AMERICAN SOCIETY FOR MICROBIOLOGY
Texas Branch

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October 29-31, 2015

Hosted by

Sam Houston State University

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AMERICAN SOCIETY FOR MICROBIOLOGY
2015-2016

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The presentation by the Keynote speaker (Dr. Brianna Burton, Harvard University) is sponsored by the American Society for Microbiology.

STAF Visit:
We would like to thank Aaron Lynne, Sibyl Bucheli, and Joan Bytheway for organizing a tour of the Southeast Texas Applied Forensic Science (STAFS) Facility at the Biological Sciences Field Station at Sam Houston State University.

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We thank the exhibitors for their generous support of our society and conference.

Texas Branch ASM
Fall 2015 Meeting Program

Quick View

Thursday Evening

03:00 – 05:00 pm	STAFS Facility Site Visit	
04:00 – 07:00 pm	Registration and Information	LSC Theater (Lobby)
05:30 – 06:45 pm	Reception	LSC 110
07:00 – 09:15 pm	Welcome and Opening Session	LSC Theater

Friday

07:00 – 08:00 am	Breakfast	LSC Ball Room A
07:30 – 12:00 pm	Poster Set Up	LSC Ball Room A
07:30 – 12:30 pm	Registration and Information	LSC 3rd Floor
08:00 – 09:45 am	Medical Microbiology (Student Session)	LSC 304
08:00 – 09:45 am	General Microbiology (Student Session)	LSC 320
09:45 – 10:15 am	Coffee Break	LSC 302
10:15 – 12:15 pm	Microbial Evolution and Bioinformatics	LSC 320
10:15 – 11:45 am	Eukaryotic Microbes, Protein Dynamics in Yeast	LSC 304
12:15 – 01:15 pm	Lunch	LSC Ball Room B
01:00 – 03:00 pm	Poster Session	LSC Ball Room A
03:15 – 05:15 pm	Clinical Microbiology	LSC 304
03:15 – 05:15 pm	Environmental Microbiology & Microbial Ecology	LSC 320
05:15 – 05:30 pm	Coffee Break	LSC Ball Room A
05:15 – 06:30 pm	Exhibitors	LSC Ball Room A
07:00 – 10:00 pm	Banquet, Awards, and Keynote Lecture	LSC Ball Room B

Saturday

08:00 – 09:00 am	Breakfast & Registration	LDB Atrium
09:00 – 10:30 am	MINI CUE Education Session	LDB 214
10:30 – 10:45 am	Coffee Break	LDB Atrium
10:45 – 11:45 am	MINI CUE: Microbrew Session A	LDB 220
10:45 – 11:45 am	MINI CUE: Microbrew Session B	LDB 207

Texas Branch ASM
Fall 2015 Meeting Program
Detail View

THURSDAY, October 29, 2015

03:00 – 05:00 pm	**STAFS Facility Site Visit**	

04:00 – 07:00 pm	**REGISTRATION and INFORMATION**	LSC Theater (Lobby)

05:30 – 06:45 pm	**RECEPTION**	LSC 110

07:00 – 07:15 pm	**WELCOME** Todd Primm, Organizing Committee John Pascarella, Dean, College of Sciences, Sam Houston State University Chad Hargrave, Chair, Department of Biological Science, Sam Houston State University Ali Azghani, President, Texas Branch ASM, UT at Tyler	LSC Theater

07:15 – 09:15 pm	**OPENING SESSION** **Microbiome and Metagenomics** Chair: Melinda Engevik, Department of Pathology & Immunology, Baylor College of Medicine Co-Chair: Todd Primm, Biological Sciences, Sam Houston State University, Visiting Professor, Baylor College of Medicine	LSC Theater
07:15 pm	**Diet and genotype interact to shape intestinal microbiome in a mouse model of phenylketonuria** Mike Allen, Center for Biosafety and Biosecurity, Dept. of Molecular and Medical Genetics, UNT Health Science Center	
07:45 pm	**Microbiome Studies: applications and considerations for simple study design through meta-analyses** Julie Cope, Texas Children's Microbiome Center, Department of Pathology, Texas Children's Hospital and Department of Pathology & Immunology, Baylor College of Medicine	
08:15 pm	**Metabolomic consequences of microbiota dysbiosis in dogs with chronic intestinal inflammation** Jan Suchodolski, Department of Small Animal Clinical Sciences, College of Veterinary Medicine and Biomedical Sciences, Texas A&M University	
08:45 pm	**TBA** Joe Petrosino, The Alkek Center for Metagenomics and Microbiome Research, Department of Molecular Virology and Microbiology, Baylor College of Medicine	

FRIDAY, October 30, 2015

07:00 – 08:00 am	**BREAKFAST**	LSC Ball Room A
07:30 – 12:00 pm	**POSTER SETUP**	LSC Ball Room A

07:00 – 12:30 pm	**REGISTRATION**	LSC, 3nd Floor lobby

08:00 – 09:45 am	**CONCURRENT STUDENT SESSION** **Medical Microbiology Student Session** Chair: Greg Frederick, Department of Biology, LeTourneau University Co-Chair: Aaron Lynn, Department of Biological Sciences, Sam Houston State University	LSC 304
08:00 am	***Lactobacilli* strains inhibit the growth and biofilm development by *Pseudomonas aeruginosa.*** T. Letbetter, Dept. of Immun. and Mol. Microbiology, Texas Tech University	
08:15 am	**Characterization of two ST179 *Enterococcus faecalis* bloodstream infection isolates** B. Schmidt, Department of Biological Sciences, The University of Texas at Dallas	
08:30 am	**Alteration in Cytokine and Chemokine expression during Staphylococcus aureus Wound infections** Kayla Bounds, Department of Biology, Texas Tech University	
08:45 am	**Redefining the 'Core' Microbiome: Understanding the Functional Role of Microorganisms in Microbial Community Assembly and Structure** Chelcy E. Brumlow, Department of Biological Sciences, Sam Houston State University	
09:00 am	**The HTLV-1 latency-maintenance factor p30II inhibits Tax-induced NF-kappa B activation and prevents Tax-mediated apoptosis** Malu Aditi, Department of Biological Sciences, Southern Methodist University	
09:15 am	**Cooperation of the HTLV-1 p30^{II} Latency-Maintenance Factor with Cellular and Viral Oncogenes during Retroviral Carcinogenesis** Tetiana Hutchison, Department of Biological Sciences, Southern Methodist University	
09:30 am	**Genome-wide analysis of the response to nitric oxide in uropathogenic *Escherichia coli* CFT073** Heer Mehta, Department of Biological Sciences, University of Texas at Dallas	

08:00 – 09:45 am	**CONCURRENT STUDENT SESSION** **General Microbiology Student Session** Chair: Bob McLean, Department of Biology, Texas State University Co-Chair: Donovan Haines, Department of Chemistry, Sam Houston State University	LSC 320
08:00 am	**Connection of the ClpXP protease to antibiotic resistance and autolytic activity in *Bacillus anthracis* and *Staphylococcus aureus*** Kevin M. Claunch, Department of Biology, Texas Christian University	
08:15 am	**Assessing the Role of the Orphan CRISPR Locus of *Enterococcus faecalis* in Genome Defense** M. Rodrigues, Department of Biological Sciences, The University of Texas at Dallas	
08:30 am	**Analysis of Gold (Au(III)) Metal Tolerance in *Rhodobacter sphaeroides*** Hannah Johnson, Department of Biological Sciences, Sam Houston State University	
08:45 am	**Circadian Regulation of Symbiotic Genes in Nitrogen-Fixing Bacteria** Dylan Parks**,** Department of Biology, University of Texas-Arlington	
09:00 am	**Transforming raw milk into safe milk using electron beam processing** Lindsay Ward, National Center for Electron Beam Research, TAMU	
09:15 am	**Spore Cortex Hydrolysis Precedes Dipicolinic Acid Release during *Clostridium difficile* Spore Germination** Michael B Francis, Texas A&M University	
09:30 am	**The impact of Dust exposure on the Growth, Sensitivity to Oxidative-Stress, and virulence potential of the Pathogenic yersiniae** Shari Galvin, Department of Biology, Texas Southern University	

09:45 – 10:15 am	**COFFEE BREAK**	LSC 302

10:15 – 12:15 pm	**CONCURRENT SESSION** **Microbial Evolution & Bioinformatics** Chair: Rajeev Azad, Departments of Biological Sciences & Mathematics, University of North Texas Co-Chair: Hyuk Cho, Department of Computer Science, Sam Houston State University	LSC 320
10:15 am	**Using Bioinformatics in Microbiology** Rajeev Azad, Departments of Biological Sciences & Mathematics, University of North Texas	
10:45 am	**Mutation Interactions cause predictably declining evolvability of experimental populations of *Escherichia coli*** Tim Cooper, Dept. of Biology and Biochemistry, University of Houston	
11:15 am	**The Microbiome and Next-generation Sequencing** Yuriy Fofanov, Department of Pharmacology and Toxicology, The University of Texas Medical Branch at Galveston	
11:45 am	**Bioinformatics and the study of the microbiome: Translating A, T, C, and G into the who and how of mixed microbial communities** Emily Hollister, Bioinformatics and Microbial Ecology, Texas Children's Microbiome Center, and Pathology and Immunology, Baylor College of Medicine	

10:15 – 11:45 am	**CONCURRENT SESSION** **<u>Eukaryotic Microbes, Protein Dynamics in Yeast</u>** Chair: Kevin Morano, Department of Microbiology and Molecular Genetics, University of Texas Medical School, Houston Co-Chair: Anne Gaillard, Department of Biological Sciences, Sam Houston State University	LSC 304
10:15 am	**An endosomal-nuclear signaling axis that coordinates membrane trafficking and cell cycle control** Vytas Bankaitis, Department of Molecular & Cellular Medicine, Texas A&M University Health Science Center	
10:45 am	**Deciphering a second code in protein targeting to the proteasome** Andreas Matouschek, Department of Molecular Biosciences, The University of Texas at Austin	
11:15 am	**Redox Control of Cytosolic Proteostasis** Kevin Morano, Department of Microbiology and Molecular Genetics, UTHSC-Houston	

12:15 – 01:15 pm	**LUNCH**	LSC Ball Room B

01:00 – 03:00 pm	**POSTER SESSION** (Posters must be removed after session)	LSC Ball Room A

03:15 – 05:15 pm	**CONCURRENT SESSION** **<u>Clinical Microbiology</u>** Chair: James Dunn, Director Medical Microbiology & Virology, Texas Children's Hospital, Associate Professor, Department of Pathology & Immunology, Baylor College of Medicine Co-Chair: Anne Stiles, Chair, School of Nursing, Sam Houston State University	LSC 304
03:15 pm	**The Evolution of the Clinical Microbiology Laboratory** Gregory Berry, Department of Pathology, University of Texas Medical Branch at Galveston	
03:45 pm	**Rapid Diagnosis of Positive Blood Cultures and CSF Infections** Morgan Pence, Clinical Microbiology and Virology, Cook Children's Medical Center	
04:15 pm	**Point of Care Testing in Clinical Microbiology: from the Bench to the Bedside** Lakshmi Chandramohan, SeqWright Genomic Services, GE Healthcare	
04:45 pm	**Understanding the Mechanism of Action for SMT19969, a Novel Treatment for *C. difficile*** Eugénie Bassères, University of Houston College of Pharmacy	

03:15 – 05:15 pm	**CONCURRENT SESSION** **<u>Environmental Microbiology & Microbial Ecology</u>** Chair: Leland Pierson, Head, Plant Pathology and Microbiology, Texas A&M University Co-Chair: Sibyl Bucheli, Department of Biological Sciences, Sam Houston State University	LSC 320
03:15 pm	**Response of Freshwater Cyanobacterial Harmful Algal Bloom (cyanoHAB) under Climate Change Scheme (Microcosm Study)** Sanghoon Kang, Department of Biology, Baylor University	
03:45 pm	**Recovery of Soil Microbial Populations, Function, and Community Composition following Reclamation of a Lignite Surface Mine** Terry Gentry, Department of Soil and Crop Sciences, Texas A&M University	
04:15 pm	**Investigating how *E. coli* grows in mixed cultures** Robert McLean, Department of Biology, Texas State University	
04:45 pm	**eDNA release promotes biofilm formation in the biocontrol strain *Pseudomonas chlororaphis* 30-84** Elizabeth Pierson, Department of Horticultural Sciences, Texas A&M University	

05:15 – 05:30 pm	**COFFEE BREAK**	LSC Ball Room A

05:15 – 06:30 pm	**VENDOR EXHIBITIONS**	LSC Ball Room A

07:00 – 10:00 pm	**BANQUET, KEYNOTE LECTURE, & AWARDS**	LSC Ball Room B
07:00 pm	**WELCOME** Madhusudan Choudhary, Organizing Committee Jaimie Hebert, Provost, Sam Houston State University	
07:15 pm	**RECEPTION & DINNER**	
08:30 pm	**DOOR PRIZES & AWARDS PRESENTATION**	
08:45 pm	**ASM DISTINGUISHED LECTURE** **<u>Genes without Borders</u>** Briana Burton, ASM Distinguished Speaker, Molecular and Cellular Biology, Harvard University	

SATURDAY, October 31, 2015

08:00 – 09:00 am	**BREAKFAST & REGISTRATION**	LDB Atrium

09:00 – 10:30 am	**MINI CUE: EDUCATION SESSION** Chair: Lee Hughes, Department of Biology, University of North Texas Co-Chair: Sonja Yung, Department of Biological Sciences, Sam Houston State University	LDB 214
09:00 am	**Small World Initiative** Lee Hughes, Department of Biology, University of North Texas	
09:45 am	**Who wants to learn human anatomy? Mechanisms to promote responsible learning.** Joni Seeling, Department of Biological Sciences, Sam Houston State University	

10:30 – 10:45 am	**COFFEE BREAK**	LDB Atrium

10:45 – 11:55 am	**CONCURRENT SESSION: MICROBREW SESSION A** Chair: Deb Scheiwe, Tarrant County College	LDB 207
10:45 am	**Utilizing Lichens to Teach About Environmental Assessment** Steve Wagner, Biology Department, Stephen F. Austin University	
11:10 am	**Mona Lisa and Microbiology** Deb Scheiwe, Tarrant County College	
11:35 am	**When STEM Education 'Gets Social' and How to Show Them Once Your Projector is No More!** Greg Frederick, Department of Biology, LeTourneau University	

10:45 – 11:55 am	**CONCURRENT SESSION: MICROBREW SESSION B** Chair: Bill Coons, Victoria College	LDB 220
10:45 am	**How not to get away with murder: Infusion of bioinformatics into microbiology** Quincy Moore, Prairie View A&M University	
11:10 am	**Teaching Genetics: Taking your students from Don't kNow Anything to DeoxyriboNucleic Acid** Sunny Yung, Department of Biological Sciences, SHSU	
11:35 am	**Extra! Extra! Extra Reading for Extra Credit** Bill Coons, Victoria College	

S.E. Sulkin Oral Presentation Award in Medical Microbiology

SES 1. ***Lactobacilli* strains inhibit the growth and biofilm development by *Pseudomonas aeruginosa.***

T. Letbetter[1], A. Ahmad[1], N. Mudaliar[1], G. Ventolini[2], and A. Hamood[1]
[1]Dept. of Immun. and Mol. Microbiol.-TTUHSC, Lubbock. [2]TTUHSC-School of Medicine at the Permian Basin

Pseudomonas aeruginosa is an opportunistic pathogen that causes serious infections in immunocompromised patients including severely burned patients. *P. aeruginosa* forms biofilms at different infection sites. Due to its resistance to numerous antibiotics, identifying alternative novel anti *P. aeruginosa* therapies is a priority. *Lactobacilli* are major ingredients in probiotics products. We hypothesized that *Lactobacilli* inhibit the growth of *P. aeruginosa*. We examined the effect of *Lactobacillus gasseri* on the growth and biofilm development by the *P. aeruginosa* strain PAO1 and the *P. aeruginosa* cystic fibrosis isolate CF05. Results of the agar plate spot assay showed that L. *gasseri* inhibited the planktonic growth of either strain. *L. gasseri*, however, did not inhibit the growth of either *Staphylococcus aureus* or *Escherichia coli*. The overnight growth of *L. gasseri* reduced the pH of LB broth to 5.42. However, LB broth adjusted to the same PH failed to inhibit the growth of either CF05 or PAO1. Using an *in vitro* biofilm assay, we examined the effect of *L. gasseri* on biofilm development by *P. aeruginosa*. Confocal laser scanning microscopy revealed the development of a mature biofilm by PAO. In contrast, PAO1 to which *L. gasseri* was added produced few scattered bacteria. These results suggest that: 1) *L. gasseri* specifically inhibits the planktonic growth and biofilm development by *P. aeruginosa*; and 2) The effect is not due to the *lactobacilli*-induced acidic environment.

