. **Blood iron binding proteins reduce *Pseudomonas aeruginosa* biofilm on intravenous catheters.**
Jena-Claire Auten and Abdul Hamood, Department of Microbiology and Immunology, Texas Tech University Health Sciences Center

Pseudomonas aeruginosa is a gram negative opportunistic pathogen that causes serious infections in severely burned patients, as well as in patients with chronic wounds. *P. aeruginosa* produces several virulence factors including Exotoxin A, siderophores, and the effector molecules of the type III secretion systems. In addition at different infection sites, *P. aeruginosa* exists within protective structures termed biofilms. The environmental conditions at the sites of infection influence both biofilm formation and the production of virulence factors. Among these environmental conditions is the variation in the levels of calcium, magnesium, and iron. Numerous previous studies have demonstrated the effect of these ions on biofilm formation, Exotoxin A production, siderophore production, and the production of type III secretion system factors. However, the level of these ions in the studies varies greatly from their level within human blood. In this study, we described a novel growth medium, Supplemented Rich Defined Medium (SRDM), in which the level of calcium, magnesium, and phosphorus resemble their level in blood. In addition, since iron exists in blood in a protein bound and not a soluble form, we supplemented SRDM further with purified iron binding proteins (holotransferrin and ferritin). Furthermore we supplied SRDM with a carbon source and adjusted its pH to reflect that of human blood. SRDM supported growth of the virulent *P. aeruginosa* strain PA14. We analyzed the effect of SRDM and the concentrations of calcium, magnesium, and iron on biofilm formation by PA14 on the surface of intravenous catheters. In comparison with a standard laboratory medium, SRDM lacking calcium, magnesium and iron binding proteins supported limited biofilm development. Biofilm development was enhanced upon the addition of either calcium or magnesium to SRDM. However the addition of either holotransferrin or ferritin to SRDM containing magnesium or calcium significantly reduced biofilm development by PA14. In contrast the addition of iron deficient transferrin (apotransferrin) had no effect on biofilm development.

SK 24. **Interactions of *Pseudomonas aeruginosa* and *Staphylococcus aureus* in dual-species biofilms**
Stephanie DeLeon[1]*, Allie Clinton[1], Kendra Rumbaugh[1], Department of Surgery, Texas Tech University Health Sciences Center, Lubbock, TX 79430[1]

Chronic wound infections have a profound effect on the morbidity and mortality of a large patient population and cost billions of dollars in direct medical costs annually in the United States. Biofilms are communities of microbes that reside in a polysaccharide shell and contribute greatly to the severity and antibacterial resistance of wound infections. Two of the most prominent and notoriously resistant bacterial species found together are *Pseudomonas aeruginosa* and *Staphylococcus aureus*. However, few studies have examined the interactions of these two species in the context of wound infections. Our goal was to create an *in vitro* model to examine the interspecies interactions between *P. aeruginosa* and *S. aureus.* We hypothesized that the two species would work synergistically to promote biofilm formation. We examined the growth of *P. aeruginosa* and *S. aureus* co-cultures in a flask, to represent free-living planktonic cell growth, and observed that *P. aeruginosa* quickly dominated the culture, presumably killing off all the *.aureus* within 8 hours. However, when the two species were grown together in conditions to simulate the wound environment, we observed a rapid formation of biofilms that remained dual-species for several days. Interestingly, we also observed that *P. aeruginosa* required the presence of *S. aureus* to form a biofilm, and these interactions were dependent of the cell-to-cell signaling systems termed 'quorum sensing'. Our data suggest that synergistic interactions between *P. aeruginosa* and *S. aureus* in wounds may contribute to biofilm formation and thus healing delays and/or antibiotic tolerance.

SK 25. **NO TITLE**
Ann Marie Scott, Texas Tech University Health Science Center, Lubbock TX

Infection with multi-drug resistant (MDR) pathogens presents a major challenge to physicians and a continuous threat to their patients. The main causes for the emergence of MDR pathogens are the indiscriminate use of antibiotics and the novel antibiotic resistance mechanisms. Therefore, extensive worldwide research efforts are focused on identifying alternative antimicrobial agents. We recently showed that selenium (Se) inhibited the development bacterial biofilms on medical devices. In this study, we tried to determine if Se solution inhibits the growth of planktonic cells of *P. aeruginosa*. Overnight cultures were diluted in a nutrient broth containing variable concentrations of Se (in form of selenocystamine solution) and grown for 16 hours to an optical density (OD600) of 0.02-0.03. The *P. aeruginosa* strain PA01 growth was inhibited at concentrations as low as .0125% Se. We obtained similar results with the three other *P. aeruginosa* virulent laboratory strains PA14, PAK, PA103, as well as *P. aeruginosa* isolates obtained from infected wounds. Selenocystamine also interfered with the development of PA01 biofilm. Initial viable count analysis (by determining the colony forming units/ml) confirmed these findings. These results suggest that: Se is a potential therapy for *P. aeruginosa* infections and can interfere with biofilm formation. Whether Se is effective against other gram-negative pathogens is yet to be determined.

SK 26. **NO TITLE**

Wail Amor, Texas Tech University Health Science Center, Lubbock TX

The skin, a physical barrier that protects the underlying tissues from invading microorganisms, is destroyed during thermal injury (burn). Thermal injury also depresses the host's immune system resulting in an immunocompromised condition. Together, these two conditions facilitate the colonization and infection of the burn wound with potentially pathogenic microorganisms. Within the wound, many of these pathogens exist within structures termed biofilms. Biofilms protect the bacteria from the host response and enhance their resistance to different antibiotics. Burn wounds are predominantly colonized by *Pseudomonas, Staphylococcus, Serratia*, *Klebsiella*, and *Enterococcus*. Some of these pathogens, especially *Pseudomonas aeruginosa* and *Staphylococcus aureus*, are highly resistant to different antibiotics. As a result, eradicating these pathogens from burn wounds by the use of antibiotics only is very difficult. Therefore, it is essential to investigate novel antimicrobial agents. Several previous studies documented the antimicrobial activity of garlic (*Allium sativum*). In this study, we utilized our recently developed *in vitro* wound biofilm model to examine the effectiveness of formulated garlic cream (G-cream) in eliminating biofilms produced by wound pathogens. We first tested the *S. aureus* strain AH133 and the *P. aeruginosa* strain PAO1-GFP. Both strains carry the gene that encodes the green fluorescent protein. Strains were individually inoculated onto nitrocellulose disks that were placed on agar plates. Equal amounts of either control cream or G-cream were evenly spread on the surface of bandages. We tested two garlic concentrations; 130 and 195 mg/cm^2. The disks were covered with either control cream or G-cream bandages. After incubation at 37°C for 24 hours, the bandages were removed and the biofilms were quantified and visualized. The numbers of microorganisms recovered from disks covered with G-cream bandages (at either concentration) were significantly less than those recovered from disks covered with control cream bandages. Confocal laser scanning microscopy confirmed these results. We also tested different wound isolates obtained from burn patients at the Timothy J. Harnar Burn Center, University Medical Center, Lubbock, TX. G-cream bandages inhibited biofilm development by all tested isolates. These results suggest garlic ointment is effective in inhibiting biofilms produced by different wound pathogens.

SK 27. **Developing an *In Vitro* Biofilm Model for Dental Caries**

Fernando Andrade[1], Carrie Graham[1], Zalman Vaksman[1], Karen Gomez[1], Gena Tribble[2], S. Ray Taylor[2] and Heidi B. Kaplan[1]. [1]Department of Microbiology and Molecular Genetics, University of Texas Medical School and [2]University of Texas Dental Branch, Houston, TX.

Dental caries is the single most common chronic disease among adults, adolescents, and children in the United States. Historically, analysis of dental microbial populations in clinical studies has been challenging due to many factors including the low sensitivity of clinical measurement tools, the variability in saliva chemistry, and the variation in microbial flora. Therefore, a defined multispecies biofilm model is needed for accurate analysis of the effects of the microbial populations on the tooth surface. Here, we have adapted an *in vitro* biofilm method previously developed in our laboratory to serve as a model for dental caries. This model includes bacterial growth on 1.2 cm discs of polymethylmethacrylate (PMMA) at 37°C under static anaerobic conditions in an artificial saliva medium supplemented with 0.2% sucrose. To simulate oral microbial communities the biofilms were inoculated with strains of *Actinomyces odontolyticus*, *Fusobacterium nucleatum*, *Streptococcus mutans*, and *Veillonella dispar* recently isolated from dental patients. The biofilms were stained BacLight LIVE/DEAD cell viability kit (Molecular Probes) and imaged with an Olympus IX81 fluorescence microscope after 48 and 96 hrs of growth. The imaging indicated that at both time points the predominant organism was *S. mutans*. Denaturing gradient gel electrophoresis and quantitative PCR was used to profile the bacterial biofilm communities and to indicate the presence of each organism. This model will be useful in analysis of primary and secondary caries.

JA 28. **Quorum sensing interaction and the effect of antibiotic on the dynamics of two types of bacteria**

Campos, S., Gauntt, S., Jackson, M., Lu, A., and *Vu, T., James Grover, PhD.; Hristo Kojouharov, PhD.

Quorum sensing plays an important role in cell-to-cell communication. Bacteria that employ this mechanism produce a signal molecule that can initiate transcription, causing phenotypic changes such as biofilm formation. In this study, we examine two types of bacteria of the same species that have different growth and mortality rates. Only one of the two types produces the signal molecule. The other type has the ability to form biofilms, allowing it to gain more protection from the antibiotic treatment. If the antibiotic can inhibit quorum sensing, the biofilm will be unlikely to form and the infection may be eliminated efficiently. Four differential equations were derived to model the effects of autoinduction and antibiotic on the quorum sensing between the bacteria. Autoinduction induces the maximum rate at which signal molecules are produced; higher signal molecule concentration implies higher possibility of converting one type of bacteria into another type capable of forming biofilm. However, the analysis and numerical simulations of this theoretical study indicate that autoinduction

plays no significant difference in the conversion. In addition, the antibiotic eliminates both types of bacteria at different rates.