SES 2. **Characterization of two ST179 *Enterococcus faecalis* bloodstream infection isolates**

B. Schmidt, X. Li, K. Hullahalli, and K. Palmer
Department of Biological Sciences, The University of Texas at Dallas, Richardson, TX

Chlorhexidine gluconate (CHG) is an antiseptic that is a staple of clinical care. Daily CHG bathing of patients is a common infection control practice. *Enterococcus faecalis* is an opportunistic pathogen primarily found in the digestive tract and often resistant to many antibiotics. A recent study reported that bloodstream isolates of *E. faecalis* from wards using CHG bathing had reduced susceptibility to CHG as compared to isolates from wards not using CHG bathing [1]. We used multilocus sequence typing to assess clonality among *E. faecalis* strains from that study, identified two strains (660 and 6358) of the same sequence type (ST179) but with different CHG susceptibilities, and used E-test assays to determine that the two strains have similar antibiotic susceptibility against seven antibiotics. To determine whether genetic variation could account for the differing CHG susceptibilities of the strains, genome sequencing was performed using Illumina MiSeq. Draft genomes were assembled in CLC Genomics Workbench and annotated using RAST (Rapid Annotation using Subsystem Technology). Whole genome alignments were performed using Mauve. The genomes differ mostly in mobile genetic elements, and these elements likely contribute to the reduced CHG susceptibility of 660 relative to 6358.

[1] Suwantarat, et al. 2014. *Infect. Cont. Hosp. Epidemiol.* 35:1183-6.

SES 3. **Alteration in Cytokine and Chemokine expression during Staphylococcus aureus Wound infections**

Kayla Bounds[1], Cassandra Kruczek[2], Matt Myntti[3], Jane A. Colmer-Hamood[4, 5], Randall Jeter[1], and Abdul Hamood[2, 5]
[1]Biology Dept., TTU. [2]Dept. of Surg., TTUHSC. [3]Next Science, Jacksonville, FL. [4]Dept. of Medical Education, TTUHSC. [5]Dept. of Immun. & Mol. Biol., TTUHSC

Chronic wounds such as diabetic foot ulcers, and venous ulcers, affect approximately 6.5 million persons with an estimated $25 billion annual cost. Wound healing occurs through specific overlapping steps that involve different cell types and extracellular matrix protein and are mediated by cytokines and growth factors. Using the murine model of wound infection, we examined the influence of infecting microorganisms on the healing process by determining the level of cytokine expression in *Staphylococcus aureus*-infected full-thickness excision wounds (compared with uninfected wound tissues). Tissues excised from the wounds at 24 hours were homogenized and total bacterial RNA was isolated. Cytokines expression was determined using RT² Profiler™ PCR Array Mouse Cytokines and Chemokines kit (QIAGEN). In uninfected wounds, the expression of numerous cytokines belonging to specific functional groups including; response to injury and tissue homeostasis; production of immune cells and hematopoiesis; and recruitment and activation of immune cells was significantly enhanced. However, in comparison with wound tissues, the level of expression these cytokines was either reduced or only slightly increased in wounded/infected tissue. These results suggest that wound infection by *S. aureus* interferes with the expression of numerous wound healing and immune response cytokines.

SES 4. **Genome-wide analysis of the response to nitric oxide in uropathogenic *Escherichia coli* CFT073**

Heer Mehta and Stephen Spiro
Department of Biological Sciences, University of Texas at Dallas

Uropathogenic *Escherchia coli* (UPEC) is the causative agent of urinary tract infections. Nitric oxide (NO) is a toxic water-soluble gas that is encountered by UPEC in the urinary tract. Therefore, UPEC likely requires mechanisms to detoxify NO in the host environment. Thus far, flavohemoglobin (Hmp), an NO denitrosylase, is the only demonstrated NO detoxification system in UPEC. Here we show that, in strain CFT073, the NADH-dependent NO reductase flavorubredoxin (FlRd) also plays a major role in NO scavenging. We generated a mutant that lacks all known and candidate NO detoxification pathways (Hmp, FlRd and the respiratory nitrite reductase, NrfA). When grown anaerobically, this mutant expresses an NO-inducible NO scavenging activity, pointing to the existence of a novel detoxification mechanism. Expression of this activity is inducible by both NO and nitrate, and the enzyme is membrane associated. The activity shows a complex pattern of regulation by NsrR, FNR, NarL and NarP. Genome wide transcriptional profiling of UPEC grown under anaerobic conditions in the presence of a source of NO highlighted various aspects of the response of the pathogen to NO. Our data suggest that exposure to a source of NO causes a reprogramming of energy metabolism in UPEC, and may contribute to increased expression of virulence-associated genes. Thus, virulence determinants may be expressed by UPEC in response to a host-generated signal, and NO may act as a signal of a suitable host environment.

SES 5. **The HTLV-1 latency-maintenance factor p30II inhibits Tax-induced NF-kappa B activation and prevents Tax-mediated apoptosis**

Malu Aditi, Tetiana Hutchison, & Robert Harrod
Department of Biological Sciences, Southern Methodist University, Dallas, TX 75275

The human T-cell leukemia virus type 1 (HTLV-I) infects CD4+ T-cells and is etiologically linked to Adult T-cell Leukemia/Lymphoma (ATLL), an aggressive and often-fatal lymphoproliferative cancer. The viral transactivator, Tax, is thought to play an important role during the early stages of CD4+ T-cell immortalization by HTLV-1. Tax has been shown to activate transcription through CREB/ATF and NF-κB, and to alter numerous signaling pathways. The activation of cellular genes is thought to be essential for Tax-mediated cell transformation. Another protein encoded by HTLV-I, p30II, has been shown to affect retroviral replication at the transcriptional and posttranscriptional levels. Little is currently known regarding the effects of p30II on the expression and nuclear export of cellular host mRNA transcript. Tax is a potent activator of HTLV-1 transcription and NF-κB. Activation of NF-κB by Tax is mediated by direct binding of Tax to the regulatory subunit of I-κB kinase (IKK), NF-κB essential modulator (NEMO), also known as IKKγ. This interaction results in constitutive activation of IKKα and IKKβ, degradation of all I-κBs, and activation of both classical and alternative NF-κB pathways. Tax mediated persistent oncogenic activation of NF-κB triggers a defense mechanism that commits cells into senescence, an irreversible state of cell cycle arrest. This checkpoint is turned on by hyper-activated p65/RelA and is mediated by two cyclin-dependent kinase inhibitors, p21Waf/Cip1 and p27Kip1, in a p53- and pRb-independent manner. The HTLV-1 Tax oncoprotein is also known to repress the transcriptional activity of wild-type p53 through its N-terminal transactivation domain. Tax competes with p53 in the binding with the coactivator CBP, and thereby represses p53 transactivation function. Jeong et al (2005) have also shown that Tax inhibits p53 functions through the activation of NF-κB signaling. Moreover, previous studies from our lab have shown that HTLV-1 p30II induces p53 and activates p53-dependent pro-survival genes associated with its cooperation with cellular oncoproteins [Romeo et al, manuscript submitted]. The *stathmin* or *OP18* (*Oncoprotein-18*) gene is negatively regulated by p53 and has been shown to play an oncogenic role in a range of invasive cancers. Stathmin is a member of microtubule dynamic destabilizing proteins. It was recently shown that Stathmin interacts with p65/RelA complexes and promotes aggressive proliferation in pancreatic cancer cell-lines. My studies have demonstrated that HTLV-1 p30II inhibits Tax-induced NF-κB activation and promotes the phosphorylation of Stathmin/OP18, which could stabilize microtubules associated with increased cell motility and contribute to ATLL disease progression. *We hypothesize that p30II may augment p53 pro-survival functions in HTLV-1-infected cells by inhibiting Tax-induced NF-κB activation, through a mechanism involving the phosphorylation of Stathmin and inhibition of Stathmin/p65 (RelA) molecular interactions*.

SES 6. **Cooperation of the HTLV-1 p30^{II} Latency-Maintenance Factor with Cellular and Viral Oncogenes during Retroviral Carcinogenesis**

Tetiana Hutchison[1], Aditi Malu[1], Averi White[1] and Robert Harrod[1]
[1]Department of Biological Sciences, and The Dedman College Center for Drug Discovery, Design & Delivery, Southern Methodist University, Dallas, TX

The human T-cell leukemia virus type-1 (HTLV-1) infects and transforms CD4+ T-lymphocytes and is the etiological agent of adult T-cell leukemia/lymphoma (ATL). ATL cells have polylobate or “flower”-shaped nuclei due to karyotypic abnormalities and genomic instability. ATL is generally resistant to most anticancer therapies. There are five HTLV-1-associated disease stages. However, the mechanism(s) of progression from chronic ATL to the more advanced stages of disease is not completely defined. The HTLV-1 proviral genome contains a highly-conserved sequence, known as *pX*, which encodes the latency-maintenance factors p30^{II}, p13^{II} and the HTLV-1 basic leucine zipper protein (Hbz). These factors suppress the Tax oncoprotein and assist evasion of detection by the host immune system. The p30^{II} protein deregulates viral gene expression, interacts with host cellular pathways that control progression of the cell cycle, and facilitates disease progression. We have previously demonstrated that the latency-maintenance factor p30^{II} promotes viral proliferation through cooperation with the host cellular oncoprotein c-MYC and enhances its oncogenic potential through acetylation on specific lysine residues. The aberrant overexpression of oncogenes can result in the accumulation of reactive oxygen species (ROS) and lead to DNA damage, cellular senescence, cytotoxicity and apoptosis. The generation of ROS can also cause mitochondrial damage, or mitophagy – a process of autolytic mitochondrial destruction. Our lab has previously shown that HTLV-1 p30^{II} induces the tumor suppressor p53. The *p53* gene is functionally inactivated or mutated in nearly half of human cancers. Interestingly, *p53* is wildtype in the majority of ATL clinical isolates. We speculate that HTLV-1 p30^{II} activation of p53 may facilitate cellular transformation and T-cell leukemogenesis. Intriguingly, our data indicate that p30^{II} also cooperates with viral oncoproteins, such as Tax and Hbz. The Tax protein is considered to be the major oncogenic determinant of HTLV-1. It promotes lymphocytic proliferation by positively regulating viral gene expression and replication. Tax has been shown to cooperate with Hbz. Hbz is a viral protein encoded from the negative strand mRNA and has been shown to attenuate cell senescence induced by Tax. It is remarkable that in many ATL cells Tax is not expressed, whereas Hbz expression is observed in all ATL cases suggesting that Tax plays a pivotal role only in early T-cell immortalization by HTLV-1. Our preliminary studies have shown that HTLV-1 p30^{II} cooperates with Tax and Hbz by mitigating cellular senescence and levels of ROS –thus protecting the cells from potential apoptosis and withdrawal from the cell cycle. The role of p30^{II} cooperation with viral proteins, such as Tax and Hbz, is yet to be fully elucidated. *Our studies allude to a pivotal role for the p30^{II} protein in the cooperation with host and viral oncoproteins during retroviral carcinogenesis.*

SES 7. **Redefining the 'Core' Microbiome: Understanding the Functional Role of Microorganisms in Microbial Community Assembly and Structure**

Chelcy E. Brumlow[1], Jeanette R. Carlson[1], Emily Hollister[2], Embriette Hyde[3], Joseph Petrosino[3], & Todd P. Primm[1]

[1]Department of Biological Sciences, Sam Houston State University. [2]Texas Children's Microbiome Center, Texas Children's Hospital, and Pathology & Immunology, Baylor College of Medicine. [3]Department of Molecular Virology & Microbiology, Baylor College of Medicine

Our model vertebrate system for studying the changes in a mucosal microbiome due to a disruption event uses the Western mosquitofish, *Gambusia affinis* (*G. affinis*). The skin of *G. affinis* contains a microbiome community that is adapted for life within a mucosal system and is also directly accessible through the water column. This allows for non-invasive (to the fish) disruptions to the mucosal microbiome and a rapid, efficient way to sample and analyze the changes occurring within the community. In order to understand what drives the microbiome community, recovery following two different disruptions was analyzed. A severe point-disruption involved the serial rinsing of a group of *G. affinis* until skin-derived bacterial culturable numbers were greatly reduced. At various time-points after the serial rinse, the skin microbiome of *G. affinis* was sampled and compared to an undisturbed, pre-treatment (PT) control sample. In a separate experiment, a group of fish were exposed to antibiotic in the water column for 3 days, and then monitored during recovery. In both experiments, culturable numbers, biochemical activity, and16S profiles were collected. After both disruptions, culturable numbers are very low, but rapidly recover to exceed the PT density, before settling to a level comparable to PT. A profile of 20 biochemical activities were strongly altered by the disruptions, but returned to patterns similar to PT. In contrast, community composition did not return to PT taxa distributions. During succession of the microbial community after the disruptive event, a single taxa (genus *Myroides* after drug treatment, family *Aeromonadaceae* after physical rinse) dominates early, then declines. This data preliminarily supports the hypothesis that the functional roles of organisms within the microbial community may be more important than the taxonomic structure. This is important when considering human conditions such as an opportunistic *C. difficle* infection where returning a particular biochemical pathway to the microbial community may have a more positive outcome than the probiotic addition of particular taxa.

O. B. Williams Oral Presentation Award in General Microbiology

OBW 1. **Connection of the ClpXP protease to antibiotic resistance and autolytic activity in *Bacillus anthracis* and *Staphylococcus aureus***

Kevin M. Claunch, & Shauna M. McGillivray
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The ClpXP protease, a global regulator of bacterial proteins, is comprised of ClpX, an ATPase that recognizes and unfolds proteins, and ClpP, the proteolytic core. Genetic and pharmacological disruption of ClpX (ΔClpX) in *B. anthracis* and *S. aureus* renders the bacteria more susceptible to antimicrobial agents that interact with the cell wall such as penicillin, daptomycin and cathelicidin antimicrobial peptides but not non-cell wall active agents. Microarray analysis also revealed that expression of the *lrgAB* operon is substantially decreased in ΔClpX and this was confirmed by QPCR. Previous research in *S. aureus* has shown that LrgA/B are negative regulators of autolysis and their disruption increases susceptibility to penicillin. Thus, we hypothesize that the decreased expression of *lrgA/B* in our ClpX mutant leads to increased autolytic activity and a corresponding increase in susceptibility to cell wall-targeting agents. We compared the rate of cell lysis and the activity of autolytic enzymes between wild-type and ΔClpX *B. anthracis*. We found no detectable difference in enzyme activity, but we did observe a small increase in autolytic activity in ΔClpX. We also found that *B. anthracis* and *S. aureus* deficient in LrgAB are more susceptible to daptomycin and antimicrobial peptides. Thus, LrgA/B and ClpX appear to be involved in resistance to many of the same antimicrobial agents. Therefore, the decrease in *lrgAB* expression in ΔClpX might contribute to increased antibiotic susceptibility via a dysregulation of autolytic activity.

OBW 2. **Assessing the Role of the Orphan CRISPR Locus of *Enterococcus faecalis* in Genome Defense**

M. Rodrigues, K. Hullahalli, K. Palmer
Department of Biological Sciences, The University of Texas at Dallas, Richardson

Enterococcus faecalis (Efs) is an opportunistic pathogen that causes hospital-acquired infections. Multidrug resistance (MDR) in Efs has been linked to an increase in horizontal gene transfer brought about by the loss of genome defense systems such as CRISPR-Cas. CRISPR-Cas systems provide adaptive immunity in bacteria by cleaving invading foreign DNA in a sequence-specific manner. The presence of CRISPR-Cas across Efs isolates worldwide is variable; however, every isolate retains an orphan CRISPR locus (designated as CRISPR2) that lacks associated *cas* genes. Since the CRISPR repeat sequences of this locus and those of a Type II CRISPR-Cas system (designated CRISPR1-Cas) present in some Efs isolates are identical, CRISPR2 may be functionally linked to CRISPR1-Cas. In this study, the gene encoding the CRISPR1 Cas9 endonuclease was introduced into the Efs V583 chromosome. V583 is a multidrug resistant strain that natively encodes only the orphan CRISPR2. Conjugation frequency into this strain was reduced when the transferred plasmid possessed a CRISPR2 target, as compared to otherwise isogenic plasmids without a target. Next, the transcriptional start site of CRISPR2 was mapped using primer extension, and the promoter for the CRISPR2 transcript was deleted. This restored conjugation frequencies to wild type levels, irrespective of a CRISPR2 target sequence. Our results confirm that CRISPR2 in the presence of CRISPR1 Cas9 is active in genome defense in Efs. Future studies will determine whether reintroduction of *cas* genes into MDR strains can cure Efs of mobile genetic elements and mitigate its virulence.

OBW 3. **Analysis of Gold (Au(III)) Metal Tolerance in *Rhodobacter sphaeroides***
Hannah Johnson, Dr. Madhusudan Choudhary
Sam Houston State University, Huntsville TX

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

OBW 4. **Circadian Regulation of Symbiotic Genes in Nitrogen-Fixing Bacteria**
Dylan Parks*, and Woo-Suk Chang
Department of Biology, University of Texas-Arlington, Arlington, TX 76019

The photosynthetic soil bacterium *Bradyrhizobium* sp. strain ORS278 forms a mutualism with *Aeschynomene* plants, resulting in root and stem nodulation. In this process, not only the bacterium provides the plant with N source via nitrogen fixation, but also it carries out photosynthesis. In a previous study, a homologous gene encoding a photosynthetic circadian feedback protein found in cyanobacteria was mutagenized and the mutant showed a nitrogen fixing deficiency in its phenotype. The nitrogenase enzyme responsible for nitrogen fixation, is inhibited by oxygen. Thus, we hypothesize that if nitrogenase activity is restricted to the night-time, the deleterious effect of oxygen production could be avoided. In this study, two genes, BRADO4470 (*labA*) encoding a putative protein that may function in circadian feedback and BRADO3946 encoding a putative sensor histidine kinase, thought to be involved in the temporal regulation of nitrogen fixation and possibly feedback control of circadian rhythm in photosynthesis, were independently mutagenized via double-homologous recombination. Then, their phenotype was compared with the wild type for nitrogen fixing ability in *Aeschynomene indica* plants. Acetylene reduction assays showed that the wild type fixed nitrogen during the night time, while the mutant strains did not. This result indicates that BRADO4470 and BRADO3946 are likely involved in the temporal regulation of nitrogen fixation through circadian feedback systems. A better understanding of the symbiotic genes could allow optimization of nitrogen fixation (e.g., in night-time) and photosynthesis (e.g., in day-time), resulting in a more productive agricultural practice for members of symbiotic nitrogen-fixing rhizobia.

OBW 5. **Transforming raw milk into safe milk using electron beam processing**
Lindsay Ward, and Suresh D. Pillai
National Center for Electron Beam Research, TAMU

Raw milk, though sterile in the udder, is a host to a vast microflora of bacteria, including foodborne pathogens. Raw milk consumption is unfortunately increasing, and illnesses associated with raw milk are also on the increase. Milk has a high pH, high water activity, and an abundance of nutrients, creating an environment that allows potential pathogens to proliferate. Raw milk harbors around 10^5 CFU/mL of aerobic bacteria along with low levels of key foodborne pathogens such as *S. aureus*, *E. coli* O157:H7, *C. jejuni*, and *L. monocytogenes*. Heat pasteurization is currently the standard practice for the milk industry. We hypothesize that Electron beam (eBeam) processing, a non-thermal food processing technology, is suitable for eliminating microbial pathogens in raw milk without causing any heat-related changes observed in heat pasteurized milk. This research challenges the paradigm that milk can be pasteurized only by heat. Our goal is to provide the milk industry with a novel tool to pasteurize milk and retain all nutrients present in raw milk. Our empirical data suggest that eBeam doses as low 2 kGy assures the microbiological safety of raw milk. Research is ongoing to validate that, along with assuring the microbiological safety, 2kGy will preserve all the nutritional and flavor profiles of raw milk. This technology can address the growing needs of some consumers who show an increasing demand for raw milk, while at the same time assuring the microbiological and public health safety of such milk. This study challenges the 200 year old benchmark of thermal pasteurization that is employed by the milk industry in the United States.

OBW. 6 **Spore Cortex Hydrolysis Precedes Dipicolinic Acid Release during *Clostridium difficile* Spore Germination**
Michael B Francis, Charlotte Allen, Joseph A Sorg
Texas A&M University, College Station, TX

Clostridium difficile is an anaerobic, spore forming bacterium that infects antibiotic-treated hosts. The vegetative form of *C. difficile* produces toxins that elicit the primary symptoms of disease but it is the spore form that is necessary for host infection. Spores are metabolically dormant forms of bacteria that are resistant to harsh environmental conditions, including antibiotics. Spores sense changes in environmental conditions and begin the transition from dormancy to vegetative growth (germination) upon sensing appropriate signals (germinants). In *B. subtilis*, the initiation of germinant receptor-mediated spore germination is divided into two genetically separable stages. Stage I is characterized by the release of dipicolinic acid (DPA) from the spore core. Stage II is characterized by cortex degradation, and stage II is activated by the DPA released during stage I. DPA release precedes cortex hydrolysis during *B. subtilis* spore germination. We investigated the timing of DPA release and cortex hydrolysis during *C. difficile* spore germination and found that cortex hydrolysis precedes DPA release. Inactivating either the bile acid germinant receptor, *cspC*, or the cortex hydrolase, *sleC*, prevented both cortex hydrolysis and DPA release. Because both cortex hydrolysis and DPA release during *C. difficile* spore germination are dependent on the presence of the germinant receptor and the cortex hydrolase, the release of DPA from the core may rely on the osmotic swelling of the core upon cortex hydrolysis. These results have implications for the hypothesized glycine receptor and suggest that the initiation of germinant receptor-mediated *C. difficile* spore germination proceeds through a novel germination pathway.

OBW 7. **The impact of Dust exposure on the Growth, Sensitivity to Oxidative-Stress, and virulence potential of the Pathogenic yersiniae**

Mohammed O. Suraju, Shari Galvin**,** and Jason A. Rosenzweig
Texas Southern University

In this study, the impact of indoor and outdoor dust exposure on the growth, oxidative stress sensitivity, and biofilm production of pathogenic *Yersinia* spp*.* (*Y. enterocolitica*, *Y. pseudotuberculosis*, and *Y. pestis*) were evaluated. Furthermore, the impact of dust exposure on the virulence potential of *Y. pestis* was investigated in a HeLa cell culture infection assay. Suprisingly, we observed species-specific growth-pattern responses following dust exposure. More specifically, *Y. enterocolitica* exhibited increased growth in the presence of indoor- and outdoor-dust in a nutrient-poor environment, while *Y. pestis*'s growth was unaffected in either condition. Interestingly, exposure to both outdoor- and indoor-dust increased sensitivity to oxidative stress in *Y. pestis*, while only outdoor-dust increased the sensitivity of *Y. pseudotuberculosis* to oxidative stress. Additionally, indoor-dust induced increased biofilm production in *Y. pestis* but suppressed biofilm production in *Y. pseudotuberculosis*. Finally, both indoor- and outdoor-dust exposure increased the pathogenicity of *Y. pestis* in a cell culture cytotoxicity assy. Collectively, these findings demonstrate a species-specific response to dust exposure and suggests that dust may increase the virulence potential of the pathogenic *Yersinia*.

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Poster Board Number and Presentation

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

Undergraduate Posters – Medical Microbiology

UP 1 (JA). **Norepinephrine Reduces Lung Epithelial Cell Adhesion of *Streptococcus pneumonia***

Anthony Bucciarelli[1], Marisa Gonzales[2], and Xavier Gonzales[2]
Department of Natural Sciences, [1]Del Mar College, Corpus Christi, TX. Department of Life Science, [2]Texas A & M University - Corpus Christi, Corpus Christi, TX

Streptococcus pneumoniae, also known as pneumococcus, is a major cause of the bacterial pneumonia in children, the elderly, and the immune-compromised. It can exist as a commensal organism in the nasopharyngeal cavity; however, if it becomes pathogenic, it can invade the lungs and blood. Invasion beyond the lungs can lead to other diseases such as meningitis, bacteremia, and in severe cases sepsis. It has been shown to kill or hinder the growth of other upper respiratory tract flora. Norepinephrine has been shown to increase the growth of bacteria. This happens because of norepinephrine's ability to bind to iron and, therefore, provides more available iron to be used by the bacteria. Studies have shown that norepinephrine increases or decreases bacterial attachment to host tissue; however, in pneumococcus it decreases its ability to adhere to lung epithelial cells. The norepinephrine regulation of pneumococcal adherence to lung epithelial cells is associated with the bacterial iron uptake mechanisms. A protein associated with both oxidation and adhesion is SpxB. It has been found to impact the iron uptake mechanism and signal transduction. **We propose that norepinephrine will inhibit pneumococcus adhesion through regulation of SpxB**. In this study we performed adhesion assays with A549 lung epithelial cells and an SpxB knockout mutant strain of *S.pneumoniae* with and without norepinephrine treatments. The adhesion assays suggest that SpxB does play a role in pneumococcus adhesion.

UP 2 (JA). **Acute viral encephalitis is associated with rapid microglial activation and production of CCL2**

Kyra Curtis
Department of Biology, Baylor University, Waco, TX

Viral infection of the central nervous system (CNS) can lead to neurological deficits such as cognitive impairment, seizures, and epilepsy that can result in death. In mice infected with Theiler's murine encephalomyelitis virus (TMEV), we have previously shown via flow cytometry and immunostaining that neutrophils and inflammatory monocytes infiltrate the hippocampus as early as 12 hours post infection (hpi), resulting in inflammation and subsequent neural injury. However, the role of brain cells such as astrocytes and microglia during acute infection is currently unknown and important in understanding the progression of acute injury. Following intracortical (i.c.) injection of TMEV we show that microglia and astrocytes have a biphasic activation pattern. Furthermore, CCL2 is a chemokine known for recruiting monocytes, but its presence has not yet been confirmed during acute time intervals in the TMEV model in mice. We show that CCL2 expression in the hippocampus begins at 6 hpi and increases along with microglial activation in mice, thus suggesting a correlation between CCL2, microglial activation, and the infiltration of monocytes in the same region.

UP 3 (JA). **Characterization and Identification of Bacterial Isolates Indigenous to South Texas from Environmental and Steer Blood Samples**

Joanna Frontera, Damien C. Seay, and Daiyuan Zhang
Del Mar College, Corpus Christi, TX

Bacteria present in the blood of healthy individuals, known as bacterial endoparasites, may or may not contribute to illnesses and are now considered a part of the human microbiome. Similarly, animals also harbor bacterial endoparasites, the diversity of which has not fully been studied. We aim to determine what, if any, endoparasitic bacteria exist in young steer's blood isolated from Banquete, Texas, and compare that to those isolated from their environment. The following experiments were performed to characterize six bacterial isolates: a) Gram staining; b) plating on differential and selective media; c) motility assays; d) antibiotic sensitivity testing; e) temperature and oxygen preference assays; f) polymerase chain reaction (PCR) and sequencing of the 16s rRNA gene; and g) transmission electron microscopy (TEM). We were able to determine the following: i) if the isolates contain peptidoglycan; ii) have preference for various sugars; iii) whether or not growth was present on MacConkey and Mannitol-Salt media; iv) if anaerobic or aerobic environments were preferred; v) whether the bacteria were psychrophilic, mesophilic, or thermophilic; vi) the bacteria's ability to move; and vii) the type of bacteria through 16s rRNA sequencing and bioinformatics analysis. We have, perhaps, shed some light on the microbiome and bacterial endoparasites present in steer in South Texas. Moreover, our findings regarding antibiotic sensitivity may offer some insight into effective treatments against possible bacterial infections, including the use of specific antibiotics or even alternatives such as bacteriophage therapy.

UP 4 (JA). **An examination of ammonia-oxidizing mircrobes and nitrification in water distribution systems in summer**

Kien Ho[1], Nikki Gordon[2], and Daiyuan Zhang[1]
[1]Del Mar College, Corpus Christi, TX. [2]Corpus Christi Water Department

Clean drinking water is a necessity for humans to live and survive. It is needed for essential functions such as providing nutrients to cells, regulating body temperature, flushing waste, and formation of saliva. Adult bodies are made up of approximately 60% water and, on average, can only survive three days without water. The importance of municipal treatment plants to provide its citizens with clean drinking water is essential. In the past, free chlorine was used for treatment; however, the US Environmental Protection Agency's stringent byproducts regulations have changed this treatment practice. Many municipal utilities have adopted a chloramine treatment practice. This practice has solved the issue of disinfection byproducts, but now the issue of potential nitrification has arisen. It is theorized that nitrification happens more readily during warm summer temperatures. By using Quantitative Real-Time Polymerase Chain Reaction (q-PCR) methods, we tested for the presence of ammonia-oxidizing archaea (AOA), bacteria (AOB), and oligotropha-like bacteria (AOC). Both microbes are catalysts during the nitrification process thus lowering water quality. The study was done during the summer when temperatures were higher. Samples were collected from sites of potential interest (SPI). Levels of nitrate and AOA rose over the course of the study but remained below EPA levels. AOA may have been favored by the extreme environment (high chlorine and ammonia) in the water distribution system. This study will be continued to provide threashold levels for PCR.

UP 5. **Characterization of Leishmania Rhomboids**

Courtney Jackson, & Ashwini Kucknoor
Hayes Biology Department, Lamar University, Beaumont, Texas

Background: *Leishmania amazonensis* is a parasitic protozoan responsible for several variants of leishmaniasis, affecting approximately 2 million people worldwide. To better understand the process of parthenogenesis in humans caused by *Leishmania,* we studied the localization and function of rhomboid proteins in the parasite. Rhomboid proteins are found in a diverse range of biological organisms and the biochemical framework of such proteins are conserved from organism to organism, yet the functions and behaviors of the structures are dependent on the species in which they are found. The hypothesis for this research project is that the Rhomboid protein is an active gene in the *Leishmania* parasite and is expressed on the surface of the parasite, enabling it to modify host proteins during infection of the host cells, particularly when amastigotes are taking up residence in the phagolysosomes.
Methods: We have cloned two potential rhomboid genes in *Leishmania amazonensis*, LaRh2 and LaRh4 into plasmid vectors with a reporter gene GFP, which will fluoresce green under UV light. The specific goal of this project is to identify the localization of LaRh2 and LaRh4 in both promastigotes and amastigotes of *Leishmania amazonensis*. A fresh clinical isolate of *Leishmania amazonensis* was grown in Schnider's drosophila medium with 10% heat-inactivated donor horse serum. Macrophage cells were used for host-parasite interaction studies and grown in Dulbecco's modified Eagle medium (DMEM), supplemented with 10% fetal bovine serum at 37°C in the presence of 5% CO2 as described before. To obtain amastigotes, parasite cultures were seeded at 1 x 106 parasites/ml and harvested at logarithmic or stationary phase of growth as defined by cell density and morphological characteristics. U937 macrophage cells were cultured in RPMI medium (Invitrogen) supplemented with 10% fetal calf serum at 37 °C and in the presence of 5% CO2 at 37 °C. LaRh2 and LaRh4 were cloned into a GFP expression plasmid –PxGFP. Purified constructs of the plasmids with Rhomboid-GFP fusions were transfected into the parasites using BTX electroporator. Following transfection, cells were grown in the presence of neomycin, and neomycin resistant cells were selected. Neomycin resistant cells that took up the plasmids were then fixed in 4% paraformaldehyde and observed under an epifluorescence microscope to detect the green fluorescence of GFP, which is in fusion with the rhomboid proteins, to detect the localization of the genes.
Results and conclusion: Only one of the rhomboid gene is expressed during the amastigote stage. Both rhomboids are expressed in promastigote, with their levels being variable. It appears that one of the rhomboids is expressed on the membrane of the internal organelles, and we are currently investigating it further.

UP 6. **Characterization of Antimicrobial Peptides for Treatment of *Pseudomonas aeruginosa***

Y. Hwang, M. Kay, and D. A. Leal
Department of Biomaterials and Environmental Surveillance, Naval Medical Research Unit San Antonio, Fort Sam Houston, TX.