SK 29. **A Biochemical Analysis of the Quorum Sensing-Like Compounds Secreted by *Chlamydomonas reinhardtii***

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Recently it has been demonstrated that cultures of *C. reinhardtii* secrete quorum sensing-like compounds that mimic and interfere with bacterial quorum sensing (QS). Previously, cultures of *C. reinhardtii* were overlaid with soft agar containing reporter strains that confirmed the secretion of both stimulatory and inhibitory compounds. Original fractionation and purification revealed about 12 different compounds containing AHL-like activity, and their physical properties (i.e. hydrophobicity and solubility characteristics), have been vaguely described. For our study, compounds will be extracted using ethyl acetate and the crude extract will be fractionated and purified utilizing a C18 reverse phase HPLC column. Fractions will then be analyzed twice via GC-MS; once as the newly purified single compound, and a second time after that same compound has been incubated in a base. Confirmed AHL-like compounds will be further analyzed utilizing ^{1}H-NMR to decipher any structural characteristics. Activity of purified extracts on the *LasR* QS pathway will be confirmed with a β-galactosidase assay.

SK 30. **Bacteria in the Fish Skin Microbiome form a Biofilm to Resist Antibiotic Action**

Leroy Brooks*[,1], Sonya Ramzanali[1], Robert Fultz[2], and Todd P. Primm[1], [1]Dept. of Biological Sciences, Sam Houston State University, [2]Cell and Molecular Biology Program, Baylor College of Medicine

The species composition and functional roles in the microbiome on humans and other animals is an area of great current interest. *Gambusia affinis* is an invasive freshwater fish common throughout much of the United States. Development of this inexpensive and hardy species for use as a model organism could expand possibilities for both an educational tool and biomedical research into microbiomes. Viewing the skin microbiome as an ecological system, antibiotics are a disruptive force. As part of this, concurrent research has found that when bacteria *in vivo* in the *G. affinis* slime layer ecosystem are subjected to high-dose rifampicin, a broad-spectrum antibiotic, there is only small change (<50%) in the total number of organisms recoverable from the skin. In contrast, when bacteria were extracted from the fish skin and cultured in the planktonic state *in vitro*, exposure to rifampicin resulted in typically large losses of viability. Hypothesizing that the bacteria on the skin are arranged in a biofilm-like state, we developed a mixed-species biofilm model *in vitro*. Treatment in this system replicated the minimal killing seen *in vivo*. Based upon this, we are further investigating the possibility that the biofilm state of bacteria in the slime layer accounts for the inhibition of rifampicin absorption and/or efficacy. This has implications to antibiotic treatment of humans, suggesting the effects on their microbiomes may be less than predicted by *in vitro* susceptibility data.

JA 31. **Biofilm formation in coral bacterial pathogens influenced by rising global temperatures**

*Joshuah Beach-Letendre[1], Whitney Mann[1], Laura Mydlarz[1], The University of Texas at Arlington

Temperature increase in the world's oceans due to climate change has increasingly become a concern to ecologists, environmentalists, and the tourism industry. These concerns stem from the correlation between oceanic temperature increase and the increasing prevalence of coral disease. Microbial biofilms form in a variety of habitats, including the surface of various coral species, and can prove to be disrupting if allowed to flourish unchecked. The formation of these intricate, layered networks provides a protective barrier against any antimicrobial compounds regularly secreted by the coral host effectively hampering immunological defense. Analogous to medical settings, where antibiotics prove ineffective in penetrating the layers of bacterial cells, the coral host cannot disrupt the ever growing matrix of bacteria. One factor which correlates with formation of these microbial mats is temperature. We suggest that there is a link between biofilm formation and coral disease due to temperature increase. The biofilm forming ability of three bacterial species implicated in coral diseases was measured over the course of several time intervals at elevated and ambient temperatures. Preliminary data trends show that temperature has a significant effect on biofilm formation in the pathogenic strains. The formation of biofilm varied over time but was variable between strains. An increased focus on host-microbe interaction during the synthesis of biofilm networks will be key to addressing the issue of temperature mediated opportunistic infections plaguing coral reef habitats.

SK 32. **Characterization of a novel IncFIB virulence plasmid in *Salmonella enterica* serovar Typhimurium**

Daniel P. Haarmann* and Aaron M. Lynne, Department of Biological Sciences, Sam Houston State University

Salmonella enterica has become an increasing problem as a pathogen for humans as well as commercial poultry farms. With the acquisition of a IncFIB virulence plasmid, *Salmonella enterica* serovar Kentucky demonstrated increased virulence similar to that found in avian pathogenic *Escherichia coli* (APEC) which carries the same plasmid. Virulence genes, such as iss, tsh, iroN, iutA, estA, estB, and cvaC, associated with the IncFIB plasmid were located on various *Salmonella enterica* serovar Typhimurium strains. The genes were confirmed to exist on an IncFIB plasmid by conjugation assay and isolated by PCR from the transconjugate. Various phenotypic expression assays will now be performed to evaluate the expression of said genes. With the acquisition of these virulence genes on a plasmid, we may see a similar increase in the ability for S. Typhimurium to cause disease, as seen in APEC.

SK 33. **Selective Pressure Potential of Antimicrobial Agents to Facilitate Spread of Resistance Plasmids in *Salmonella enterica***

Maria Garcia*, Keri Kershaw*, and Aaron M. Lynne, Department of Biological Sciences, Sam Houston State University, Huntsville, TX 77381

Salmonella enterica is the leading cause of bacterial food-borne disease and is a public health concern. According to the CDC, *Salmonella enterica* serovar Typhimurium is one of the most commonly isolated serovars. Often, antimicrobial resistance genes are found on large, conjugative plasmids. The goal of this study was to assess the ability of exposure to antimicrobial agents to increase the conjugation rates of large resistance plasmids in *S.* Typhimurium in vitro. Conjugation pairs were mated under varying concentration of antimicrobials, plated on double antibiotic plates to select for transconjugants and conjugation rates were calculated. Initial results indicate an increased rate of conjugation of resistance plasmids in *S.* Typhimurium.

34. **Searching for genes required for *Salmonella* Typhimurium to withstand inflammation.**

T. Endicott[1*], S. Porwollik[2,3] C. Shields[1], H. Yang[1], P. Cui[1], M. McClelland[2,3], and H. Andrews-Polymenis[1], [1]Texas A&M Health Science Center, College Station, TX, [2]Univ. of California, Irvine, CA, [3]Vaccine Res. Inst. of San Diego, San Diego, CA

Upon infection with non-typhoidal *Salmonella enterica* serotype Typhimurium (STm) the primary response of the host is a large infiltration of neutrophils and the production of anti-microbial peptides by the intestinal epithelium. STm is able to thrive in this highly inflammatory environment whereas other enteric organisms are susceptible to it. As a result the host's own intestinal flora is diminished, giving STm a growth advantage. Using two libraries of STm mutants that have undergone targeted deletion of either large portions of the STm chromosome (multi-gene deletion or MGD) or single genes (single gene deletions or SGD) we have inoculated pools of mutants from each collection and used them to inoculate bovine ligated ileal loops. Using this approach we will identify those mutants that are not able to withstand attack by the host response in the intestine during infection. We have identified several genes of interest as under selection in the inflamed intestine using this approach. For two of these mutants, we have verified each mutant's susceptibility to inflammation through competitive infection in Strep-treated mice.

35. **Gene Knockouts in the Pyruvate Metabolism Pathway of *Salmonella typhimurium* Increase *SPI1*-Virulence Expression and Invasion of Host Cells**

Jason Abernathy*[1], Carolina Corkill[1], Carolee Hinojosa[1], Xianyao Li[1] Helene Andrews-Polymenis[2]and Huaijun Zhou[1], [1]Department of Poultry Science, [2]Department of Microbial and Molecular Pathogenesis, Texas A&M University, College Station, TX 77843

Salmonellosis remains one of the most widespread and prevalent foodborne diseases worldwide. Yet a complete understanding of the human-*Salmonella* interactome has thus far not been realized. Two *Salmonella* genes in the pyruvate metabolism pathway, pyruvate formate lyase I (Δ*pflB*) and acetaldehyde-CoA/alcohol dehydrogenase (Δ*adhE*), were significantly up-regulated in our previous host-*Salmonella* whole genome gene expression profiling study. Therefore, knockout mutants of these *Salmonella genes* were generated by the lambda red recombinase system to assess the effects of these genes on host infectivity. A time-course infection study was performed on cell line HCT-8, human intestinal epithelia, as well as a macrophage cell line THP-1. Intracellular bacteria were counted at 1h, 4h, 18h, 24h, and 48h post-infection of host cells with the wild-type parent strain or either mutant using the gentamicin protection assay. The results show that

epithelial cells infected with either mutant strain contained significantly (p<0.01) higher numbers of intracellular bacteria than the wild-type, beginning at 4h post-infection and lasting through the duration of the study at 48h. Mutant strains, with or without opsonization, were also elevated inside macrophages compared to the wild-type. We then assessed the expression of invasion genes as a potential basis for the increased infectivity. A total of 19 *Salmonella* pathogenicity island 1 (*SPI1*) genes were examined by qPCR. Multiple *SPI1* genes were up-regulated in the mutant strains, ranging from 3-4 genes measured from cultures and 7-13 genes measured within invaded HCT-8 host cells at various times post-infection. *SPI1* genes have been shown to be regulated by formate, a metabolite common in the mammalian intestinal tract. Our data suggest induction of invasion gene expression is potentially due to an imbalance of metabolites in the mutants. We hypothesize that invasion gene expression can be differentially regulated through multiple carbohydrate metabolism pathways and that infectivity of the host changes as these pathways are perturbed.