Background: Oral infections including gingivitis and periodontitis are major issues for deployed military personnel. Data collected by Navy dentists in Iraq during 2008 and 2009 indicate that over 30% of dental emergencies in Navy and Marine Corps personnel were due to gingivitis or periodontitis. *P. aeruginosa* is an opportunistic pathogen and is associated with periodontal infections. Because of its persistent nature, *P. aeruginosa* is often difficult to eradicate.
Antimicrobial peptides (AMPs) are short peptide sequences that have unique antibacterial properties. The use of AMPs is emerging as a novel avenue to effectively treat persistent multidrug resistant (MDR) *P. aeruginosa* infections that lead to emergencies involving the periodontium.
Objective: The objectives of this study were to screen antimicrobial activity of AMPs against *P. aeruginosa*.
Materials/Methods: Antibacterial activities of multiple AMPs were tested against several clinical MDR *P. aeruginosa* strains. Their minimal inhibitory concentration and minimal biofilm inhibitory concentration were assessed using 4 different culture media. Student's t-test was used to determine the level of significance ($p < 0.05$).
Results: Significant difference observed between effectiveness of AMPs in different culture media.
Conclusions: AMPs are generally more effective in minimal media. MDR *P. aeruginosa* strain 105765 was more susceptible to AMPs. Dermaseptin derivatives and K6L9 were more effective. Further testing and characterization are underway to identify additional promising AMPs for the treatment of periodontal pathogens.

UP 7 (JA). **Understanding Community Interactions in a Fish Model of Mucosal Microbiomes**

Corbett French, Annie B Leonard, and Todd P Primm
Department of Biological Sciences, Sam Houston State University

Mucosal microbiomes are complex microbial communities that have major effects on the host organism's health. Typically, in these microbiomes, when non-native bacteria are introduced they fail to colonize or are outcompeted. This suggests specific control of community members, which likely comes from both host factors and the microbial members of the community themselves. To better understand interactions between members of the community, we use a fish (*Gambusia affinis*) skin model. The skin is easy to manipulate and sample from, and the microbiome is relatively simple, with 5-7 dominant bacterial organisms. *Acinetobacter* is a native organism often found as one of the dominant genera in the skin microbiome. In contrast, *E. coli* is rarely recovered from the fish, and when it is, is a rare (less than 0.1% abundance) organism. To determine microbiome invasion and persistence of these two organisms, fish are exposed to high concentrations of them in the water column, and then the microbiome extracted and bacteria quantified using selective, differential media. We predict that the native organism will invade and persist at a higher rate than the non-native. This model system should allow future explorations on mechanisms behind microbiome community selection.

UP 8 (JA). **Assessment of Bactericidal Effects of Food Contact Materials Containing Silver Nanoparticles and Expression of Silver Resistance Genes during Subchronic Exposure**

Luis Valencia[1], Katherine Williams[1], Kuppan Gokulan[1], Raul Trbojevich[2], Carl Ceniglia[1], and Sangeeta Khare[1]
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Silver for centuries has been utilized specifically for medicinal and health additive purposes due to their well-known potent bactericidal properties against pathogenic bacteria. The influence of nanotechnologies to the food industry has become the innovation of the 21st century to enhance consumer products safety and quality. Manipulation of particles at a nano scale has led to the ingenuity of engineering nanoparticle embedded products known as Food Contact Materials (FCM). Silver nanoparticles (AgNPs) are one of the widely used metal nanoparticles for FCM due to its antimicrobial properties. The antimicrobial and toxicological properties of AgNP are particle size, shape and dose –dependent.
Like many antimicrobial agents, growing public health concerns is if the antimicrobial property of AgNP may also cause the antimicrobial resistance in microbes. It has been reported that some bacterial strains have the capability to become silver resistant when exposed to high concentrations of silver. The silver resistance is known to be plasmid mediated (pMG101) or in some particular cases also on bacterial chromosome. This plasmid encodes for silver ion binding proteins (SilE and SilF), efflux pumps ATPase (SilP) and chemiosmotic cation/ proton antiporter (SilCBA). This study was initiated to determine if subchronic exposure of AgNP may promote silver resistance to the gut microbiome.
In this study, antimicrobial activity was measured by identifying the reduction in bacterial bio-burden caused by the exposure to FCM (food storage containers, food wrapping material, and cutting board) that advertise antimicrobial activity. Furthermore, studies were conducted to assess if subchronic exposure of silver nanoparticles may induce the silver resistance in gut mucosa associated commensal bacteria. To achieve this, gut mucosa DNA obtained from male and female Sprague-Dawley rats that were orally gavaged to three sizes and three doses of AgNP for 13 weeks was used to analyze the expression of silver resistance genes.Our findings from FCM trials suggest very limited capacity for the bactericidal effect against tested bacterial species. Experiments are currently underway to test for silver resistant genes to identify and validate (i) if the development of the resistance to silver is nanoparticle size and dose dependent; (ii) how the expression of genes encoding for efflux pump and antiporter system is affected by the different size and dose of AgNP.
Public Health Concern: To identify if consumer exposure to FCM products containing silver nanoparticles alters the intestinal microbiome and gastrointestinal tract functions. The safety of nano silver in nanotechnology derived products that are used in the food industry still remains an open question.

UP 9 (JA). **Population-Level Analysis of Programmed Cell Death in *Chlamydomonas***

Matthew R. Breuer, Allyson R. Keathley, Anne R. Gaillard
Department of Biological Sciences, Sam Houston State University, Huntsville, Texas, 77341

Programmed cell death (PCD) is any form of cell death which is activated by a genetic program. PCD is essential to normal processes in multicellular organisms, such as the shaping of digits during development. In these cases, PCD plays a distinct role in the organism. However, PCD has also been observed in many unicellular organisms. In these cases, the purpose of such a programmed death is unclear. Using the model organism *Chlamydomonas*, a unicellular algae, we hope to shed light on the seemingly illogical notion of a benefit to PCD in the unicellular world. The purpose of our more recent experiments has been to differentiate the processes of PCD and necrosis. Previous experiments have shown that *Chlamydomonas* cells undergo PCD when faced with a mild stressor, but have failed to distinguish PCD and necrosis. We seek to differentiate PCD and necrosis at the cellular and the population levels. This presentation emphasizes our work at the population level. Here, we present two hypotheses. The first hypothesis is that PCD provides a benefit to cells that survive the stressor. To test this, we grew fresh cells in media from PCD and necrotic cells. To measure the growth rates of the cultures, we took daily absorbance readings of the cultures using a spectrophotometer. The second hypothesis presented is that cells which are surrounded by genetically-similar kin will be more likely to undergo PCD than cells which are not surrounded by kin. To test this, we measured the relative amount of death in genetically homogenous and genetically heterogeneous cultures using Evans blue, a dye retained only by dead cells. Our experiments are currently ongoing and conclusions from our results have yet to be made.

UP 10 (JA). **Engineering NADPH production in *E. coli* with modified lipoamide dehydrogenase**

Christa D. Frontera[1], John F. Ramirez[1], Jamie L. Vulgamore[1], J. Robert Hatherill[1], Victor Chubukov[2], Daiyuan Zhang[1], & Aindrila Mukhopadhyay[2]
[1]Del Mar College, Corpus Christi, TX. [2]Fuels Synthesis Division, Joint BioEnergy Institute, Lawrence Berkeley National Laboratory, Berkeley, CA

The use of fossil fuels as the primary source of energy scares environmentally concerned citizens and nations. Concerned citizens reduce our carbon footprint by carpooling as a source of transportation, using wind turbines or solar panels as renewable resources, and composting or recycling as a means of waste reduction. Scientists are also investigating biofuels as a renewable source of energy. Researchers at the Joint BioEnergy Institute (JBEI) are using *Escherichia coli* as a potential candidate in biofuel research. Our research group studied *E. coli* growth activity on various carbon sources. *E. coli* was transformed with the maqu2220 plasmid containing an alcohol reductase. Gas-chromotagraph-mass-spectrometry was used to detect the presence of fatty alcohols. We obtained mutant strains of the enzyme lipoamide dehydrogenase (Lpd) and redesigned its active site. We determined the growth of our Lpd mutant strains on different carbon sources. In conclusion, our preliminary data demonstrate fatty alcohol production in *E. coli*. Such fatty alcohol production is an important step for developing advanced biofuels.

UP 11 (JA). **Distinguishing Between Programmed Cell Death and Necrosis in *Chlamydomonas***

Allyson R. Keathley, Matthew R. Breuer, and Anne R. Gaillard
Department of Biological Sciences, Sam Houston State University, Huntsville, TX 77341

Chlamydomonas is a single celled eukaryotic alga that is commonly used in experimental studies. Here, we used *Chlamydomonas* to show the morphological and physiological characteristics that distinguish programmed cell death (PCD) from necrosis. PCD is the controlled, stepwise death of a cell that occurs in response to a mild stressor, while necrosis is the sudden accidental death of the cell. We have studied various facets of *Chlamydomonas* cells undergoing PCD and necrosis to help characterize the two processes: phosphatidylserine flipping, reactive oxygen species (ROS) accumulation, and the breakdown of DNA. Here, we used two dyes to show the differences of PCD and necrosis: Alexa fluor 488 Annexin V and non-membrane permeable DAPI. Alexa fluor 488 Annexin V binds to phosphatidylserine, which becomes externalized during programmed cell death processes. Non-membrane permeable DAPI binds to DNA, but only if the plasma membrane has become compromised. We also used the fluorescent dye CM-H_2DCFDA to show the accumulation of ROS. Our results show that both PCD and necrosis have compromised membranes, phosphatidylserine flipping, and ROS accumulation, but they occurred at different rates and different intensities. Also, DNA laddering is only present in PCD cultures. This shows that there are distinct morphological and physiological differences between PCD and necrosis. In the future, we plan on studying other areas of these two death processes to further define the two as separate events, as well as studying what causes differences in PCD and necrosis.

UP 12 (JA). **Searching for a bacterial factor that triggers the Nod factor-independent symbiosis between nitrogen-fixing *Bradyrhizobium* and the tropical legume *Aeschynomene***

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Bradyrhizobium sp. BTAi1 is able to form nitrogen-fixing nodules on the roots and stems of the tropical legume *Aeschynomene indica,* although the bacterium does not have essential *nod* genes responsible for nodulation (Nod) factor production in its genome. In this study, we investigated the differential gene expression of BTAi1 exposed to *A. indica* root exudates using RNA-Seq technique to seek for key genes involved in the Nod factor-independent nodulation. The differentially expressed genes (DEGs) were analyzed by the RNA-Seq data analysis tools EDGE-PRO and DESeq2. Of the 158 DEGs by the root exudate treatment, 41 genes were up-regulated and 117 down-regulated with q value <0.05. One of the most highly expressed genes encodes CobW protein (BBta_2276) involved in cobalamin biosynthesis, suggesting that cobalamin probably plays a role in the Nod factor-independent nodulation. In addition, all genes in the nitrous oxide reductase (Nos) gene cluster (*nosRZDFYLX*) were upregulated. Our RNA-Seq results would help us discover a bacterial factor that triggers the Nod-independent symbiosis between BTAi1 and *A. indica*.

UP 13 (JA). **Construction of *recA* deletion and analysis of SOS response in *Rhodobacter sphaeroides***

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RecA mediates the regulation of the SOS response, a prokaryotic DNA-damage repair system. While LexA binds to the promoter of the SOS regulon under normal conditions and represses the transcription of over 40 genes, RecA is activated by UV-damaged DNA and de-represses the transcription of the SOS regulon through the induction of autoproteolysis of LexA. RecA also regulates error-prone DNA synthesis that bypasses DNA lesions. An abundance of gene duplications that exist in the *Rhodobacter sphaeroides'* fully-sequenced genome are the result of RecA-mediated homologous recombination. In addition to the above characteristics, *R. sphaeroides'* ability to grow under extreme soil and marine environments (aerobic, anaerobic or photosynthetic) makes *R. sphaeroides* a model bacterium to study the SOS response and homologous DNA recombination. This study employs the construction of an in-frame deletion (Δ*recA*) and comparisons of the cell survival and growth characteristics including colony morphology under various UV exposures between the wild-type and the Δ*recA* mutant. We have successfully constructed and confirmed the Δ*recA* strain of *R. sphaeroides.* Results revealed that wild type and the mutant strains displayed similar growth characteristics under both aerobic and photosynthetic conditions. Furthermore, as UV exposure increased, the survival rates of the wild type and the mutant strain differentially decreased with the mutant strain being more affected than the wild type strain. UV exposure beyond 20 mJ had lethal effects on both wild type and mutant strains. Differential gene expression patterns between wild type and Δ*recA* strains will be examined to provide a better understanding of the genes involved in the SOS response.

UP 14 (JA). **Use of the Yeast Estrogen Screening Assay to Detect the Reduction of Estrogenic Bioactivity in Effluent Through Electron Beam Irradiation**

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A concern with recycled water has been the increase of emerging contaminants such as pharmaceuticals and chemicals, specifically estrogenic compounds, due to industrialization.Over exposure to exogenous estrogenic compounds has been shown to lead to cancers and diseases such as reproductive disorders.
electrons to yield rapid reduction and oxidation reactions, and has been shown to break down estrogens in wastewater biosolids. Therefore, it is important to employ a method that will accurately depict the amount of estrogens present throughout the treatment of effluent with Electron Beam (eBeam) irradiation. The yeast estrogen screening assay (YES Assay) incorporates a recombinant yeast, *Saccharomyces cerevisiae*, which was modified to have a human estrogen receptor encoded into its chromosome. Our hypothesis is that the YES Assay will be sensitive enough to detect the reduction of estrone (E1), 17-β-estradiol (E2), and bisphenol A (BPA), in wastewater effluent after utilizing eBeam irradiation at doses between 2 kGy and 15 kGy. The detection limits of the YES Assay were found to be 25pM for E1 and E2 and 0.25pM for BPA. Reduction of estrogenic bioactivity was seen in E2 at an absorbed dose of 15kGy. However, reproducible reduction of estrogenic bioactivity for E1 and BPA could not be determined from irradiation trials. The YES Assay was originally developed with E2 and therefore it is possible that it is not an optimal assay to detect reduction in bioactivity for E1 and BPA.

UP 15. **Isolation, Characterization and Antibiotics Susceptibility Testing of Halophilic Bacteria from the Grand Saline Salt Marsh**

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Halophilic bacteria have the ability to survive in hypersaline environments and are of interest because of the unique mechanisms they utilize to survive in high salt concentrations. However, environmental halophiles have not been well characterized in the past, especially regarding antibiotic susceptibility testing. A few recent studies have been conducted that indicate the presence of antibiotic resistance genes in bacteria that have been around since before the widespread use of antibiotics (Martinez 2012; Dennis 2014). By examining the antibiotics susceptibility of halophilic bacteria isolated from the Grand Saline Salt Marsh, this could give further clues as to how the purpose of antibiotic resistance genes found in nature have changed over time. For this project, three wet soil samples were collected at different sites around the salt marsh. Isolates were assessed with 16s rRNA gene sequencing, biochemical testing, and optimum salt tolerance determination in order to identify the isolates. Antibiotics susceptibility testing of the isolates was performed using the Kirby-Bauer method.

UP 16 (JA). **Quantitation of Cell-Density Effects on Twitching Motility in *Myxococcus xanthus***

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Twitching motility is type IV pili-mediated flagella-independent group movement on a solid surface by gram-negative bacteria, including *Pseudomonas aeruginosa* and *Myxococcus xanthus,* a pathogen and a non-pathogen respectively. Currently, the most accepted mechanism thought to drive this motility is the binding of type IV pili to a neighboring cell's surface, causing pilus retraction, which pulls the cell forward towards the next cell. We support an alternative hypothesis, that the pili attach to exopolysaccharide (EPS) secreted onto the surface by the cell. This predicts that high-density colonies expand more rapidly and at a constant rate as more cells produce more EPS, allowing these cells to move earlier and ultimately farther. A mathematical model devised to represent this mechanism was tested experimentally. Liquid cultures of *M. xanthus* DK1218, a strain exhibiting only twitching motility, were grown, diluted to different densities, and 3 µl spots of each density were placed onto agar plates. The plates were incubated at 32˚C in a humid environment for up to 96 hrs. Each spot was imaged with a camera attached to a dissecting microscope, and the distance the edge of each colony moved was measured at least twice a day. Under standard conditions (1% casitone in 0.5% agar plates), as expected, the higher densities colonies expanded more rapidly and as a result traveled farther. However, at all densities the colonies slowed down over time, deviating from our prediction. To eliminate the slowing down, two types of conditions were tested: variations in the agar content of the plates (0.3%, 0.4%); variations in nutrient composition (0.5%, 1.5%, 2% casitone, and 1% casitone with 0.2% yeast extract). Only the high nutrient plates (0.2% casitone, 1% casitone with 0.2% yeast extract) resulted in constant expansion. Future experiments will confirm these results, allow for more in depth statistical analysis, and expand testing our studies with *P. aeruginosa* and the *M. xanthus* rippling motility by twitching only cells.