JA 36. **The Mode of Action of Thioridazine against Growing and Non-growing *Staphylococcus aureus***

Johnathan Adamson, Julian G. Hurdle, University of Texas at Arlington, Department. of Biology

S. aureus is a leading cause of hospital-acquired infections. In addition to possessing genetic resistance to antibiotics, *S. aureus* also exhibit phenotypic tolerance to antibiotics by residing in a dormant state (slow or non-growing). These infections persist in spite of treatment. Analyzing the response of *S. aureus* to antimicrobials shown to display anti-dormancy activity may be a way forward to obtaining new agents or understanding the physiology of *S. aureus* in its dormant state. Recently, thioridazine (THZ) was shown to kill dormant *M. tuberculosis*, though the exact mode of action (MOA) is unknown. We performed this study to explore if THZ also kills dormant *S. aureus* and to define its MOA against *S. aureus*. After determining the MIC of THZ in TSB, the bactericidal activity of THZ against logarithmic, stationary phase and nutrient-starved cultures of *S. aureus* N315 were determined, alongside effects on ATP biosynthesis using BacTiter-Glo. Mutants resistant to THZ were generated for genotyping studies. THZ rapidly killed actively growing *S. aureus* at concentrations above its MIC (12.5ug/mL), causing a 3-log reduction in 3h. Cell killing was accompanied by reduced ATP biosynthesis. Like many other agents, THZ was ineffective against stationary phase cultures even at high concentrations (200 g/mL). However, since the physiologies of cells change with different conditions, studies are ongoing to determine if non-growing nutrient starved cells are also insusceptible to THZ killing. Spontaneous resistance to THZ was limited, but mutants could be obtained following chemical mutagenesis. This may imply that THZ has multiple targets or resistance development is difficult. Putative NDH Type 2 enzymes identified in *S. aureus* through BLAST searches are being sequenced to determine if these proteins are involved in the MOA of THZ.

SK 37. **Incorporation of Hyaluronic Acid into *Staphylococcus aureus* Biofilms**

*Kathryn Tierney[1], Fernando Andrade[2], Zalman Vaksman[2] and Heidi B. Kaplan[2], [1]The University of Dallas, [2]The University of Texas Medical School at Houston

Staphylococcus aureus is a leading cause of nosocomial surface-associated infections termed biofilm infections. In our laboratory's *in vitro* model, robust biofilms are observed when hyaluronic acid (HA), a high molecular weight extracellular polysaccharide found in all mammalian connective tissues, is a major component of the growth environment. We hypothesized that *S. aureus* incorporates environmental HA into the biofilm matrix and as a result, hyaluronidase (HAase) will function as a dispersant. *S. aureus* biofilms were grown statically on polymethylmethacrylate (PMMA) discs in 24-well plates at 37°C in a synthetic interstitial fluid (SIF) medium developed by our lab with 0.2% glucose. This biofilm medium was supplemented with HA or methylcellulose (MC) to increase viscosity. After the biofilms were grown for five days in SIF, HAase was added to half of the wells and the plate was incubated 4 hr to overnight. Then all the biofilms were stained with the BacLight Live/Dead Viability Stain Kit (Molecular Probes) and visually analyzed with an OlympusIX81 fluorescence microscope. The results indicate that the presence of HA in SIF medium allowed for almost twice the biomass compared to biofilms grown in MC. Furthermore, HAase dispersed about 80% of the biofilms grown in the presence of HA and had much less of an effect on the biofilms grown in the presence of MC. These results support our hypothesis and indicate that *S. aureus* incorporates environmental HA into its biofilm matrix. Understanding how HA contributes to biofilm formation should lead to new methods for treating biofilm-based infections by preventing adherence or increasing their dispersal.

SK 38. **Nasopharyngeal aggregate formation by *Streptococcus pneumoniae* and contributions of serum-derived host factors to biofilm formation**

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Streptococcus pneumoniae is a Gram-positive, commensal bacterium that colonizes the nasopharynx and causes opportunistic diseases including otitis media, pneumonia, and meningitis. Biofilms are now known to play a critical role in chronic infections with biofilm-related structures serving as a mechanism for persistence *in vivo*. For *S. pneumoniae*, a role for biofilms in colonization of the nasopharynx is understudied. Herein, we demonstrate biofilm formation within the murine nasopharynx by a number of *S. pneumoniae* clinical isolates. Notably, the size of biofilm aggregates recovered from the nasopharynx increased over time, whereas CFU counts decreased. Biofilm aggregates were observed up to 6 weeks after inoculation, despite the fact that bacteria were non-culturable after 2 weeks. Thus, the amount of biofilm within the nasopharynx increased and the associated bacteria were present in a non-culturable state. Additionally, we have determined the presence of an active factor within serum that contributes to biofilm formation in a strain-dependent manner. This interaction appears to be unaffected by deletion of the bacterial adhesins CbpA, PavA, and PsrP; the neuraminidase NanA; and the cell wall hydrolase LytA. Our findings thus far suggest that duration of colonization extends beyond the point of isolation of culturable bacteria from the nasopharynx and that host factors contribute to biofilm formation *in vivo*.

SK 39. **Pneumococcal cytotoxicity to cardiomyocytes**

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Streptococcus pneumoniae (the pneumococcus), a Gram-positive bacterium, accounts for approximately 40% of all cases of community-acquired pneumonia (CAP). Individuals hospitalized for pneumococcal CAP are at an increased risk for sudden death as a result of adverse cardiac events. Herein, we begin to examine events occurring during pneumococcal pneumonia that may adversely affect cardiac function using an *in vitro* culture system with HL-1 cardiomyocytes. Infection of HL-1 cells with *S. pneumoniae* at 10^7 CFU/ml caused >70% death after 4 hours. Cell death decreased in a bacterial dose-dependent fashion with exposure to 10^7 heat-killed bacteria having no impact on mortality. Treatment of HL-1 cells with pneumolysin, a cholesterol dependent pore-forming toxin, for 4 hours with 2 µg/ml, a dose 6 times greater than that necessary to lyse 50% of red blood cells, killed only 10% of cardiomyocytes. No cytotoxicity was observed following treatment with pneumococcal cell wall alone. Unexpectedly, the modest pneumolysin toxicity observed was reduced in HL-1 cells simultaneously treated with bacterial cell wall. We conclude that high numbers of *S. pneumoniae* are capable of killing HL-1 cells, but that cardiomyocytes are intrinsically resistant to pneumococcal products. Studies are ongoing to characterize how despite protection, there continues to be adverse cardiac events in humans with pneumonia.

SK 40. **Modulation of IL-18 secretion from the gut epithelium by *Yersinia* toxins**

Josephine W Thinwa*, Rumu Bose, and Peter Dube. Department of Microbiology and Immunology, University of Texas Health Science Center San Antonio, 7703 Floyd Curl Dr., San Antonio, TX 78229.

Yersinia enterocolitica, a food-borne gastrointestinal pathogen, is highly adapted to invading the gut epithelium while simultaneously evading host innate immune responses. The primary immune evasion mechanism of *Yersinia* involves the intoxication of host cells with virulence proteins called *Yersinia* outer proteins (Yops). These Yops disable innate immune defense mechanisms including the production of proinflammatory cytokines. Interleukin-18 is an important cytokine for the clearance of the bacterium and is among the cytokines inhibited by *Yersinia*. The secretion of IL-18 is tightly regulated by a multi-protein complex called the inflammasome, which controls the activation of caspase-1 and subsquent proteolytic maturation of IL-18. Thus far, little is understood on the mechanism of inflammasome activation in epithelial cells, although the gut epithelium has been identified as an important source of IL-18 during inflammation. In our studies, the infection of the intestinal epithelial cell line Caco-2 leads to the activation of the NLRP3 inflammasome and IL-18 secretion. However, we found that the *Yersinia* virulence protein YopH, which functions as a protein tyrosine phosphatase, acts as a potent inhibitor of IL-18 secretion from infected cells. Furthermore, we observed that IL-18 secretion is dependent on p38 MAPK. The inhibition of p38 MAPK resulted in the down-regulation of the inflammasome receptor NLRP3, thus disrupting the inflammasome function during *Yersinia* infection. Overall, our studies have identified p38MAPK signaling as a critical pathway for inflammasome activation during infection, and a novel YopH-mediated immune evasion mechanism of *Yersinia*, which targets IL-18 secretion from intestinal epithelial cells.

SK 41. ***Mycoplasma pneumoniae* CARDS toxin induces pulmonary eosinophilic and lymphocytic inflammation.**

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Mycoplasma pneumoniae causes acute and chronic lung infections in humans producing a variety of pulmonary and extra-pulmonary diseases. Research suggests *M. pneumoniae*-associated exacerbation of asthma to be a significant source of morbidity. The mechanism by which *M. pneumoniae* causes pathogenesis is not well understood, however, it was recently shown to produce a toxin, termed the Community Acquired Respiratory Distress Syndrome (CARDS) toxin. This toxin has unique properties in that it is both an ADP-ribosylating enzyme and vacuolating toxin. Our studies demonstrate that CARDS toxin associated-asthma in mice has many features associated with human asthma. These data reveal an increase of cellular infiltration; including lymphocytes and a significant eosinophilic component. Additionally, exposure of mice to CARDS toxin induces expression of Th2 cytokines associated with asthma, as well as increased airway hyperreactivity. These findings are significant because CARDS toxin might represent a Mycoplasma virulence factor that induces and/or exacerbates asthma in humans.