UP 17 (JA). **Benchmarking the Minimum Electron Beam (eBeam) Dose needed to Achieve Sterility of Space Foods**

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The safety, nutrition, acceptability, and shelf life of space foods are of paramount importance to NASA, especially on long-duration missions. Since food and mealtimes play a key role in reducing stress and boredom of prolonged missions, the acceptability of food in terms of appearance, flavor, texture and aroma can have significant psychological ramifications on astronaut performance. The FDA, which oversees space foods, currently requires a minimum dose of 44 kGy for irradiated space foods. The underlying hypothesis is that commercial sterility of space foods could be achieved at significantly lower doses. Lowering the minimum dose can positively impact the visual appearance, sensory attributes, nutrient content, and overall acceptability of space foods. The focus of this project was to use beef fajitas (a NASA approved space food) and employ eBeam processing to benchmark the minimum eBeam dose required for sterility. Furthermore, accelerated shelf life studies (simulating a 5 years shelf life) to determine the sensory and nutritional qualities after 5 years are planned. The long-term goal of this project is to collect empirical data to enable NASA to petition the FDA to lower the minimum dose from 44 kGy to significantly lower doses.

UP 18 (JA). **CRISPR2 as a Rapid Tool for Small-Scale Epidemiological Investigations in *Enterococcus faecalis***

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Clustered, Regularly Interspaced Short Palindromic Repeats and their associated Cas proteins (CRISPR-Cas) provide prokaryotes with a mechanism for defense against mobile genetic elements (MGEs). A CRISPR locus is a molecular memory of MGE encounters. It contains an array of short sequences, called spacers, that have sequence identity to MGEs. Three CRISPR loci have been identified among strains of the opportunistic pathogen *Enterococcus faecalis*. CRISPR1 and CRISPR3 are associated with the *cas* genes necessary for blocking MGEs, but these loci are present in only a subset of *E. faecalis* strains. The orphan CRISPR2 lacks *cas* genes and is ubiquitous in *E. faecalis*, although its spacer content varies across strains. Because CRISPR2 is a variable locus occurring in all *E. faecalis*, comparative analysis of CRISPR2 spacers may provide information about the clonality of *E. faecalis* strains. We examined CRISPR2 sequences from 228 *E. faecalis* genomes in relationship to subspecies phylogenetic lineages (sequence types; STs) determined by multilocus sequence typing (MLST) and to a genome sequence based phylogeny. We found that specific CRISPR2 sequences are associated with specific STs and branches on the genome tree, indicating that CRISPR2 is a meaningful phylogenetic marker in some cases. We then evaluated 14 *E. faecalis* bloodstream isolates using CRISPR2 analysis and MLST. CRISPR2 analysis identified two groups of clonal strains, an assessment that was confirmed by MLST. We conclude that CRISPR2 analysis, while not a replacement for MLST, is an inexpensive method to assess clonality among *E. faecalis* isolates. Taken together, our analysis suggests that CRISPR2 can be used as a meaningful epidemiological tool for small scale investigations.

UP 20. **Genetic Mapping of Wg/Wnt Pathway Suppressors in *D. melanogaster***

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The fruitfly, *Drosophila melanogaster*, is useful for analyzing developmental processes because of its powerful genetics and simple body plan. The Bejsovec lab uses Drosophila to study the Wingess (Wg)/Wnt pathway. This pathway generates pattern in all animals, and is associated with cancer in humans. Forward genetic screens in Drosophila have revealed critical genes involved in this pathway. Our lab used a sensitized background, wg^{NE2}, to isolate mutations that suppress the weak *wg* mutant phenotype. These suppressors may disrupt gene products that negatively regulate the pathway. Because of the high dose of mutagen used, each suppressor chromosome may carry several lethal mutations, only one of which is likely responsible for the suppression. The goal of my project was to identify the chromosomal location for each lethal mutation. Deficiency lines, each with a defined portion of the 2nd chromosome deleted, were crossed to the suppressor lines to test for failure to complement the lethality. I have screened 808 crosses, and identified 6 lethal regions in 3 different suppressor lines.

Graduate Student Posters – Molecular Microbiology

GP 1 (SK). **Development of a SybrGreen qPCR for the analysis of cluster 2 frankiae in soils**

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Members of the genus *Frankia* are usually characterized as nitrogen fixing bacteria that are capable of forming root nodules with some woody plants in specific host infection groups. Four clusters of frankiae are described with clusters 1, 2 and 3 representing frankiae of the *Alnus*, the *Dryas* and the *Elaeagnus* host infection groups, respectively, and cluster 4 representing generally non-nitrogen fixing frankiae. Information on frankiae is usually obtained by molecular analyses including comparative sequence analyses of rRNA gene fragments or PCR/qPCR based detection and quantification in nodules and soils. While a considerable amount of data is available on the diversity and ecology of frankiae of clusters 1 and 3, information on frankiae of clusters 2 and 4 is scarce. The goal of our research was to develop qPCR based quantification procedures that would allow us to analyze *Frankia* populations in soils. Similar to previous studies on frankiae of clusters 1 and 3, 23S rRNA gene fragments of frankiae of cluster 2 were targeted to develop specific primers for a SybrGreen-based qPCR. The primers were tested for specificity on PCR generated 23S rRNA fragments of pure cultures and uncultured populations in root nodules, reaction conditions optimized, and the optimized protocol used to quantify uncultured *Frankia* populations of cluster 2 in soils with agricultural and silvicultural history.

GP 2 (SK). **Statistical Model of Protein Abundance in *Rhodobacter sphaeroides* 2.4.1**

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Engineering genetic regulatory networks to express a given protein at a desired level in the cell requires a quantitative description of the system. Among others, factors like Shine-Dalgarno sequence (SDS) usage, mRNA expression levels and mRNA stability are important predictors of protein availability in the cell. This study aims to model protein expression in *Rhodobacter sphaeroides* 2.4.1. To this end, a sliding window analysis of the last 100 nt of 5' UTR and the first 100 nt of ORFs for protein-coding genes was done using Vienna. Also, a protein stability index as well as a coding score which estimates the influence of SDS, GC content and codon bias, were calculated for each gene. Then, proteomic data for 84 photosynthetic genes was used to create a multiple linear regression model. Results revealed that on average, mRNAs without SDS have significantly lower MFEs compared to those with SDS. However, there is no significant difference in mRNA stability around the start codon. Also, there is a weak but significant correlation between mRNA expression and protein expression. Lastly, the coding score is a significant contributor in estimating protein abundance, while the level of protein stability seems to be a statistically insignificant predictor in the model. Future studies involve creating a non-parametric regression model with other variables like hybridization affinity between mRNA and the ribosome, and mRNA decay rate added to adequately predict protein abundance in the cell.

GP 3 (SK). **Disruption of the Skin and Gut Mucosal Microbiomes in a Vertebrate Fish Model after Exposure to a Broad Spectrum Antibiotic**

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Antibiotics are heavily employed for human and agricultural animal health; however, this consequentially affects the commensal and symbiotic bacteria that inhabit the organism's natural microbiota. Dysbiosis promotes the onset of many diseases such as Antibiotic-Associated Enterocolitis. This study uses a small fish, *Gambusia affinis*, as a vertebrate model. Samples of two mucosal microbiomes, the skin and gut, were collected before, during, and after exposure to the broad spectrum antibiotic rifampicin. Total plate counts of the skin microbiome dropped quickly at 17.5 hrs of antibiotic exposure, followed by a complete return of total bacteria numbers and rapid appearance (by 38.5 hrs) of rifampicin resistance. This phenomenon is observed as well in the gut, but the response is more delayed than the skin microbiome. Community compositional analysis within the microbiota via 16S rRNA profile sequencing showed that a dominance by the genus *Myroides* occurs during antibiotic exposure in both the skin and gut microbiomes, but it is not stable. After 7 d of antibiotic exposure both microbiomes exhibit altered compositions, which do not return to the untreated structure after 1 week of recovery. The difference between untreated and treated skin microbiomes was further tested functionally via biochemical pathway profiling. The treated community lost 8 (of 20 examined) enzymatic activities and 4 gained compared to untreated. Antibiotic susceptibilities were examined that displayed 3 d after antibiotic exposure the treated microbiome became completely resistant to rifampicin as well as two other drugs. This study illustrates an in-depth analysis of how the microbiota is altered taxonomically and functionally after exposure to an antibiotic in a fish model. Our findings open future investigations, one in particular is the relevant importance of taxonomic organism community distribution versus the biochemical functional components of the microbiota.

GP 4. **Development of novel *Clostridium difficile* genetic tools**

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Genetic tools are important when analyzing the physiology of an organism. *Clostridium difficile* is a significant concern as a nosocomial pathogen. The tools available to genetically manipulate this organism are limited, but include: TargeTron (ClosTron) system, Allelic-Coupled Exchange/CodA systems and Mariner transposition. Anecdotal reports and our own experiences with the allelic exchange systems indicate that these systems do not work as described in the original publications, which prevents the efficient introduction of site-directed, non-polar mutations. The most widely used system is TargeTron system which relies on the re-targeting of mobilizable group II introns and the use of retrotransposable activated markers (RAM). Though RAM markers allow for the easy identification of potential mutants, unfortunately, the TargeTron system only creates insertion mutations which prevent us from introducing more precise mutations into the *C. difficile* genome. Our lab is currently trying to apply other genetic systems to *C. difficile* research with the hope of rapidly introducing site-directed insertions or deletions into the *C. difficile* genome.

GP 5 (SK). **Importance of Decarboxylation in the Biosynthesis of the Lantibiotic, Mutacin 1140**

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Posttranslationally modified (PTM) peptide antibiotics contain numerous modifications which aid in in biosynthesis and bioactivity. Lantibiotics are one such class of postranslationally modified peptide antibiotics. These antibiotics contain numerous modifications important for activity, such as lanthionine rings and dehydrated residues. Mutacin 1140, produced by *Streptococcus mutans* JH1140, is a lantibiotic which has shown promising bioactivity against a broad spectrum of Gram-positive bacteria. Unique to mutacin 1140 and some lantibiotics is the presence of a 2-Aminovinyl-Cysteine at the C-terminus of the antibiotic, which is a result of decarboxylation and lanthionine ring formation. Although a previous decarboxylase deletion mutant resulted in an inactive strain, further characterization of the strain was still required. We hypothesized that this modification is important for the bioactivity of mutacin 1140 and not biosynthesis. Deletion of the *mutD* decarboxylase resulted in a strain that produced an inactive product. Further characterization of this product, indicated a fully modified mutacin 1140 with an intact C-terminal carboxyl group, which we called mutacin 1140-COOH. To confirm if the lack of decarboxylation resulted in the loss of activity, the C-terminus was capped with small organic amines using HOAt/EDC coupling. Conjugation with methylamine restored full activity to the compound. Capping the C-terminus with a fluorophore showed that mutacin 1140 binds to its cell wall target in a patch-like manner. Using purified MutD, we demonstrated *in vitro* activity of the enzyme against a synthesized peptide substrate, but it was not capable of decarboxylating mutacin 1140-COOH. This is the first evidence indicating that decarboxylation occurs in a coordinated manner with other PTM's. This work has yielded a variant of mutacin 1140 that can be further modified to produce a peptide with novel applications and provides a basis for solid phase peptide synthesis of mutacin 1140.

GP 6 (SK). **Deciphering Horizontal Gene Transfer in *Guillardia theta***

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Guillardia theta is a unicellular alga belonging to group Cryptophyta. *G. theta* is unique in many ways, for example, it harbors a plastid with four outer membranes rather than just two membranes as observed in other groups. It also harbors a special remnant nucleus, called nucleomorph, which indicates a red algal ancestry as a consequence of a secondary endosymbiotic event. The nuclear genome of *G. theta* has ~87 Mbp, whereas the chloroplast and nucleomorph have 121.5 Kbp and 551.2 Kbp respectively. We analyzed the composition of the *G. theta* genome using an integrative recursive segmentation and agglomerative clustering method. The largest genome segment cluster thus obtained was identified as harboring the native or ancestral genes. Other clusters harbored genes of atypical composition and are likely acquired through horizontal gene transfer during the course of evolution. The alien clusters were comprised of nucleomorph genes and a small portion of nuclear genes. This is in agreement with previous studies that reported nucleomorph to have arisen from secondary endosymbiosis. The novel alien genes were further analyzed in this study, in context of their contributions to the adaptive evolution of *G. theta*.

GP 7 (SK). **Identification and Characterization of Replication Origin of Two Chromosomes in *Rhodobacter sphaeroides***

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The completely sequenced multipartite genome of *Rhodobacter sphaeroides* 2.4.1 is approximately 4.5 Mb, and it consisted of two chromosomes, Chromosome I (CI), Chromosome II (CII), and five endogenous plasmids. The primary and secondary chromosomes are comprised of 3.18 and 0.94 Mb, respectively, which necessitates synchronized and coordinated replication and segregation during cell division. In order to fully understand the synchronization of cell cycle, the origin of replication of both chromosomes must be identified. This study aims to ascertain and characterize the origin of replication for each chromosome of *R. sphaeroides*. Genomic and bioinformatics tools such as Orifinder, GC-Skew, and Z-curve analysis were used to identify putative origins of replication based on nucleotide disparities and DnaA binding sites along the chromosomal sequences. To further confirm the true origin biologically, these putative origins were amplified and cloned into a pLO1 plasmid. *E. coli* cells containing pLO1 recombinant plasmid were conjugated with *R. sphaeroides*, and resulting transconjugants were further examined for autonomous replication and stable maintenance of these plasmids in *R. sphaeroides*. Results demonstrated that a single true-origin of replication exists on each chromosome in *R. sphaeroides*. Future molecular and biochemical analyses of the two origins will further help elucidate the synchronization of chromosomal replication and segregation processes during the cell division of the multipartite genome.

GP 8 (SK). **Characterization of a Novel Quorum Sensing System in *Sinorhizobium meliloti* Rm2013**

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Sinorhizobium meliloti is a bacterium capable of forming a symbiotic relationship with the legume *Medicago sativa*. Successful bacterial invasion of plant roots results in the formation of a new plant organ, nodules, which house the nitrogen fixing bacteria. Bacterial cell-cell communication, or quorum sensing (QS), functions in a population density dependent manner and is required to coordinate the interactions between bacteria and plant. To date, two QS systems have been observed in *Sinorhizobium meliloti*: the Sin/ExpR and the Tra systems. The Sin/ExpR regulates the *Sinorhizobium*-legume interaction by the production of long chain *N*-acylhomoserine lactones (AHLs). The second QS system, the Tra system, produces short chain AHLs and controls the conjugative transfer of an accessory plasmid, pRME41a. *S. meliloti* Rm2013 is a wild isolate that exhibits a unique AHL production profile composed of all short chain AHLs. This strain appears to have two QS systems: a non-functional Sin/ExpR system and a novel system that produces short chain AHLs. Though Rm2013 does not produce the Sin/ExpR AHLs, it is capable of efficiently invading *M. sativa.* Rm2013's novel system will be identified by screening for AHL deficient mutants. The effects of the presence of this novel system will be assessed in expression studies and with plant assays. Once the genes involved in the Rm2013 novel quorum sensing system have been characterized, we will determine the system's functional role and if the presence of this quorum sensing system confers a selective advantage.

GP 9 (SK). **Transcriptional responses of the nitrogen-fixing bacterium *Bradyrhizobium japonicum* to heat stress**

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Bradyrhizobium japonicum, a nitrogen-fixing bacterium, can establish a mutualistic relationship with the soybean plant. As a result, nodules are formed on soybean roots; thereby the bacteria become bacteroids within nodules to fix atmospheric nitrogen into ammonia. One variable that affects the growth and persistence of *B japonicum* necessary for initiating the infection process and building this symbiotic association is temperature, specifically heat stress. To reveal transcriptional and physiological responses of *B. japonicum* to heat stress, we employed microarray technology and site-specific mutagenesis. The microarray data showed that out of the 8,453 open reading frames, 623 genes were upregulated while 517 genes were downregulated during heat stress (i.e., 42°C). Interestingly, blr7731, encoding inositol monophosphatase (IMPase), is the most upregulated gene (29 fold). To further study the role of IMPase during heat stress, we mutated the blr7731 gene by deletion mutagenesis using the red recombinase system. We also constructed a complementary strain to confirm any mutation-derived phenotype changes. The mutant strain showed a longer generation time compared to the wild type. The survivability test at 42°C also revealed that the mutant was more susceptible than the wild type when exposed to heat stress for 20 min. The IMPase enzyme assay demonstrated not only that the enzyme activity of the wild type was higher at 42°C compared to 30°C, but also that the wild type showed higher enzyme activity than the mutant during heat stress.