SK 42. **Extracellular Superoxide Dismutase Inhibits Innate Immune Responses and Clearance of *Listeria monocytogenes***

Timothy J. Break*, Sujung Jun, Mohanalaxmi Indramohan, Karen D. Carr, Amy N. Sieve, Ladislav Dory, and Rance E. Berg, Department of Molecular Biology and Immunology, University of North Texas Health Science Center, Fort Worth, Texas, USA, 76107.

Reactive oxygen species (ROS) play an important role during immune responses to bacterial pathogens. Extracellular superoxide dismutase (ecSOD) regulates extracellular concentrations of ROS and protects tissues during inflammatory insults. The participation of ecSOD in immune responses seems intuitive, yet is poorly understood. In the present study, we utilized mice with varying levels of ecSOD activity to investigate the involvement of this enzyme in immune responses against the Gram-positive, intracellular bacterium, *Listeria monocytogenes*. Surprisingly, our data demonstrate that ecSOD activity negatively impacted host survival and bacterial clearance. Paradoxically, increased ecSOD activity was accompanied by increased percentages and numbers of neutrophils in the livers or mice that were left uninfected, or infected with *Listeria monocytogenes.* Increased ecSOD activity was accompanied by decreased co-localization of neutrophils with bacteria, as well as increased neutrophil apoptosis, which reduced overall and neutrophil-specific TNF-alpha production. Neutrophil depletion studies revealed that high levels of ecSOD activity resulted in neutrophils with no protective capacity, whereas neutrophils from mice lacking ecSOD provided superior protection compared to neutrophils from wild-type mice. Taken together, our data demonstrate that ecSOD activity regulates innate immune responses and provides a potential target for therapeutic intervention.

SK 43. **Inflammatory monocyte recruitment is regulated by IL-23 during *Listeria monocytogenes* infection**

Mohanalaxmi Indramohan*, Amy N. Sieve, Timothy J. Break, and Rance E. Berg, Department of Molecular Biology and Immunology, University of North Texas Health Science Center, Fort Worth, Texas 76107, USA.

Listeria monocytogenes (LM) is a Gram-positive intracellular pathogen that causes meningitis and septicemia in immunocompromised individuals, and spontaneous abortion in pregnant women. The innate immune reponse against LM is primarily mediated by neutrophils and monocytes. IL-23 is an important pro-inflammatory cytokine well known for its role in neutrophil recruitment in various infectious diseases. Previously, we have shown that IL-23 is required for resistance against LM and for neutrophil recruitment to the liver, but not the spleen, during infection. Despite efficient neutrophil recruitment to the spleen, IL-23p19 knockout (KO) mice have an increased bacterial burden in this organ, suggesting that IL-23 may regulate the recruitment/function of another cell type. Here, we show that the specific depletion of neutrophils abrogated the differences in bacterial burden in the livers, but not the spleens of C57BL/6 and IL-23p19 KO mice. Moreover, LM-infected IL-23p19 KO mice had fewer monocytes in the spleen, as well as a reduction in the monocyte-recruiting chemokines, CCL2 and CCL7, compared to C57BL/6 mice. This lack of efficient monocyte recruitment resulted in decreased production of monocyte-derived TNF-α and $NO^{\cdot}$, leading to increased bacterial burdens in the spleens of LM-infected IL-23p19 KO mice. Collectively, our data establish that IL-23 is required for the optimal recruitment of TNF-α and $NO^{\cdot}$-producing inflammatory

monocytes, thus revealing a novel mechanism by which this pro-inflammatory cytokine provides protection against bacterial infection.

SK 44. **Characterization of the Effect of 4-hydroxyacetophenone on Chlamydomonas reinhardtii Motility**

Travis Hardcastle*, Todd P. Primm, Anne R. Gaillard, Department of Biological Sciences, Sam Houston State University, Huntsville, TX 77341-2116

Every cell in the human body possesses at least one cilium during its lifetime. These cilia are divided into two categories: motile and primary (nonmotile). Defects in motile cilia lead to reduced fertility, hydrocephalus, and diseases such as Kartagener's syndrome. However, little is known about the exact mechanisms by which motility is produced. Wild-type Chlamydomonas reinhardtii exhibits positive phototaxis. Previous studies indicate that 4-hydroxyacetophenone induces negative phototaxis in wild-type Chlamydomonas reinhardtii cells. This study aims at providing additional insight into the inner workings of motile cilia by identifying the flagellar target of 4-hydroxyacetophenone. Reactivation of cell models was performed with and without the presence of 4-hydroxyacetophenone. Preliminary data indicates a significant difference in swimming speed and percent motility between the two groups. Additional research is being conducted in which flagellar protein extracts are isolated and separated by 4-hydroxyacetophenone affinity chromatography, followed by SDS-PAGE. Separated proteins will be excised and sequenced via MALDI-TOF mass spectrometry. These peptide sequences will be compared with gene models of Chlamydomonas to identify the proteins present. Identifying the flagellar target of 4-hydroxyacetophenone will help further develop our understanding of mechanisms involved in controlling ciliary motility.

45. **Nitric oxide detoxification by uropathogenic *Escherichia coli* CFT073**

Heer Mehta* and Stephen Spiro, University of Texas at Dallas

Uropathogenic *E. coli* (UPEC) is an extraintestinal pathogen which colonizes the urinary tract of its host. As a defense mechanism, the host releases nitric oxide. Nitric oxide (NO) is a toxic molecule that can attack metal centers of proteins, thiol groups, DNA and lipids, thus restricting microbial proliferation. Bacteria have evolved to resist nitric oxide. The enzymes flavohemoglobin (Hmp), flavorubredoxin (FlRd) and periplasmic nitrite reductase (Nrf) can convert nitric oxide to less toxic products, thereby protecting the pathogen. Hmp oxidizes NO to nitrate under aerobic and microaerobic conditions. FlRd reduces NO to nitrous oxide under anaerobic conditions and also shows aerobic NO detoxification activity. Nrf shows strictly anaerobic NO detoxification activity and catalyzes the 5 electron reduction of NO to ammonia. These activities are NO inducible. NsrR is a nitric oxide sensitive repressor which regulates expression of the genes encoding Nrf and Hmp. The genes encoding Flrd are regulated by the NO-sensitive activator NorR. We observed that a strain lacking the three known NO detoxification systems is still capable of NO detoxification. This activity is anaerobically induced by NO and nitrate. We wish to identify this activity and test its effectiveness in protecting UPEC within the urinary tract.

JA 46. **In vitro Fitness Cost of Rifaximin Resistance in *Clostridium difficile***

*Uyen Dang, Xiaoqian Wu, Julian G. Hurdle, University of Texas at Arlington

C. difficile infections (CDIs) are a leading cause of death in hospitalized elderly patients in the US. Due to limited treatment options, rifaximin (RFX), a semisynthetic derivative of rifampicin (RMP) that binds to RpoB, is undergoing development as a therapy for CDI. However, a high frequency of RMP-resistance is found among clinical isolates in some hospital centers. To explain this, we sought to determine if a fitness cost is associated with rifamycin resistance in C. difficile and define RFX resistance alleles. Methods: RFX-resistant mutants were selected at 4 and 32 x its MIC against C. difficile BAA-1875. The MICs of RFX were re-determined against mutants. The fitness cost was estimated by standard mixed-culture competition assays. Results: RFX-mutants arose at a frequency of 10-8, and had MICs ranging from 128 - >256 g/mL. Point mutations were detected in RpoB at position 550 i.e. S550Y/F. This allelic site has been shown to confer RMP resistance in S. aureus. Our present studies indicate that RFX resistance did not impose a significant fitness cost. Discussion: Mutations at position 550 also cause RFX resistance in C. difficile, without imposing a fitness burden. Future studies will need to evaluate additional RFX and RMP-selected resistance alleles for their fitness costs and impact on virulence.

SK 47. **Environmental factors affect the distribution of *Burkholderia psudomallei* in soils.**

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Burkholderia pseudomallei is the causative agent of melioidosis. This Gram-negative bacillus is a saprophytic organism found in soils of endemic areas such as Southeast Asia and northern Australia. Therefore, soil is believed to be an important reservoir for infection in humans and animals. We used a grid system to collect 25 soil samples with the goal of investigating the presence of *B. pseudomallei*. Results from direct culturing showed positive results for 24 soil samples but one. The number of colonies in each grid sample varied from $1x10^4$ to $6.6x10^6$ colonies/g of soil. In order to account for non-culturable *B. pseudomallei,* Real-time PCR with primers targeting the spacer region between ribosomal RNA genes was used. A total of six soil samples showed a higher number of DNA copies in comparison to that for *B. pseudomallei* isolated colonies. Soil physicochemical factors such as pH and moisture content were found to be correlated with the presence of *B. pseudomallei*. The pathogen showed an increase in cell numbers with pH values of 4.2-4.5 and moisture contents of 2-3 %. In addition, our results suggest that *B. pseudomallei* is unevenly distributed in soil, limiting the efficacy of possible control methods for this pathogen.

SK 48. **Investigation of Novel Virulence Factors in *B. anthracis***

Elizabeth Franks[1]*, Celia Ebrahimi[2], Raffi Aroian[2], Victor Nizet[2] and Shauna M. McGillivray[1,2], Texas Christian University, Fort Worth TX[1]; University of California, San Diego, La Jolla CA[2].