GP 10 (SK). **Role of *CSA1*, *CSA2* and *PGA7* in the Secondary Utilization of Hemoglobin-derived Iron by *Candida albicans***

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Candida albicans, though normally a commensal member of the human microbiota, is an opportunistic pathogen. *C. albicans* is the most frequent cause of nosocomial fungal bloodstream infection, which is associated with a 49% mortality rate. A major factor contributing to the virulence of pathogenic microorganisms is the ability to acquire iron from host tissues. *C. albicans* exploits host hemoglobin (Hb) as an iron source using Hb-binding proteins like Rbt5p and Rbt51p, members of a protein family containing CFEM (common in several fungal extracellular membrane) domains. Knockout of these genes severely impaired Hb utilization. However, a double knockout strain, KC100, was able to internalize and use Hb at higher Hb concentrations. We hypothesized that other members of the CFEM family, *CSA1*, *CSA2* and *PGA7*, play a secondary role in Hb utilization and are up-regulated when Hb is the sole iron source. KC100 and control cells were grown in iron-depleted medium supplemented with Hb. Semi-quantitative gene expression analysis demonstrated that while *PGA7* expression was similar in both strains, *CSA1* expression was increased in KC100 cells relative to control. *CSA2* was expressed only in KC100. These results support our hypothesis that Csa1p and Csa2p may compensate for lack of Rbt5p/Rbt51p. Future studies will determine the specific role these proteins play in Hb utilization by *C. albicans*.

GP 11 (SK). **Clade-specific modification systems in commensal and clinical *Enterococcus faecium***

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Enterococcus faecium (Efm) is an opportunistic pathogen that colonizes the human intestine. The species is composed of two phylogenetic clades, A and B. Clade A consists primarily of multidrug resistant clinical isolates, while Clade B consists primarily of fecal isolates. Restriction-modification (R-M) systems act as barriers to genetic exchange by allowing bacteria to distinguish self from non-self DNA. Little is known about R-M in Efm. We hypothesize that R-M barriers exist between Clades A and B, contributing to the observed population structure. We used bioinformatics analyses to identify clade-enriched R-M systems in 73 Efm strains. This revealed different Type I R-M systems enriched in Clades A and B. SMRT sequencing was performed on Clade A representative 1,231,502 and Clade B representative 1,141,733 and identified different Type I modification motifs in each of the strains, providing support for our hypothesis. In order to show that the methylation patterns detected by SMRT sequencing were due to the activity of the Type I R-M systems identified by our bioinformatics, *E. faecalis* (Efs) was used as a heterologous expression host. The predicted R-M systems were cloned into Efs strains using single-site integration. Expression of the genes was then confirmed prior to SMRT sequencing. The results of the SMRT sequencing show that Efs acquired methylation patterns identical to the ones identified in Efm. This shows that the Type I systems methylate the genome of Efm strains in unique ways. Future work will focus on identifying a function for these methylation patterns in either genome defense or gene regulation.

GP 12 (SK). **Towards more robust metagenome profiling**

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Characterization of the microbial diversity of a microbiome begins with the sequencing of its DNA complement, namely, the metagenome. Methods for the taxonomical profiling of metagenomic sequences rely on the microbial sequence database, which however represents a tiny fraction of microbes dwelling the earth. Therefore, attempts are made to systematically profile the metagenomic sequences, beginning with the species level towards higher taxonomic level until a “match” with the sequence of interest is found. Construction of signature models of sequenced microbial genomes underlie the current state-of-the-art in the field. We posit that these “static” models are inherently limited in exemplifying the microbial dynamism that shapes the genomes, resulting in chimeras with segments of different ancestries or origins. We, therefore, propose a segmental genome model, where a genome is represented by an ensemble of signatures derived from segments of apparently different ancestries or origins. By incorporating segmental signature models within a variable order Markov model framework for scoring metagenomics sequences, we could achieve a more robust metagenome profiling. The proposed method was assessed on synthetic and real metagenomes, and compared with the popular methods for metagenome classification. This new approach for metagenome characterization will be presented with a focus on future directions in this meeting.

GP 13 (SK). **Intestinal Mucus as a Maturation Factor for Dendritic Cells**

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Background: Gastrointestinal (GI) epithelial cells are covered by a thick layer of mucus which acts as a barrier, shielding the host epithelium from the luminal contents. Mucus consists of highly glycosylated mucin proteins, primarily MUC2. Intestinal dendritic cells (DCs) are professional antigen presenting cells capable of extending projections through the epithelium to sample antigen from the mucus layer and the lumen. In this way, DCs are capable of interacting with antigens and metabolites from the intestinal microbiome. DCs act as sentinels in the gastrointestinal tract by capturing and processing antigen, undergoing maturation, and regulating an immune response via migration to secondary lymphoid organs or production of cytokines. Previous studies have demonstrated that DCs are capable of extending processes through the mucus layer or acquiring antigen from antigen-presenting goblet cells. Little is known about the effect of mucus recognition by DCs and how this affects interaction with the microbiome. **Methods**: To assess the role of mucus in DC maturation and cytokine production the human monocyte cell line THP-1 were matured to DC by stimulation with IL-4 and GM-CSF. Alternatively THP-1 cells were stimulated with crude human stool mucus or the mucus producing human cell line HT29-MTX secreted mucus. Mucus-stimulated THP-1 cells or IL-4, GM-CSF DCs were subjected to bacterial lipopolysaccharide (100 ng/ml LPS) for 1 hrs. Cells were analyzed by flow cytometry for mature DC markers CD11c and HLA and supernatant was examined for TNF production by ELISA**. Results**: Flow cytometry data demonstrated that THP-1 cells could be stimulated towards a DC-like phenotype with IL-4, GM-CSF based on increased expression of CD11c and HLA. Introduction of LPS resulted in an enhanced secretion of TNF in DCs compared to unstimulated THP-1 cells (8.6-fold higher TNF). Interestingly, the presence of either stool or HT29-MTX cell-derived mucus produced an increase in the $CD11c^{hi}HLA^{lo}$ and $CD11c^{hi}HLA^{hi}$ DC populations from unstimulated THP-1 cells. This increased DC population was also observed in gross morphology as mucus stimulated THP-1s presented with increased diameter and projections. The ability of DCs to uptake bacteria in the presence of mucus was assessed using fluorescent carboxyfluorescein diacetate succinimidyl ester (CFDA-SE) labeled bacteria. HT29-MTX cell-derived mucus did not alter TNF production in unstimulated or LPS-treated THP-1 or in unstimulated or LPS-treated DCs. However HT29-MTX mucus did increase the $CD11c^{hi}HLA^{hi}$ population in mucus and LPS-treated. **Conclusions**: Together, this data indicates that intestinal mucus may act as a DC maturation factor which has far-reaching implications for immune interactions with the host and the intestinal microbiome.

Graduate Student Posters – Gram Positive Microbiology

GP 14 (SK). **Re-examining the germination phenotypes of several *C. difficile* strains**
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C. difficile is a Gram-positive, spore forming, anaerobic bacillus, known for causing nosocomial diseases associated with antibiotic use. Due to the strictly anaerobic nature of *C. difficile* cells, only spores can survive for extended periods of time outside the host. Inside a host, spores germinate to the vegetative form which produces toxins that elicit the primary symptoms of the disease. Previous work by our lab, and others, has shown that *C. difficile* spores germinate *in vitro* in response to bile acids and glycine. Previous work in our lab demonstrated that cholic acid-derivatives initiation spore germination whereas chenodeoxycholic acid-derivatives (CDCA) act as competitive inhibitors of cholic acid. Recently, some *C. difficile* clinical isolates have reported to germinate in media alone or to not be inhibited by CDCA. Here, we aim to study the variability in the specificity of the reported clinical strains to explain the behavior by observing the apparent affinity of the bile acids for the *C. difficile* spore, the abundance of DPA in the spore and levels of germination specific proteins (the bile acid germinant receptor, CspC, the germination specific protease, CspB, and the cortex hydrolase, SleC). Upon re-examining these strains we determined that all strains tested had the requirement for bile acids to stimulate germination, are inhibited by CDCA and exhibit variability in the germinant receptor complex. Finally, the in-depth analysis of these strains led to the proposal of an inhibitor function for the bile acid germinant receptor, CspC, during *C. difficile* spore germination.

GP 15 (SK). **Regulation of Histamine Production by Lactobacillus Reuteri by Other Bacterial Metabolites**
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Amino acid metabolism by the gut microbiome is critical for human health. The decarboxylation of L-histidine to histamine by *Lactobacillus reuteri* reduces the production of the pro-inflammatory molecule, TNF. This work examines histamine and histidine metabolism by *L. reuteri*, as well as the effects of microbial histidine metabolism on the host. Supernatants from bacterial cultures with $^{13}C_6{}^{15}N_3$ L-histidine were processed and NMR was used to identify novel bacterial metabolites. We have determined that the addition of carnosine (β-alanyl-L-histidine) in the media significantly increases histamine production. We have also determined that media concentrations of acetate and folate have effects on viability, histamine production and TNF production by THP-1 cells treated with bacterial supernatants. Currently, we are working to determine the mechanism by which carnosine, folate, and acetate affect histamine production. Our plans include the oral administration of $^{13}C_6{}^{15}N_3$ L-histidine to mice and co-culture of *L. reuteri* with human ileal enteroids. These studies will determine the effects of *L. reuteri* and histidine/histamine metabolism on the human intestinal epithelium. Together the data will facilitate understanding of bacterial metabolites produced by this probiotic and their effects on the mammalian intestine.

GP 16 (SK). **Chlorhexidine induces VanA-type vancomycin resistance genes in enterococci**

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Chlorhexidine is a bisbiguanide cationic antiseptic that is routinely used in clinical practice. Daily chlorhexidine gluconate (CHG) bathing is a mechanism for hospital infection control. After bathing, CHX can persist on the skin of patients, with a concomitant decrease in antimicrobial activity. *Enterococcus faecium* (Efm) is a gram-positive bacterium that natively inhabits the human gastrointestinal tract and is among the leading causes of hospital-acquired infections. It is likely exposed to chlorhexidine at sub- and supra-inhibitory concentrations; however, the impacts of this stress on Efm are unknown. We used Illumina RNA sequencing to investigate how Efm responds to MIC-level CHG exposure. Among the genes up-regulated ≥10-fold after 15 minutes CHG exposure were those encoding VanA-type vancomycin resistance (*vanH$_A$AX$_A$* and *vanY$_A$Z$_A$*). We determined that this gene expression response to CHG is not strain- or species-specific by querying other VanA-type Efm and *E. faecalis* clinical isolates using quantitative reverse transcription-PCR. A fusion of the *vanH$_A$* promoter with *lacZ* was responsive to sub-MIC CHG in Efm, as was production of the VanX$_A$ protein. Using deletion analysis, the phenomenon was found to be VanR$_A$-dependent. Finally, VanA-type VRE are more sensitive to ceftriaxone antibiotic in the presence of sub-MIC chlorhexidine. We conclude from our results that MIC and sub-MIC CHG induces VanA-type vancomycin resistance gene expression and show synergism with ceftriaxone against VanA-type VRE. Overall our results indicate that the impacts of chlorhexidine exposure on Efm should be monitored.

GP 17 (SK). **Identifying the glycine germinant receptor involved in *C. difficile* spore germination**

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Clostridium difficile is a Gram-positive, spore-forming bacterium and is a leading cause of healthcare associated diarrhea. Antibiotic treatment is the greatest risk factor for *C. difficile* infection (CDI) because antibiotics alter the normal ecology of the gut microbiota. Due to the strict anaerobic nature of the vegetative form, *C. difficile* cannot survive in the aerobic environment outside a host. Thus spores are important for transmission between hosts. Once in a host, spores much germinate to the vegetative form in order to cause disease. *In vitro C. difficile* spore germination is triggered by bile acids (e.g., taurocholic acid [TA]) and glycine. Recently, we identified the bile acid germinant receptor. The receptor with which glycine interacts is unclear but, based on previous data, we hypothesized that i is localized near the spore cortex. To identify the glycine germinant receptor, we used chemical mutagenesis to introduce random mutations into *C. difficile* cells and the cells were allowed to form spores. These spores were germinated in the presence of TA and betaine (a glycine analog that normally is not a co-germinant). We predict that any spore that can germinate in the presence of TA and betaine could have a mutation in the glycine receptor allowing it to recognize betaine as a co-germinant. By far, the most frequent phenotype isolated from this procedure spores that germinate in presence of TA only without the requirement of glycine. Sequencing the known germinant receptor *cspC* had no mutation, suggesting that mutation in some other gene is responsible for the TA only phenotype. Currently, we are working to identify the mutations leading to this phenotype.

GP 18 (SK). **In vitro efficacy of hospital disinfectants on the spores of *Clostridium difficile***

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Background: *C. difficile* is a leading cause of hospital acquired infection in the US and worldwide. Ubiquitous presence of *C. difficile* spore at hospital environment coupled with inadequate knowledge regarding disinfectant efficacy is a challenge to the hospital infection control practice. The objective of this study was to investigate the in vitro efficacy of common hospital disinfectants on the spores
Materials & methods: Nine EPA registered hospital grade chemical disinfectants with active ingredients as formaldehyde, ortho-phthaldehyde, hydrogen peroxide, Iodophors, phenol, sodium hypochlorite, ethanol, chlorine dioxide and Hypochlorous acid were tested against 10 clinical strains of *C. difficile* isolates. Both high and low concentration spores were exposed to liquid hospital disinfectants based on recommended contact time or 30 minutes, washed, plated on blood agar plates and anaerobically cultured to count the colony forming units (CFU). All the experiments were done in presence and absence of organic substrate (BHI) with 2 replicates of each study.
Result: At full strength of disinfectant and low concentration of spores, there was 2 to 3 log10 reduction in CFU count for hydrogen peroxide, ortho-phthaldehyde, sodium hypochlorite, formaldehyde and hypochlorous acid containing disinfectants. This effect was sustained at half and one-fourth dilution for all except hydrogen peroxide containing disinfectant. The rest disinfectants were not effective with some increasing the spore count by 24.1 to 1314%. On further exposure of effective disinfectants to high concentration spores, there was approximately 4 to 6 log10 reduction in CFU count. In presence of organic substrate, sodium hypochlorite, ortho-phthaldehyde and chlorine dioxide containing disinfectants were rendered ineffective. Hydrogen peroxide and formaldehyde containing disinfectants were effective irrespective of organic substrate. There was also some inter strain variation with respect to disinfectant activity.
Conclusion: Hospital grade disinfectants are mostly effective against *C. difficile* spore with more than half rendered ineffective in presence of organic substrate and some facilitating microbial growth. This result may have implication on hospital cleaning protocols.

GP 19 (SK). **Identifying alternative mechanisms conferring daptomycin resistance in E. faecium**

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Viewed as a drug of last resort, Daptomycin (DAP) has been prescribed off label to combat Vancomycin-resistant-Enterococci infections. Unfortunately, many cases have developed resistance to this last line of defense and it is paramount that the mechanisms behind this resistance be understood. Our lab identified the main mode of resistance acquired in *Enterococcus faecalis*: mutations in *liaFSR* which remodel the cell membrane to divert DAP away from the division septum. Unlike *E. faecalis*, *Enterococcus faecium* account for over 70% of Enterococcus infections, are intrinsically more resistant to a variety of antibiotics, and are much more difficult to eradicate. *E. faecium* also develops different resistance mechanisms to various antibiotics than what is observed in *E. faecalis.* These differences show that what is true for *E. faecalis* may not be definitively applied to *E. faecium.* While DAP resistant clinical isolates of *E. faecium* have been observed to acquire mutations in *liaFSR*, many clinical cases have shown that *E. faecium* achieves resistance through an additional pathway involved in cell wall turnover and cell division: *yycFG.* How these two pathways function in *E. faecium* remains unknown. Our collaborators isolated 2 separate *E. faecium* strains considered DAP tolerant, one of which harbors a *yycG mutation,* and knocked out *liaR* (the most commonly mutated gene in resistant isolates) from their genomes. The lack of *liaR* caused both strains to become DAP hypersensitive. By adapting these two strains to high DAP concentrations and sequencing the genomes throughout adaptation, I will be able to determine when mutations in alternative pathways appear and how they confer DAP resistance. These alternative mechanisms can then be used as drug targets to be used in combination with *liaR* inhibitors.

GP 20 (SK). **Control of Iron Regulation and Uptake by Quorum Sensing in *Sinorhizobium meliloti***

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Sinorhizobium meliloti is a gram negative soil bacterium that establishes a symbiotic association with the legume *Medicago sativa*. *S. meliloti* uses Quorum Sensing (QS), a cell-cell based communication mechanism for the successful invasion of plant root nodules. The Sin/ExpR QS system controls the process of symbiotic nitrogen fixation which requires an interplay of many factors; one of which being iron. At physiological pH iron is poorly soluble and unavailable. Therefore, microbes have adapted strategies for iron acquisition; including the use of siderophores, secreted under iron limited conditions. *S. meliloti* produces rhizobactin 1021 as its predominant siderophore. Recent studies identified RirA, a regulator in *S. meliloti* that controls iron responsive genes under iron limitation. However mutants of *rirA* formed effective root nodules suggesting that *rirA* might not be involved in regulating the availability of iron during the symbiotic nitrogen fixation. In addition, the role of *rirA* in siderophore mediated iron uptake still remains unclear. Studies conducted in our laboratory on plants inoculated with a wild type QS strain *vs*. a QS mutant under low iron conditions show that, a wild type QS strain is more efficient in invading the root nodules than a QS mutant. In addition, the genes involved in the synthesis of rhizobactin 1021 were found to be differentially regulated in a QS wild type vs. a QS mutant. This suggest that the presence of a QS system enhances the ability of *S. meliloti* to cope with iron stress and fix nitrogen for the plant.

GP 21 (SK). **Salmonellae in the Intestines of *H. plecostomus* in the San Marcos River**

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Heavy rainfall events have been associated with outbreaks of many waterborne diseases including salmonellosis. Salmonellosis is caused by members of the genus *Salmonella* that can enter water systems through sewage contamination, runoff after heavy rainfalls, or flow-through channels through manure fields after heavy rains or flooding. Currently, salmonellae are not closely monitored in regards to water quality. In this study, *Hypostomus plecostomus*, an invasive, algae consuming fish, was sampled from the San Marcos River (San Marcos, TX), the intestines analyzed for the presence of salmonellae by quantitative real-time polymerase chain reaction (*q*PCR) after semi-selective enrichment, and results related to precipitation for the river area. Salmonellae were detected in the intestines of *H. plecostomus* in 40-100% of the fish, with higher percentages after precipitation. Salmonellae were not consistently detected in environmental samples (i.e. water and sediments). This leads us to believe that *H. plecostomus* is ingesting salmonellae through their food sources and that the amount of salmonellae present in those food sources may be increasing after large rainfall events but is not dependent on these events. Salmonellae isolated from positive samples are characterized by repetitive polymerase chain reaction (rep-PCR), evaluated for unique banding patterns, and uniqueness confirmed using the Agilent 2100 Bioanalyzer.

GP 22 (SK). **Whole-genome assembly of multi-resistant *Pseudomonas aeruginosa* isolate**

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P. aeruginosa is the leading opportunistic pathogen that causes life threatening infections in patients with cystic fibrosis. Its pathogenicity is due to multitude of virulence and antibiotic resistance mechanisms encoded in its genome. We aim to sequence the genome of a *P. aeruginosa* isolate CDN118 obtained from a bacteremia patient. CDN118 is extremely resistant to clinically used antibiotics. The CDN118 genome was sequenced using the Illumina and PacBio sequencing platforms. The *de novo* assembly using the Illumina reads was done using CLC Genomics software. SMRTPortal software was utilized to create a hybrid assembly by using the long PacBio and Illumina draft genome. The PacBio reads were used to close the gaps of the Illumina de novo assembly. We identified phage insertions and several genomic islands harboring antibiotic resistant genes. To verify chromosomal inversions, specific primers were used to amplify the ribosomal RNA clusters using PA14 as a control. This study will help understand the pathogenicity of this strain and direct the course of treatment.

GP 23 (SK). **Role of Glucose metabolism in swarming motility of *Escherichia coli.***

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Swarming is a poorly understood form of flagella-dependent movement of bacteria on semisolid media. Glucose is present in swarm media at a concentration of 0.5% and its absence does not support swarming in E. coli. High intracellular cysteine is needed for swarming in S. enterica. Glycolysis provides intermediates for synthesizing cysteine and hence is proposed as one of the important functions of glucose. We examined the glucose requirement in model genetic organism *E. coli*. The first branch of glycolysis was examined by analyzing deletion mutants *pfkA* (phosphofructo kinase) and *zwf* (glucose 6-P dehydrogenase). *pfkA* failed to swarm while *zwf* swarmed uninhibited. This suggests the importance of glycolysis instead of oxidative arm of the pentose pathway, opening up a new approach to study the role of glucose. Other branches in glycolysis may be explored to study the role of intermediates of the pathway. Mutants lacking enzymes at 3-P-glycerate (for serine-cysteine synthesis), pyruvate (for alanine synthesis), and acetyl-CoA (for TCA cycle vs sub level phosphorylation) were also analyzed. Only *SerC* (serine synthesis) showed inhibited swarming. We attempted to replace glucose with a single compound or combinations. The combined effect of serine and pyruvate on swarming suggests that glycolysis is needed mainly for cysteine as well as pyruvate synthesis. Analysis of the media further showed that spent swarm media was acidic while media void of glucose was alkaline. These results indicate multiple functions of glucose in swarming motility in *E. coli.*

GP 24 (SK). **Antibiotic Resistant Bacteria in an Urban Watershed**

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Methods for treating antibiotics (ABs) in wastewater are undeveloped, and may place selective pressure on exposed bacteria for the proliferation of AB resistant traits. To characterize resistance of surface water bacteria in an urban stream setting, *E. coli* isolates and total heterotrophic bacteria (THB) were cultured from 6 sites in the Carter Creek watershed of College Station, TX, and evaluated for resistance to select ABs. THB were cultured on R2A amended with selected ABs, and compared to culturable populations on AB-free media. *E. coli* were isolated using EPA M.1603 and tested for resistance to 7 ABs using the Kirby-Bauer method. THB counts produced a wide variation in the ratio of viable cultures between control and amended media, likely due to variability in the watershed. All *E. coli* isolates displayed susceptibility to imipenem; however, the majority (82%) showed resistance to cephalothin, and lesser resistance to each remaining AB tested. A substantial fraction (12%) of *E. coli* isolates were multidrug resistant, and nearly all were obtained downstream of a wastewater treatment plant. Initial results suggest the possibility of a variable resistance profile moving downstream through the watershed, and may indicate an influence of urbanization on bacterial resistance characteristics.

GP 25 (SK). **Metabolomic Analysis of the response of Escherichia coli O26 to acid stress**

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Non O157 Shiga toxin producing E.coli strains such as E. coli O26 are being implicated in a number of foodborne illnesses. It is well known that acid preservation of food is an age-old practice in the food industry, however, the continued cases of illnesses in high acid foods begs the question as to why pathogens such as E.coli O26 can survive acid stress. Though there are number of studies related to the genomic responses during stress, our understanding of the metabolites produced during acid stress is still rudimentary. Our hypothesis was that metabolomics analysis of E.coli O26 during acid stress will reveal hitherto unknown metabolic responses. Escherichia coli O26 cells were exposed for 24 hours in a pH buffer of 3.6 to evaluate its response to acid stress. Although the acid caused significant reduction in culturable cells (as evidenced by inability to grow in culture media), fluorescent microscopy revealed that a large percentage of the cells were viable (based on membrane integrity). An untargeted metabolomic analysis was performed .A total of 292 metabolites unique to acid stress were identified. The most significant impact of acid stress was observed in the metabolite phosphate, stearic acid and hydroxylamine. All the metabolites that were statistically different ($p<0.01$) in the two treatment groups were used in metabolic pathway analysis. The results indicate that 21 metabolic pathways are triggered as a result of acid stress, proving that E.coli O26 is still metabolically active. The key metabolic pathways were identified to be; pepditodglycan biosynthesis, purine metabolism, D-Glutamine and D-glutamate metabolism, Nitrogen metabolism, Biosynthesis of unsaturated fatty acids and Inositol phosphate metabolism.
Keywords: Acid stress, E.coli O26, Metabolomics, Shiga toxin producing E.coli

GP 26 (SK). **Effect of polyamines on swarming motility in *Escherichia coli***
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Swarming is a surface motility behavior exhibited by some bacteria including *Escherichia coli, Proteus mirabilis* and *Salmonella enterica*. Basic features of swarming include metabolic differentiation, hyper-flagellation, cell elongation and sometimes oscillatory movement. Results from our lab suggest that swarming motility is a behavior that responds to various environmental factors. One of these factors is polyamines. Polyamines are aliphatic cations known to interact with nucleic acids and phospholipids playing a role in gene regulation by affecting chromosomal and ribosome structure, mRNA interactions and protein and nucleic acid elongation rates. Previous studies suggest the polyamine putrescine is required for swarming in *P. mirabilis* and spermidine may be required in *E. coli*. We examined various polyamine deficient mutants of *E. coli* and found that putrescine, but not spermidine, is required for swarming in *E. coli*. The effects of exogenous polyamines support these conclusions. We propose that polyamines control flagella synthesis and metabolism. This work can help present a basic understanding on swarming and how bacteria behave in response to various environmental factors and how they regulate their behavior.

GP 27. **Fitness of Escherichia coli when in Mixed Culture**
Avry Stolzman
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Escherichia coli coexists with many different species, such as Enterococccus faecalis, in the gastrointestinal tract of many animals. However, it may encounter other organsims, such as Vibrio cholera, during some diseases. There is currently little known about the mechanism of how E. coli is able coexist under these conditions. Using a genetic screen and mutant collection of E. coli, we will be able to observe specific genes within E. coli that effect its fitness when in mixed culture. We will grow the organisms up together and observe the fitness of the E. coli mutant. We will then identify a specific gene or pathway essential to E. coli's survival when in mixed culture. This could lead to future research that would allow for the alternative treatment of E. coli without the use of antibiotics.

Graduate Poster – Others

GP 28 (SK). **Sandy Loam Soil Resistance and Resilience to Biodiesels**
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This study focuses on different base oils used in drilling fluids and compares the effects of petroleum diesel and three biodiesels on soil health. In this study, microbial communities were tracked for 180 days to find the degradation rate of contaminants and the effects on the diversity, physiology and function of soil microbial communities. Results suggest the physiology of microbial community in different treatments may return to normal level within 6 months; and that biodiesels are not statistically different from petroleum diesel in adverse impacts on soil microbial physiology. Site restoration potential was evaluated by seed germination and plant growth over 35 days. ANOVA results show all groups have similar plant germination rates while diesel treatments exhibit significantly shortened total plant length. One biodiesel, castor methyl ester, has a statistically similar yield of plant dry weight compared with plants grown in the untreated soil control. Our results suggest that the chemical structure of biodiesels may determine whether biodiesels are environmentally benign, and that biodiesels should not be considered as a group to be environmentally harmless.

GP 29 (SK). **Biochar feedstock type has diverse effects on CO_2, N_2O and CH_4 flux in a laboratory incubation study**
Swastika Raut, and Sanghoon Kang
Department of Biology, Baylor University

Biochar has been demonstrated as an effective soil amendment and greenhouse gas (GHG) mitigation tool. However, the degree and mechanism of reduction in GHGs emission upon biochar addition remains poorly understood. Here we perform a laboratory incubation study to assess the effect of biochar feedstock type on CO_2, N_2O and CH_4 flux using gas chromatography. Numerous studies have reported reduction in GHG emission upon biochar addition to soil. Based on previous research on biochar, we hypothesized that biochar application would reduce the flux of all three GHGs. In contrast the cumulative CO_2 flux increased with mesquite biochar treatment whereas switchgrass biochar and control treatments showed similar response in CO_2 flux. Furthermore, the overall CH_4 and N_2O flux were indistinguishably low for all three treatments. Even though woody biochar tends to reduce CO_2 emission, fresh mesquite had higher CO_2 flux than aged switchgrass biochar. Fresh biochar could potentially add more labile carbon to soil and increase CO_2 flux through priming effect. No significant effect of biochar feedstock type on CH_4 and N_2O flux could be due to the lab incubation conditions not optimal for denitrification (soil moisture <60% WFPS). Our results suggest a further need of systematic investigation involving multifactorial treatments to examine the degree and mechanism of various biochar feedstocks on GHG emissions. In order to evaluate the microbial response to GHG emission, we are currently investigating functional genes using qPCR.

GP 30 (SK). **Estrogen and Triclosan Effects on Soil Microbial Diversity**

Ezinne Osuji, Deborah Carr, Ph.D., Eduardo Velo
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Pharmaceutical and personal care products (PPCPs) associated with land farming of municipal waste water effluent potentially may persist in soil and alter soil microbial community processes. Estrone (E1) and the anti-microbial agent, Triclosan, were examined for their potential to persist and disrupt soil microbial community function. Community level physiological profile was examined using BIOLOG® EcoPlates™. Soil with decade-long exposure to these chemicals and naive soil (not previously exposed), was spiked with estrone, triclosan, and a 1:1 mixture of estrone: triclosan, incubated for 90 days and analyzed for the ability of their microflora to utilize ecologically relevant carbon sources. Control samples consisting of unspiked exposed and unexposed soil were included in the analysis. At day 90, there was a significant increase in substrate activity and substrate richness in all treatments except in the naive soil treated with a mixture of the estrone and triclosan. Principal component analysis (PCA) of the data showed the community utilized different carbon substrates at day 90 whereas they had exhibited similar substrate utilization at day 0. Microbial communities in the previously exposed soil samples adapted more quickly to the contaminants.

GP 31 (SK). **Identifying the Cellular Target of the Novel Antifungal Compound Occidiofungin**

Akshaya Ravichandran, and Dr. James Smith
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Due to the rampant occurrence of strains of fungi that are resistant to the common classes of antifungals, such as azoles, echinocandins and polyenes, there is a rising need to identify novel antifungal agents. Occidiofungin is a non-ribosomally synthesized glycolipopeptide, produced by *Burkholderia contaminans,* which is a soil bacterium. Occidiofungin has a base mass of 1200 Da and consists of eight residues which include standard and non-standard amino acids. The compound is rapidly fungicidal against a wide spectrum of fungal species and has been shown to cause minimal toxicity in murine models. Previously, we demonstrated that occidiofungin triggers apoptosis in target fungal cells. The exact molecular target that the compound interacts with to cause programmed cell death is yet unknown. In this study, we attempt to use click chemistry to track the localization of occidiofungin inside a fungal cell. Occidiofungin, functionalized with a reactive alkyne group is used to treat the fungal cells. Subsequently, the compound is probed using an azide functionalized fluorophore to detect areas of localization in yeast. Colocalization with organelles and time course tracking of occidiofungin within the cells were done. A unique pattern of localization within the yeast cells was observed. Utilizing the alkyne functionalized occidiofungin, pulldown assays were carried out to detect proteins that bind to occidiofungin. LC-MS/MS was used to detect proteins pulled down using alkyne functionalized occidiofungin and comparing to those pulled down using unmodified occidiofungin to eliminate random binding. Further studies such as comparing the sensitivity of null mutants and over expression mutants of the protein hits to occidiofungin will be done.

Postdoctoral Fellow Oral Presentation

Understanding the Mechanism of Action for SMT19969, a Novel Treatment for *C. difficile*

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SMT19969 is an antimicrobial with targeted activity against *C. difficile* (CD) currently undergoing clinical trials whose mechanism of action (MOA) has not been fully elucidated. This study aimed to assess the pharmacologic activity of SMT19969 and determine a potential MOA.

Time-kill curves were performed using the strain R20291 (BI/NAP1/027) at supra- (4x and 40x) and sub-MIC (0.125x, 0.25x, and 0.5x) concentrations of SMT19969. Following treatment, CD cells were collected for CFU counts, toxin A and B production, and morphologic changes using scanning electron and fluorescent microscopy. Caco-2 cells were co-incubated with SMT19969-treated CD growth media to determine the effects on host inflammatory response.
Treatment at supra-MIC concentrations of SMT19969 resulted in a reduction of vegetative cells over 72 hours (4 log difference, $P<0.01$) compared to controls without effect on spore formation. These results correlated with a 75% and 96% decrease in toxin A and B production, respectively ($P<0.05$, each). At 0.5X MIC, toxin A and B production were reduced by 91% ($P<0.01$) and 100% ($P<0.001$), respectively, which resulted in an attenuation in inflammatory response as measured by a 74% reduction in IL-8 release compared to controls ($P<0.05$). 0.5xMIC treated cells formed filamentous structures and were drastically longer in size than control cells. Following fluorescent-labeling, we determined that the cell septum was not forming in sub-MIC treated cells, yet the DNA was dividing.
These results suggest that SMT19969 has potent killing effects on CD that reduces toxin production and attenuates human inflammatory responses. SMT19969 also elicits effects on cell division, which likely abrogates these downstream effects.