Bacillus anthracis is a rod-shaped, gram-positive, spore forming bacteria, which is the causative agent of the deadly disease Anthrax. *B. anthracis* possesses a chromosomal genome that contains ~5500 genes, as well as two large extra chromosomal plasmids. *B. anthracis* has two large bacterial plasmids that are known to carry key virulence genes, however we wanted to examine the role chromosomal genes may play in pathogenesis. Using a transposon mutagenesis system, we created random mutations in the chromosomal genes in the attenuated Sterne strain of *B. anthracis*. This transposon library was then screened using a *C. elegans*, model system of infection to identify mutants unable to infect *C. elegans*. One mutant was found to have a disruption in an operon containing multiple tellurium resistance genes. Additional genetic mutants were constructed to further investigate the role of this operon in evading host defense. Using in vitro assays, we have found that loss of tellurium resistance genes renders *B. anthracis* more susceptible to reactive oxygen species, such as H_2O_2, which are critical to host defense. Our results suggest a novel role of the tellurium resistance operon in *B. anthracis* virulence.

SK 49. **Regulation of Antibiotic Production in *Streptomyces acidiscabies***

Gus Wilson*, Caitlin Spaulding, and Dr. Frank G. Healy, Trinity University

The genus *Streptomyces* is responsible for over two-thirds of clinically relevant antibiotics. These antibiotics are usually assembled modularly by operons encoding enzymes necessary for the creation of the complex molecules. *Streptomyces acidiscabies* produces the secondary metabolite WS5995B, the production of which seems to be regulated by three genes SabA, SabR, and SabS. These enzymes encode the hierarchical regulatory system based on γ-butyrolactone (GBL) production. SabR and SabS have shown sequence homology to GBL-regulated DNA-binding proteins, while SabA shows sequence homology to enzymes necessary for GBL production. Because it appears that this regulatory operon serves to regulate other operons, particularly those involved in sporulation and antibiotic production. Using an optimized SELEX assay and gel shift, we have isolated sequences from a synthetic database and the genomic library of *S. acidiscabies* and are working to characterize the binding conditions of these transcription factors with said sequences.

SK 50. **Origin and Evolution of PE and PPE in *Mycobacterium***

Ashay Bavishi*, Madhusudan Choudhary, and Todd Primm, Sam Houston State University, Huntsville, Texas 77341

The PE and PPE families have been extensively studied in several *Mycobacterium* species. To better understand the origin and evolution of these protein families, several bioinformatic analyses were employed among both *Mycobacterium* species and other closely related species. Protein homology searches determined the highest matches among the chosen PE and PPE proteins. Pairwise identity tables constructed for the *Mycobacterium* organisms exhibited varying levels of protein identities among different groups of organisms. Similarly, structural constraints analyses conducted revealed that these genes experience differing levels of selection within these organisms. In conjunction, these findings shed light on the diverse functions and pathogenicity of the PE/PPE proteins among these organisms. In addition, it was discovered that in contrast to previous studies and findings, PE and PPE proteins are not limited to the genus *Mycobacterium*. More specifically, organisms within the genera *Amycolicicoccus*, *Rhodococcus Segniliparus*, and *Tsukamurella* were found to possess genes with significant homology to several PE/PPE genes in *Mycobacterium* species. Detailed phylogenetic analyses suggest a possible common ancestral origin of these genes or the presence of significant horizontal gene transfer among these organisms.

51. **New Bornaviruses Renew Interest in a Puzzling Neuropathic Virus**

S. Payne*, S. Hoppes, J. Heatly, D. Smith and I. Tizard, Department of Veterinary Pathobiology, Texas A&M University

Borna disease (BD) is a fatal meningoencephalitis of horses that was first described in and around Borna Germany in the1800s. The infectious nature of BD was demonstrated in the 1920s and small animal models of infection were established. The virus was finally determined to be a highly cell-associated negative-sense RNA virus in the 1990s. Behavioral anomalies in BDV-infected rodent models prompted searches for an association between human neuropsychiatric disease and BDV. Some evidence of an association was reported, but those studies were controversial, and remain so today. In 2008 a new bornavirus was identified as the causative agent of proventricular dilatation disease (PDD) of captive parrots, an unusual and devastating neurologic disease that emerged in the mid 1970s. Avian bornaviruses (ABVs) are found in association with captive parrots world-wide, and the viruses show genetic diversity that does not correlate with geographic location, avian species infected, or outcome of infection. Parrots experimentally infected with cultured ABV in development of PDD in about 100 days. We have recently found bornaviruses in Canada geese (CG), mute swans, and domestic duck embryos. These viruses are distinct from other ABVs in both genome arrangement and sequence and have some surprising similarities to BDV. The detection rate was as high as 40% in flocks of apparently healthy birds. There is also a correlation between 'encephalitis of unknown cause' and high levels of ABV antigen in archived goose and swan brain samples, although it cannot be determined if bornavirus infection caused disease The presence of bornaviruses in domestic duck embryos also raises questions about use of duck embryo fibroblasts in research and vaccine manufacture.

2:30 -3:30 Plenary Session: Palo Pinto and San Saba

Plenary

Population Genomics and Molecular Pathogenesis: The Flesh-Eating Model

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Many bacterial species that cause infections in humans, animals, and plants co-exist with the host in more benign relationships, including asymptomatic carriage. We have used the human pathogenic bacterium group A Streptococcus (the flesh-eating pathogen) as a model system to probe the population genomics and molecular pathogenesis of severe invasive infections, pharyngitis, and asymptomatic carriage. Whole-genome sequencing of large populations of strains cultured from patients with well-defined clinical syndromes, together with animal model experimental work, have provided substantial new information about the molecular basis of pathogen-host interactions. Recent discoveries arising from this work will be discussed.

3:45 – 5:35
Invited General Microbiology Session (concurrent)
Red River, 2nd floor Hereford Center
Moderated by: Laura Mydlarz, University of Texas at Arlington

3:45 **The Co-existence of Insects and Bacteria**
Blake Bextine, Department of Biology, University of Texas at Tyler, Tyler, TX 75799

4:05 **Host defense and pathogen counter-defense: a cross-kingdom molecular battle on the leaf surface**
Maeli Melotto, Department of Biology, University of Texas at Arlington, Arlington, TX 76019

4:25 **Nitrogen Cycling on the West Florida Shelf as Determined by Stable Isotope Probing and Functional Microarray Analysis**
Boris Wawrick, Department of Botany and Microbiology, University of Oklahoma, Norman, OK 73019

4:45 **Eubacterial and archaeal diversity in nuisance cyanobacterial mats: can Archaea stimulate nitrogen fixation?**
Dmitri Sobolev, Biology Department, University of Houston-Victoria, Victoria, Texas

5:05 **Harnessing Microbial Ecology for Sustainable Bioproducts**
Michael Allen, Department of Biology, University of North Texas, Denton, TX 76203

3:45 – 5:35
Invited Medical Microbiology Session (concurrent)
Concho, 2nd floor Hereford Center
Moderated by: Julian Hurdle, University of Texas at Arlington

3:45 **The Changing Face of *Clostridium difficile* Infection**
Zhi-Dong Jiang, School of Public Health, The University of Texas Health Science Center at Houston, Houston, Texas

4:05 ***Helicobacter pylori* versus the host: remodeling of the outer membrane is required for colonization of the gastric mucosa**
Stephen Trent, Molecular Genetics and Microbiology, The University of Texas at Austin, Austin, Texas

4:25 **SOS-inducible biofilm formation and vesiculation of *Pseudomonas aeruginosa***
Tao Wei, Department of Biology, University of Texas at San Antonio, San Antonio, Texas

4:45 **The Role of Neutrophils During Bacterial Infection**
Rance Berg, Department of Molecular Biology and Immunology, University of North Texas Health Sciences Center, Fort Worth, Texas

5:05 **Bacterial manipulation of the immune system: lessons learned from Staphylococci**
Gabriella Bowden, University of Houston, Downtown, Department of Natural Sciences, Houston, Texas

6:00 -7:15 Awards and Dinner

Rio Grande Ballroom, Hereford Center

7:20 ASM Lecturer and Keynote Speaker

The Planetarium

ASM Lecturer

"Now, about that cantaloupe...." Microbiological food safety issues in today's marketplace.

Kathryn J. Boor
The Ronald P. Lynch Dean
College of Agriculture and Life Sciences
Fellow of the American Academy of Microbiology
Fellow of the International Academy of Food Science and Technology
Fellow of the Institute of Food Technologists.
Cornell University

Microbiological food safety issues are of concern throughout the world, ranging from foodborne disease outbreaks and costly food product recalls in the developed world to food- and waterborne diarrheal illnesses that cause numerous deaths in developing countries. A 2011 CDC study estimates that, on an annual basis, domestically acquired foodborne illnesses in the US cause 48 million cases of gastrointestinal illnesses, 128,000 serious illnesses resulting in hospitalizations, and 3,000 deaths. The three pathogens most frequently associated with deaths due to foodborne illnesses are *Salmonella*, *Toxoplasma*, and *Listeria monocytogenes*. Modern microbiological approaches, specifically, molecular biology based subtyping ("DNA fingerprinting") methods have greatly enhanced our collective ability to detect foodborne disease outbreaks and identify microbial contamination sources throughout the food chain. The recent application of improved detection methods for foodborne disease outbreaks, and especially, detection of outbreaks that previously may have gone unnoticed, may lead to the perception that food safety problems are also increasing. However, application of effective DNA fingerprinting methods has enhanced the safety of our food supply. Early detection of outbreaks can reduce the number of disease cases in a given outbreak. Molecular biology-based source tracking efforts have reduced pathogen contamination of foods from farm to table. Thus, on-going efforts to improve the safety of the food supply provide outstanding examples of social benefits from basic and applied research endeavors.

Saturday, 12 November 2011

7:00 – 8:00 Breakfast and Coffee

2nd floor Hereford Center
Carlisle Suite

Mini-CUE Session
Academy, 2nd floor Hereford Center
Moderated by: Todd Primm

8:00 **Undergraduate Microbiology Curriculum Guidelines from ASM**
Jackie Reynolds, Richland College, Houston, TX

8:45 **Integrating Research Methodology in the Large Lab Setting**
Madhusudan Choudhary, Sam Houston State University, Huntsville, TX

9:30 **Educational Usage of Games in Science**
Kristi Bowling, Rice University, Houston, TX

10:15 **Break**

10:30 **Panel Discussion**
Robert Bauman, Amarillo College, Amarillo, TX

Abstracts of Contributed Oral Presentations

A Tale of Two Chromosomes in Prokaryotes

Cheramie Trahan[1*], Hyuk Cho[2], Madhusudan Choudhary[1]
[1]Department of Biological Sciences, [2]Department of Computer Science, Sam Houston State University, Huntsville, Texas

Since the explosion of genome sequencing, the identification of bacterial species possessing multiple chromosomes has rapidly increased. Most bacteria with a complex genome structure possess two chromosomes, where the primary chromosome is denoted CI and the accessory chromosome is denoted CII. The majority of the bacteria with two chromosomes are members of the *Proteobacteria* group.
The existence of multiple chromosomes in these bacterial species is thought to allow for increased genome plasticity as CI encodes for essential proteins necessary for cell survival and proliferation while CII expands the metabolic flexibility. Whole-scale genome alignments revealed a rapid divergence of the accessory chromosome. There could be several mechanisms that could lead the rapid evolution of CII. These mechanisms include positive selection pressure, differential mutation rates, or/and genes acquired by horizontal gene transfer (HGT) from other species.
Results revealed that CI and CII experience similar selection pressure, as well as similar amounts of HGTs regions, and therefore these two factors together could not account for the rapid evolution of CII. Global genome mutagenesis and microarray analysis further reveal that CI and CII are essential and ancient partners of *R. sphaeroides* genome. The flexibility and divergence of CII may allow for increased adaptability to specialized environmental and nutritional niches that possession of a single chromosome may not fully permit.

Characterization of the Transcriptional Regulator NrdR in *Escherichia coli*

Brandon McKethan* and Stephen Spiro
University of Texas at Dallas

Ribonucleotide reductases (RNRs) are critical enzymes that are required for the *de novo* synthesis of deoxyribonucleoside triphosphates (dNTPs) from ribonucleotides. Transcriptional regulation of RNR levels is believed to be coordinated with the cell cycle, and involves a number of regulatory proteins. *Escherichia coli* expresses a transcriptional repressor, NrdR, that has been shown to regulate all three *nrd* operons that encode three RNR isoenzymes. The repressor activity of NrdR is believed to be stimulated by elevated dNTP concentrations, though there is no direct evidence for this model. In this study, we sought to elucidate the underlying biochemical mechanism by which NrdR regulates *nrd* expression according to the abundance of (d)NTPs. We determined that ATP and dATP bind to NrdR in a negatively cooperative fashion, such that neither nucleotide can fully occupy the protein. Binding activity was shown to be dependent upon the oligomeric state of the protein, which in turn is influenced by the binding of (deoxy)nucleotides. We further found that as-prepared NrdR contains a mixture of (deoxy)nucleoside monophosphates and triphosphates, and that the DNA binding activity of NrdR is inhibited by ATP/dATP and stimulated by AMP/dAMP. We propose a model in which NrdR selectively binds nucleoside triphosphates, which are hydrolyzed to their monophosphate counterparts in order to regulate DNA binding. The key features of this model are currently being tested using *in vitro* transcription assays with NrdR proteins in various states of (deoxy)nucleotide occupancy.

Characterization of a novel surface modification in *Campylobacter jejuni,* linking the assembly of flagella and lipooligosaccharide

Thomas W. Cullen[a*], James A. Madsen[b], Petko L. Ivanov[a], Jennifer S. Brodbelt[b], M. Stephen Trent[a,c]
[a]Section of Molecular Genetics and Microbiology, [b]Department of Chemistry and Biochemistry, and [c]The Institute of Cellular and Molecular Biology, The University of Texas at Austin, Texas 78712

Gram-negative bacteria assemble complex surface structures that interface with the surrounding environment and are involved in pathogenesis. Two major surface features, the outer membrane lipooligosaccharide and flagella, are highly variable and are often targets for modification. In this work, we identify a gene encoding a phosphoethanolamine (pEtN) transferase (EptC) in *Campylobacter jejuni* that serves a dual role in modifying not only the lipooligosaccharide lipid anchor lipid A with pEtN, but also the flagellar rod protein FlgG. Generation of a mutant in *C. jejuni* 81-176 by interruption of *eptC* resulted in the absence of pEtN modificaitons on lipid A as well as FlgG. The *eptC* mutant showed a 20-fold increase in sensitivity to cationic antimicrobial peptide, polymyxin B, as well as a decrease in flagella production and motility. Furthermore, we characterize the unique post-translational pEtN modification of FlgG using collision induced and electron transfer dissociation mass spectrometry to map the site of modification. Specifically, we show that FlgG is modified with pEtN at a single site (threonine) by EptC and demonstrate enzyme specificity by showing that EptC is unable to modify other amino aicds (e.g. serine, tyrosine). Most intriguing, this research identifies a pEtN transferase showing preference for two periplasmic substrates linking membrane biogenesis and flagellar assembly. Characterization of EptC and its enzymatic targets expands on the increasingly important field of prokaryotic modification of surface structures and the unidentified role they may play in the pathogenesis.

Molecular genetics studies of a- mating type loci of *Sporisorium reilianum*, the head smut fungus in maize and corn.
Ghada L Radwan and Clint W Magill, Department of Plant Pathology & Microbiology, Texas A&M University, College Station, TX

Sporisorium reilianum (Kühn) Langdon & Fullerton, *a dimorphic, basidiomycetous fungus,* causes head smut on maize and sorghum. Phylogenetic analysis of ITS and ribosomal large sub- unit DNA shows that *S. reilianum* is closely related to *U. maydis,* the common smut pathogen in maize but *S. reilianum* differs from *U. maydis* in disease etiology. Although *S. reilianum* has long been known as a pathogen on maize and sorghum, molecular characterization is lacking. Mating type genes play an important role in formation of the infectious filamentous form and subsequent pathogenicity. We are characterizing mating type loci in isolates that differ in pathotype through sequence analysis and expression levels as part of an effort verify the ploidy level of the cultures and to determine if crosses can be made between pathotypes. Sequences of the 3 alleles of the 'a' mating-type locus (*a1*, *a2*, and *a3)* of *S. reilianum* are being compared to gene bank public sequence databases. The entire sequence and alignments for the three alleles is being done using a transposon sequencing system. Among 40 strains, including 7 different pathotypes, only four isolates (two sets) showed a positive mating test result. Pheromone genes are designated *mfa1.2* and *mfa1.3* for the a1 allele, *mfa2.1* and *mfa2.3* for the *a*2 allele and *mfa3.1* and *mfa3.2* for the a3 allele. The pheromone receptor genes are designated *pra*1, *pra*2 and *pra*3 for the respective alleles. The expression level of mating type genes in compatible strains and mating types locus belong to each strain have been confirmed using QRTPCR.

Isolation and Identification of Chromosomal I-origin of replication in *Rhodobacter sphaeroides*
Bat-Erdene Myagmarjav*, Jonathan Stone, and Madhusudan Choudhary
Department of Biological Sciences, SHSU, Huntsville, TX 77341

A number of bacterial species possess multiple chromosomes, which presents a question of how multiple chromosomes regulate and coordinate chromosomal replication and segregation of during the cell cycle. *Rhodobacter sphaeroides* is a model system as its genome consists of two chromosomes (CI and CII), and the genome is completely sequenced and fully annotated.
Preceding studies have found three sites on chromosome I as putative replication origins, OriI-1, OriI-2 and OriI-3. In present study, all three of these sequences have been biologically tested, to confirm if one of these putative origins is the true chromosomal origin. The putative sequences have been amplified from the *R. sphaeroides* genomic DNA and cloned into a suicide vector, pLO1. The cloned sequences were confirmed by DNA sequencing. The recombinant plasmids were transferred from *E. coli* to *R. sphaeroides* cells by conjugation, and the resulting exconjugants were selected on Sistrom plate with Kanamycin. The recombinant plasmids were purified and analyzed via PCR.
OriI-1 was confirmed to be the true origin of replication as it autonomously replicate in *R. sphaeroides* and gets transfered with a relatively high frequency. The genes flanking the Ori-I regions from other α-proteobacteria were conserved suggesting further that this is the true origin of replication of CI.

EVALUATION OF SOLID MEDIUM BASED METHODS FOR ISOLATION OF NITROGEN FIXING MICROORGANISMS
*Babur Mirza and Jorge Rodrigues, University of Texas at Arlington

The isolation of free living diazotrophic microorganisms relies on nitrogen free semisolid medium based methods. We tested three different isolation strategies comprised of the solid under low O_2 concentration, semisolid and combination of both conditions for the isolation of free living diazotrophic microorganisms from soil. A total of 790 isolates, minimum of 250 per treatment were differentiated and grouped based on the Rep-PCR. Strains representing unique Rep-profiles (206 isolates) were identified by 16S rRNA gene sequencing. These strains belonged to four bacterial phylum including *Proteobacteria*, *Firmicutes*, *Actinobacteria*, and *Bacteriodetes*. The most abundant bacterial genera detected with solid based methods were *Pseudomonas*, *Burkholderia*, *Rhizobium*, *Sinorhizobium,* and *Bacillus*, whereas *Pseudomonas* corresponded to 78% of total isolates in the semisolid method. This newly devised solid method increased the number of putative nitrogen fixers by 85% as compared to the conventional semisolid based methods. All isolates were able to grow on the nitrogen free medium and 79% of them did show the presence of *nif*H gene. We performed acetylene reduction assays (ARA) and approximately 43% of the isolates were tested positive under 2% oxygen concentrations. Our results indicate that a combination of nitrogen free solid medium and low O_2 concentration increases the diversity of free living diazotrophic microorganisms isolated from soil.

Degradation of *Escherichia coli* Produced Indole by *Pseudomonas aeruginosa* in Mixed Cultures
Ernesto Valenzuela Jr[1]*, T.R. Zere[2], J. Gollihar[1], M. Whiteley[3], T.K. Wood[4], and R.J.C. McLean[1].
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Within a controlled environment single bacterium can be isolated, inoculated and cultured as pure planktonic-cultures. However in nature bacteria are commonly found in mixed species, surface-attached biofilm communities. Two microorganisms found in intestinal and aquatic habitats are *Pseudomonas aeruginosa* and *Escherichia coli.* We recently found that *E. coli* indole production enables this organism to compete against *P. aeruginosa* in mixed culture by inhibiting N-acylated homoserine lactone (AHL)-regulated competition factors such as pyocyanin. *P. aeruginosa* AHL mutants were also unable to inactivate indole. In the present study, we are investigating the effect of other quorum regulated genes on *P. aeruginosa* competition and indole degradation including two genes (*pqsA* and *pqsH*) associated with the *Pseudomonas* quinolone signal, two strains with defects in alginate regulation (*algR*), and comparing them to the AHL mutants (*lasI rhlI*) and (*rhlI*) studied earlier. While AHL-mutants have been associated with indole degradation and competition by *P. aeruginosa*, we have observed that other regulatory networks may also be involved.

Diatom species traits vary in response to inorganic versus organic acidity in Adirondack streams

Katrina L. Pound* and Sophia I. Passy, Department of Biology, University of Texas Arlington

Streams in the Adirondack region of New York are acidified by both inorganic acid deposition and organic acidity in the form of dissolved organic carbon (DOC), but the impacts of these two types of acidity on biological communities are not well understood. Acid deposition mobilizes aluminum in streams to toxic levels, but DOC mitigates this process by forming biologically unavailable complexes with aluminum. The ratio of inorganic to organic aluminum (Alim:Alom) is correlated with the proportion of inorganic to organic acidity in streams, and high ratios of Alim:Alom are considered stressful to biota. Stressful environments are predicted to select for smaller and short-statured species which tend to exhibit higher stress tolerance. We tested the predictions that higher inorganic acidity would associate with a shift toward smaller and short-statured species, higher proportion of acidobiontic species with optimal occurrence at pH <5.5, and lower species richness. Diatom cell size, ecological guilds, proportion of acidobiontic species, and species richness were examined in acidified streams with a broad gradient of Alim:Alom, ranging from 0.09 to 5.7. Ecological guild classifications, based on position in the periphyton mat, included low profile, high profile, and motile species. Our results showed that average cell size was significantly smaller in streams with preponderance of inorganic acidity (i.e., Alim:Alom >1), which had greater abundance of small, low profile and acidobiontic species. Conversely, streams with higher organic acidity (i.e., Alim:Alom<1) had significantly higher species richness and greater abundance of high profile and motile species, which may be indicative of lower stress or higher nutrient input associated with the transport of DOC into streams. These results support predictions that diatom traits respond to stressful environments and provide evidence that different types of acidity (inorganic versus organic) may have different impacts on community composition. Future research should focus on developing indices to distinguish inorganic from organic acidity, which would be important to acid-sensitive regions, such as the Adirondacks.

Single cell predator- prey interactions: Simple or complex?

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Nanoflagellates provide an important link in aquatic food webs by consuming bacterial biomass and subsequently providing nutrients to higher trophic levels by becoming prey themselves. Although the protist-bacterial interaction is recognized as important to aquatic ecology, little is known about the mechanism of prey selection. In a series of single prey feeding experiments, a phylogenetically diverse group of fifteen bacterial species were offered to *Ochromonas danica*, a common aquatic nanoflagellate. Bacteria were grown to mid-exponential phase and late-stationary phase in R2A broth batch cultures under similar environmental conditions. *O. danica* was grown on *Ochromonas* Medium in a chemostat and was harvested immediately prior to each feeding experiment to ensure physiologically similar cells. Bacterial mortality rates and *O. danica* growth rates were tracked over time using flow cytometry and ingestion rates for each type of prey were determined. Results suggest that *Ochromonas danica* is a relatively indiscriminant consumer of bacterial prey, but the efficiency of ingestion differs among prey. Four different patterns describing bacterial mortality were identified. These patterns suggest that *O. danica* adapts its feeding behavior (efficiency) based on type of prey.

Indole inhibition of AHL-mediated quorum signaling in Chromobacterium violaceum and Janthinobacterium lividum.

Benjamin Hidalgo-Romano* and Robert JC McLean Dept. Biology, Texas State University, San Marcos TX *-presenting author

In the last several years research groups have demonstrated that gram-negative bacteria use an array of quorum-sensing systems, such as: the luxS/AI-2, indole, and the luxI/luxR/N-acylated-homoserine lactone (AHL) systems. These signaling systems are involved in intra- and interspecies communication, based on population density. Quorum sensing depends on the synthesis of small molecule that diffuse in and out of bacterial cells. We recently found that indole production by E. coli helps this organism compete with Pseudomonas aeruginosa in co-culture, by interfering with the P. aeruginosa AHL-regulated pyocyanin. Although indole-inhibition of P. aeruginosa AHL had been reported previously, its effect on other AHL-regulated systems has not been shown. Here, we tested the effects of indole on AHL-regulated violacein production in

two gram-negative soil isolates, Chromobacterium violeaceum and Janthinobacterium lividum. C. violaceum and J. lividum grown at different concentrations of indole (0.5 µm, 1.0 µm, 1.5 µm and 2.0µm) and E .coli supernatants show a significant reduction of biofilm formation, production of exopolysaccharide, and different levels of inhibition of violacein production. Indole-mediated inhibition of AHL quorum signaling appears to be a general phenomenon and not exclusive to P. aeruginosa.

Ruminal *Clostridium* species potentially implicated as primary contributors to ruminal lipolysis Holly Edwards*[1], Robin Anderson[2], Stephen Smith[1], Rhonda Miller[1], T. Matthew Taylor[1], Nathan Krueger[2], David Nisbet[2]; Texas A&M University, College Station, TX, USA[1], United States Department of Agriculture/Agricultural Research Service, Southern Plains Agricultural Research Center, Food & Feed Safety Research Unit, College Station, TX, USA[2]

Lipolytic activity by mixed populations of ruminal microbes is markedly inhibited by supplemental glycerol. Recently, several lipolytic *Clostridium* species have been found at high numbers in the rumen but sensitivity of their lipase to glycerol has yet to be determined. To further characterize ruminal lipase-producing bacteria populations, ruminal microbes obtained from a cannulated cow were cultured with 5% olive oil, in the presence of glass beads (used as a support matrix) and 6 ml rumen fluid based media. Four sets of tubes were inoculated (in triplicate) and subjected to one of the following treatments non-heat and heat treatments (the latter to enrich for heat resistant *Clostridium*) and non-glycerol and 6% (vol/vol) glycerol supplementation. The heat treatment consisted of 10 min incubation at 80°C and all tubes were then incubated for 48 h at 39°C under CO_2. Free fatty acids were measured colorimetrically. Results revealed that lipolysis activity was less ($P < 0.05$) for the heat-treated than the non heat-treated populations (14.9 ± 15.83 vs. 56.2 ± 2.44 nmol ml^{-1} h^{-1}, respectively). The heat-treated populations were insensitive to glycerol whereas lipolysis by the non heat-treated populations was decreased ($P > 0.05$) during culture by the presence of glycerol (10.0 ± 9.14 and 0.2 ± 0.35 nmol ml^{-1} h^{-1}, respectively). In conclusion ruminal *Clostridium* species may contribute appreciably to ruminal lipolysis but other prominent glycerol-susceptible lipolytic bacteria have yet to be identified. Insight into ruminal bacterial populations and their contributions to lipolysis is crucial for the development of strategies to protect heart healthy unsaturated fatty acids from ruminal biohydrogenation.

High Relative Humidity Compromises Stomatal Innate Immunity against *Pseudomonas syringae* and *Salmonella enterica* but not *Escherichia coli*

Debanjana Roy*, Shweta Panchal, Maeli Melotto, Department of Biology, University of Texas, Arlington, Texas, 76019, \

Stomata, the natural openings present in the aerial parts of the plants, not only allow connection between the internal and the external environment but also provide a port of entry for bacteria during plant microbe interaction. Recent studies have shown that stomatal closure is a functional output of the plant's innate immunity that minimizes bacterial invasion. It is not known, however, how guard cells prioritize their response to multiple stimuli. In this study, we focused on the effect of relative humidity on stomatal immunity of both *Arabidopsis thaliana* and lettuce (*Lactuca sativa*) against the plant pathogenic bacterium *Pseudomonas syringae* pv. *tomato* (*Pst*) DC3118 and the human pathogens *Escherichia coli* O157:H7 and *Salmonella enterica* serovar Typhimurium (STM) strain SL1334. Our study shows reduced stomatal closure under >95% relative humidity (RH) in the presence of *Pst* DC3118 and STM SL1334 allowing for increased bacterial penetration and survival inside Arabidopsis as well as lettuce. However, O157:H7-triggered stomatal closure, and bacterial penetration and survival inside the plant does not change significantly under >95% RH. Furthermore, these three bacteria induce differential transcriptional responses in plant cells. These results suggest that some human pathogens may be more adapted to the plant environment than others. The understanding of the initial contamination process is paramount in preventing losses in crop yield and food borne diseases with major implications to both crop diseases and food safety.

Light-independent coronatine biosynthesis activation on the leaf surface

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Coronatine is a non-host specific phytotoxin produced by several pathovars of the plant pathogenic bacterium *Pseudomonas syringae*. This toxin contributes to disease developments in plants at both early and late stages of infection. A prominent role of coronatine is to facilitate penetration of bacteria into leaves by overcoming stomatal immunity. Specifically, coronatine re-opens pathogen-triggered stomatal closure. It is well known that stomatal movement follows a daily cycle, in which stomata open during the day maximizing the CO_2 diffusion into the apoplast for photosynthesis and close during the night minimizing the water loss from in most plants. An intriguing question is whether coronatine facilitates bacterial penetration as a virulence strategy even during the night. In fact, we found that coronatine, both in pure form and biologically produced by *P. syringae* pv. *tomato* (*Pst*) strain DC3000, is effective in opening dark-closed stomata of Arabidopsis leaves. Using a combination of approaches, including microscopy, reporter gene construct, bacterial mutants, and HPLC analysis, we have also observed that coronatine biosynthetic genes are activated on the leaf surface indicating possible recognition of the leaf environment by *Pst* DC3000 in preparation for the virulent, endophytic phase of its life cycle. These findings suggest epidemiological advantages for coronatine-producing bacteria and also elucidate further functional attributes of coronatine.

The effect of temperature on pathogen virulence of the Caribbean sea fan, *Gorgonia ventalina*

*Whitney Mann[1], Joshuah Beach-Letendre[1], Laura Mydlarz[1], The University of Texas at Arlington[1]

Caribbean sea fans (*Gorgonia ventalina)* are important members of coral reef communities providing, food and shelter for many reef inhabitants and serving as source of carbon sequestration. *Aspergillus sydowii* is a common fungal pathogen of *G. ventalina* and is causing perturbing rates of mortality for sea fan populations. The virulence of a pathogen depends on its ability to colonize and infect a host. Several factors of virulence can be characterized such as growth rate and enzymatic activity. We hypothesize that rising sea surface temperatures associated with global climate change, are a main driving force for promoting *A. sydowii* virulence and affecting disease prevalence of *G. ventalina* across the Caribbean. As factors of virulence, protease, antioxidant, and growth rate activities were measured in several strains of *A. sydowii* grown at ambient and elevated temperatures. Significant temperature effect was observed in protease and catalase activity of all strains but no effect was seen in growth rates. These data suggest an up regulation or activation of enzymatic activity driven by temperature enabling for better colonization or defense against the host. As oceanic temperatures are expected to rise, the monitoring of pathogen virulence of reef organisms is ever critical for mitigation and monitoring of disease on coral reefs.

REGULATION OF *CLOSTRIDIUM DIFFICILE* TOXIN PRODUCTION BY A QUORUM SIGNALING SYSTEM

***Charles Darkoh[1,3], Heidi B. Kaplan[1-2], Herbert L. DuPont[1-4]**
[1]The University of Texas Graduate School of Biomedical Sciences, [2]The University of Texas Medical School, [3]The University of Texas School of Public Health-Center For Infectious Diseases, [4]Baylor College of Medicine.

C. difficile is the leading definable cause of nosocomial diarrhea worldwide due to its virulence, multi-drug resistance, spore-forming ability, and environmental persistence. Virulent strains of *C. difficile* possess a 19.6-kb pathogenicity locus that contains the *tcdA* and *tcdB* genes, which encode toxins A and B, respectively. These toxins are critical virulence factors and have the same enzymatic cleavage activity, which is due to N-terminal glucosyltransferase domains that monoglucosylate low molecular weight GTPases of the Rho family in the host cytosol using cellular uridine diphosphoglucose as the glucose donor. Currently little is known about the regulation of *C. difficile* toxin production, except for two points. First, there is CodY-dependent regulation of *tcdA* and *tcdB* suggesting a possible involvement of nutrient limitation in toxin production. Second, *C. difficile* encodes a $luxS_{cdiff}$ gene and mid-log phase supernatant from cultures of *C. difficile* contain autoinducer-2 (AI-2_{cdif}) that is able to induce bioluminescence in a *Vibrio harveyi luxS*-reporter strain. Importantly, it is not known whether a $luxS_{cdiff}$-dependent quorum signaling system controls toxin expression, because there are no *C. difficile luxS* mutants and because the addition of AI-2 to *C. difficile* cells did not affect toxin production. However, we propose that *C. difficile* toxin production is regulated by a quorum sensing mechanism. Two lines of physiological evidence support this hypothesis. First, small heat-sensitive soluble molecules (< 4.5 KDa) with toxin-inducing activity are released by and accumulate in the medium of high-density *C. difficile* cells. This conditioned medium when incubated with low-density *C. difficile* cells causes them to produce early and elevated levels of toxin. Second, *C. difficile* cells grown in dialysis bags in unchanged medium produced early and elevated levels of toxin. However, cells grown in dialysis bags in medium that is changed every 4 hrs delay toxin production. Current experiments are aimed at identifying the signal and the signal transducers involved in this intra-species cell-cell communication network. It is anticipated that understanding the quorum-dependent regulation of *C. difficile* toxin production will lead to promising therapeutic approaches for the prevention and/or treatment of these infections.

***Pseudomonas aeruginosa* biofilm-associated infections contribute to chronicity and increased antimicrobial tolerance in the diabetic wound environment**

Chase Watters[1]*, Urvish Trivedi[1], Katrina DeLeon[1], Trevor Dalton[1], Mark Lyte[2] and Kendra Rumbaugh[1],
Department of Surgery, Texas Tech University Health Sciences Center, Lubbock, TX 79430[1] Department of Pharmacy Practice, Texas Tech University Health Sciences Center, Lubbock, TX, 79430[2]

Diabetes affects 23.6 million people in the U.S., or 7.8 of the population, and these numbers are even higher in developing countries. Diabetic patients are more susceptible to the development of chronic-wounds with debilitating bacterial infections than non-diabetics. Our project was to test the **hypothesis that bacterial biofilms, or sessile communities of bacteria that reside in a complex matrix of exopolymeric material, contribute to the severity and chronicity of diabetic wounds.** To test this hypothesis we developed an *in vitro* media emulating the diabetic environment, and an *in vivo* diabetic chronic-wound model to investigate *P. aeruginosa* biofilm-associated infections. We found that insulin increased *P. aeruginosa* biofilm formation in the presence of glucose *in vitro* and, that diabetic mice given *P. aeruginosa*-infected chronic wounds displayed impaired bacterial clearing and wound closure than did their non-diabetic littermates. We also observed that more biofilm was present in the wounds of diabetic mice and this correlated to an increased tolerance to antimicrobial treatments. In addition, treating diabetic mice with insulin did not restore their ability to recover from *P. aeruginosa*-infected chronic wounds. Finally, we observed a considerable increase in antibiotic tolerance in the insulin-treated versus untreated diabetic mice. These data suggest that the diabetic wound environment promotes the formation and persistence of antibiotic tolerant *P. aeruginosa* biofilms, which results in more chronically-infected wounds.

The ability of saponins from a variety of natural sources to block virus replication

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Natural compounds from plants have been the source of leads for the development of various drugs. Saponins are a diverse group of glycosides that are widely distributed within plant kingdom and have an equally broad range of biological properties. Saponins exhibit cell membrane–permeabilizing properties and have been investigated for their therapeutic potential. *Tribulus terrestris* and *Yucca schidigera* plant extracts containing saponins were tested for in vitro antiviral activity against two viruses, rhesus rotavirus (RRV) and reovirus serotype 3 (ST3) strain Dearing (MRV-3DE). Antiviral activity was evaluated and involved, pre-treatment with saponins prior to infection, cotreatment and post treatment regimes. Cytotoxicity of the saponin-containing plant extracts was determined using cell lines that support the growth of rhesus rotavirus, rhesus monkey kidney MA-104 cells and reovirus, mouse L929 fibroblasts. The $CCIC_{50}$ for the Yucca extract ranged from 0.12-0.38 mg/ml and for the Tribulus extract from 0.38 to 2.96 mg/ml. Pre-treatment or post treatment of cells alone was not sufficient to block virus infection and subsequent replication. Cotreatment with either saponin containing extracts was able to reduce rhesus rotavirus and reovirus infectivity. *Tribulus terrestris* demonstrated a more robust activity, about 93 % inhibition against reovirus and 90% inhibition against rhesus rotavirus. These results indicate that *Tribulus terrestris* has inhibitory antiviral activity against rhesus rotavirus and could be exploited for its therapeutic effect.

Finding appropriate transfection normalization technique in the context of MCMV infection

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Worldwide 60 to 100 % of adults are infected with human cytomegalovirus (HCMV), but it causes no problems in people with a healthy immune system. However HCMV causes serious illness or death in immune-compromised people, such as organ transplant recipients or AIDS patients and it is a major cause of birth defects. HCMV can only infect humans, limiting the studies done with this virus to tissue culture of limited cell types. The closely related mouse cytomegalovirus (MCMV) has similarity in both sequence and diseases caused, thus can act as a model to study function and regulation of conserved genes. One such conserved gene in MCMV is m142. The m142 gene product is essential for viral growth by inhibiting a cellular antiviral defense pathway. Our long term goal is to identify regulatory mechanisms for m142 promoter. The traditional method for normalization of transfection efficiency is to co-transfect cells with a second reporter gene, controlled by a different promoter. In our experiment, we will infect cells with MCMV after transfection to study the effect of viral transcription factors on m142 promoter regulation. A frequently used promoter comes from human cytomegalovirus. Expression from this promoter is up-regulated by MCMV infection. Testing with SV40 promoter also showed up-regulation by MCMV. Transfection efficiency was detected by alternate method of q-PCR for plasmid DNA. This method of normalization could be used for normalization whenever treatment may affect expression level of a control.

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