Faculty and Post-Doctoral Posters

FPP 1. *Lactobacillus reuteri* Alters Tumor Adherent Mucins and Enhances Chemotherapeutic Susceptibility

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Dysregulation of mucin homeostasis, especially cell-adherent mucins, is a hallmark of several cancers. Mucinous adenocarcinomas, a histological subtype of colorectal cancer, exhibit increased adherent (MUC1, MUC3, and MUC4) and secreted (MUC2 and 5AC) mucus. Mucinous adenocarcinoma patients typically have a poorer prognosis than other non-mucus secreting cancers due to resistance of several chemotherapeutic interventions. The biochemical and stoichiometric nature of mucins are predicted to protect mucinous cancer cells from chemotherapeutic agents. Several members of the gut microbiota are capable of altering mucin production and may represent potential treatment to increase mucinous adenocarcinomas chemotherapy susceptibility. The mucus producing adenocarcinoma cell line HT-29-MTX-E12 which expression both adherent and secreted mucins was incubated with conditioned media from 16 human gut microbiota species. Analysis of *MUC* expression revealed that several *Bifidobacterium* spp., *Clostridium* spp., and *Lactobacillus* spp. were capable of decreasing adherent and secreted expression. Of the *Lactobacillus* members, *L. reuteri* ATCC 6475 was capable of altering total adherent mucins, as determined by alcian blue and biotinylation. To assess whether bacterial-specific changes in mucin expression correlated with increased cancer cell susceptibility to drug treatment, HT-29-MTX-E12 cells were incubated with conditioned media followed by the chemotherapeutic agent 5-FU. Metabolic activity and mucus production were assessed via resazurin assay and Alcian Blue staining. *L. reuteri* conditioned media increased susceptibility to 5-FU treatment resulting in significant decreases in cancer cell viability. This susceptibility correlated with decreased adherent mucins. This effect was independent of pH or lactate. The *L. reuteri* secreted factor was found to be a >10 kDa heat stable protein. To establish the ability of *L. reuteri* to promote tumor 5-FU susceptibility *in vivo*, C57BL/6J-*Apc*^{Min}/J mouse tumors were excised and *ex-vivo* treated with *L. reuteri* conditioned media followed by 5-FU. Addition of conditioned media resulted in enhanced susceptibility in male and female intestine tumors as assessed by resazurin. C57BL/6J-*Apc*^{Min}/J mice colonized with *L. reuteri* bacteria had significantly decreased adherent Muc1 tumor masses, consistent with the HT29-MTX cell line data. Together this data suggests that bacterial-induced modifications of cancer-driven mucus in combination with traditional chemotherapeutic agents may provide new therapeutic strategies for the treatment of mucinous adenocarcinomas.

FPP 2. **Characterization of Antimicrobial Peptides for Treatment of Pseudomonas aeruginosa**

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Background: Dental infections including gingivitis and periodontitis are major dental issues in the military. In Iraq, 30% of dental emergencies involved gingivitis or periodontitis. *P. aeruginosa* is one bacteria causing dental infection. Because traditional periodontal treatment is time consuming and requires repeated dental visits, innovative antimicrobial and antibiofilm compounds need to be discovered for the effective prevention of the formation of biofilm and eradication of existing bacterial biofilms in dental plaques. Antimicrobial peptides (AMPs) have novel modes of antibacterial activity which allows effective treatment of biofilm and drug resistant bacteria, emerging as an attractive research area.
Objective: The objectives of this study were to screen the antimicrobial activity of AMPs against *P. aeruginosa,* and test their stability in saliva and serum for the clinical application in dental infection.
Materials/Methods: *P. aeruginosa* strains, PA14, PA15, PA16, ATCC BAA-2110, ATCC 27853 and PAO1, were purchased from ATCC. Two AMPs, 1018 (VRLIVAVRIWRR) and Oncocin 10 (VDKPPYLPRPRPPRRIYNR) were synthesized by 9-fluorenylmethoxycarbonyl/*tert*-butyl solid-phase peptide synthesis method with >98% purity and acetate salt form. Antimicrobial activities of AMPs were assessed using the minimal inhibition concentration (MIC) values in 1% tryptic soy broth medium and the LIVE/DEAD bacterial viability assay. The stability in commercially available artificial saliva, human saliva and serum was determined by liquid chromatography mass spectrometry with incubation at 37°C for 0.5, 1, 2 and 4 hours. Student's t-test was used to determine the level of significance ($p < 0.05$).
Results:
Oncocin 10 was active against all *P. aeruginosa* strains tested including a multidrug resistant BAA-2110 strain with MIC in range from 2 to 8 µg/ml. Peptide 1018 showed a MIC in range from 32 to 64 µg/ml. Oncocin 10 and 1018 were relatively stable in artificial and human saliva with a half-life of approximately 60 minutes. In human serum, their half-life was less than 30 minutes.
Conclusions:
Oncocin 10 and 1018 demonstrated significant antibacterial activity against numerous *P. aeruginosa* strains and were relatively stable in saliva, but more quickly degraded in serum. Further testing and characterization are underway to identify additional promising AMPs for the treatment of periodontal pathogens.

FPP 3. **Prevalence and characteristics of toxigenic *Clostridium difficile, C. perfringens* and *Enterococcus* on shoe-bottoms from a hospital system**

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Background: Healthcare associated infections (HAI) are common everywhere of the world. Environmental surfaces are cleaned regularly, but may re-contaminated from shoes. Shoe-bottom surfaces could be highly contaminated with pathogenic bacteria from a diverse sources. Our recent studies on community house shoe-bottom surface swab samples were found frequently contaminated with toxigenic *C. difficile*. Our objectives of this pilot study were to investigate the prevalence *C. difficile, C. perfringens*, and *Enterococcus* of shoe-bottom surface swabs samples from a large hospital source.
Materials and Method: We collected 20 shoe-bottom swab samples from a hospital system and cultured for the bacteria using standard methods. Isolates were characterized by molecular methods. *C. difficile* and *C. perfringens* were cultured anaerobically by enrichment and selective agar plates (CCFA and Perfringens agar). *Enterococcus* counts were determined by Enterolert kit method.
Results: All the samples (20/20; 100%) were positive for *C. perfringens*, and 9 (45%) for toxigenic *C. difficile* (tcdA and tcdB genes). *Enterococcus* counts were between 25 and >12000 cells/swab for all the samples. Vancomycin resistant *Enterococcus* spp were recovered from all the samples by selective culture using mEnterococcus agar.
Conclusions: Overall, hospital shoe-bottom samples were highly contaminated with potential human pathogens. Further studies are needed to control shoe-source HAI in and around our health systems.

FPP 4. **An amino-terminal signal peptide of Vfr protein negatively influences RopB-dependent SpeB expression and attenuates virulence in *Streptococcus pyogenes***.

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Streptococcal pyrogenic exotoxin B (SpeB) is an extra-cellular cysteine protease that is a critical virulence factor made by the major human pathogen group A Streptococcus (GAS). *speB* expression is dependent on the regulator of proteinase B (RopB) and is up regulated with increasing cell density and during infection. Because computer modelling suggested significant structural similarity between RopB and peptide- sensing regulatory proteins made by other Gram- positive bacteria, we hypothesized that speB expression is influenced by RopB–peptide interactions. Inactivation of the gene (*vfr*) encoding the virulence factor related (Vfr) protein resulted in increased *speB* transcript level during the exponential growth phase, whereas provision of only the amino-terminal region of Vfr comprising the secretion signal sequence in trans restored a wild-type *speB* expression profile. Addition of the culture supernatant from a Vfr signal peptide-expressing GAS strain restored wild-type *speB* transcript level to a *vfr*-inactivated isogenic mutant strain. A distinct peptide in the Vfr secretion signal sequence specifically bound to recombinant RopB. Finally, overexpression of the Vfr secretion signal sequence significantly decreased speB transcript level and attenuated GAS and virulence in two mouse models of invasive infection. Taken together, these data delineate a previously unknown small peptide-mediated regulatory system that controls GAS virulence factor production.
Keywords: *Streptococcus pyogenes*, Quorum sensing, SpeB, RopB, Vfr

FPP 5. ***Lactobacillus reuteri* derived-histamine suppress interleukin-6 by inhibiting H1-receptor downstream signaling in germ-free mice.**

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Probiotics may beneficially affect the disease course of patients with chronic immune-mediated disorders like inflammatory bowel disease and colorectal cancer (CRC) via modulation of the host immune responses. A recent pangenomic study showed that human-derived clade II *L. reuteri* strains contained a complete chromosomal histidine decarboxylase (*hdc)* gene cluster (genes *hdcA*, *hdcB*, *hdcP*) and have the genetic capacity to convert histidine to histamine. In our lab we found *L. reuteri* 6475 (clade II) derived-histamine suppressed TNF production in human myeloid cells. In addition, we also showed administration of *L. reuteri* to HDC knock-out mice bearing inflammation-associated colon cancer (IaCC) showed suppression of inflammatory cytokines mainly IL-6, IL-22, IL-1α and TNF. Apart from suppression of inflammatory response, $CD11b^{+}Gr\text{-}1^{+}$ immature myeloid cell (IMCs) populations were reduced in spleen and bone marrow of $hdcA^{+}$ *L. reuteri* administered to HDC KO mice bearing IaCC compared to HDC KO IaCC mice with $hdcA^{-}$ *L. reuteri*. These changes correlated with reduced tumor numbers in the HDC KO mice. However, *L. reuteri* with mutant *hdcA gene* were unable to produce histamine and neither protected the HDC KO mice from IaCC. However, *L. reuteri* 6475 derived histamine and its ability to suppress inflammation and reduce IaCC are not well understood. So, we hypothesized that *L. reuteri* derived-histamine down-regulates histamine receptor 1(H_1R) and allows H_2R activation, thereby suppressing inflammation. We used Swiss-Webster WT and BALB/c WT germ-free mice mono-associated with *L. reuteri* wild-type or the *L. reuteri hdcA* mutant strain. The result showed significant diminution of IL-6 expression in the GF mice receiving *L. reuteri* WT and *hdcA* mutant strain. In addition, we found that both WT and mutant strain can produce Diacylglycerol kinase (DAGK). DAGK can inhibit DAG signaling downstream. Therefore, we speculate that IL-6 is suppressed due to DAG inhibition of H_1R downstream signaling and regulates the H_2R signaling by *L. reuteri* histamine.

FPP 6. **Diguanylate cyclases control nitric oxide metabolism via NNR in *Paracoccus denitrificans***

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Cyclic di-GMP is a bacterial secondary messenger first described for its role in controlling cellulose synthesis in *Gluconacetobacter xylinus*. In other bacteria cyclic di-GMP is known to regulate diverse phenotypes including the cell cycle, virulence, biofilm production, adhesion, developmental transitions, and motility. Cyclic di-GMP is synthesized by a diguanylate cyclase (characterized by a conserved GGDEF motif) and degraded by phosphodiesterases (containing EAL or HD-GYP domains encoding genes). Synthesis and degradation of cyclic di-GMP is typically sensitive to environmental cues. The GGDEF domain is often found fused to or co-expressed with other domains and proteins involved in sensing extracellular or intracellular signals. *Paracoccus denitrificans* is an alpha proteobacterium which has been used as a model for the study of denitrification. Denitrification is an anaerobic respiratory pathway in which nitrate and nitrite are used as terminal electron acceptors and are reduced sequentially to nitric oxide (NO), nitrous oxide and dinitrogen. The *P. denitrificans* genome encodes two GGDEF and two EAL domain proteins. One of these GGDEF proteins is encoded

in a predicted transcription unit with the gene encoding a nitric oxide sensing H-NOX protein. In order to understand the role of cyclic di-GMP in *P. denitrificans*, we have initiated a genetic and physiological characterization of the signaling system. A mutant lacking both GGDEF domain proteins consumes NO more slowly than the wild type, while an H-NOX deficient mutant reduced NO more rapidly. In the denitrification pathway, NO is reduced by a respiratory NO reductase encoded by genes in the *nor* operon. The *nor* promoter is activated in response to NO by NNR, a transcriptional regulator from the FNR-CRP family. A *norC-lacZ* reporter fusion showed a lower activity in the GGDEF double mutant compared to the wild type and the H-NOX mutant. We showed that NNR binding to DNA can be stimulated by cyclic di-GMP *in vitro*. Our data are consistent with a model in which NO stimulated synthesis of cyclic di-GMP leads to NNR-mediated activation of the *nor* operon promoter. Several lines of evidence suggest that activation by cyclic di-GMP is not the only mechanism by which NNR activity can be controlled in response to NO.

Education Session

ES. 1 **Who wants to learn about Human Anatomy? Mechanisms to promote responsible learning.**

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How can one keep students engaged in a large, lower-level biology class of non-major students? In particular, how is this best done in a course in which the material is very detailed and complex? I have incorporated numerous innovations over several years to promote student learning in a human anatomy course. The use of clickers promotes a more active classroom. Students use clickers to answer questions that I pose during lectures, followed by discussions between pairs of students and then with the instructor. In addition, students use clickers for exams. The use of clickers in lectures and on exams provides students immediate feedback. To further engage students, I began the use of adaptive homework. Adaptive homework is a service available with many textbooks that uses computer programs to query students on a particular topic until they master it. This is a direct way for students to confirm their knowledge base and pinpoint areas in which they need additional study. Most recently, I have instituted student-run review sessions, in which each student prepares their own review notes, and several students are randomly selected to lead each review session. This provides an incentive for students to prepare for their exams ahead of time, as students do not know if they will be selected until the time of the review session. Each of these innovations provides a framework upon which students can build their knowledge base, promoting progressive knowledge gains rather than last minute cramming. Moreover, the responsibility for learning is put in the students' hands. Overall, test scores have increased, as well as students' mastery of human anatomy.

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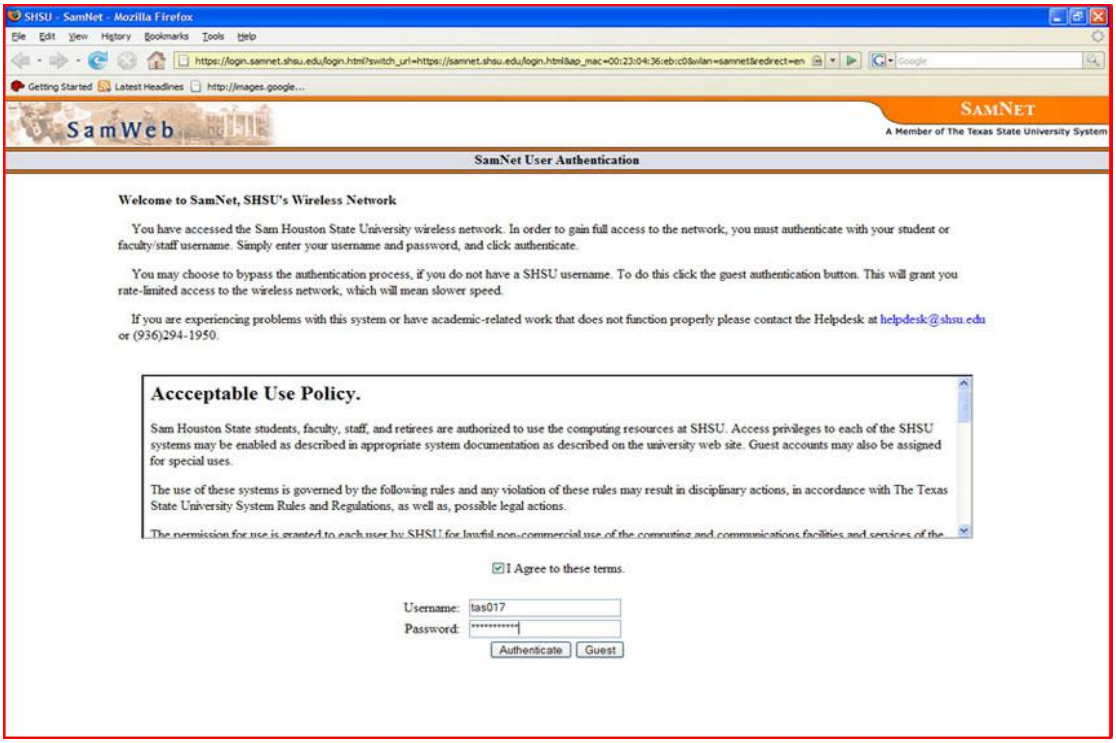

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Fax: (936) 294-3417
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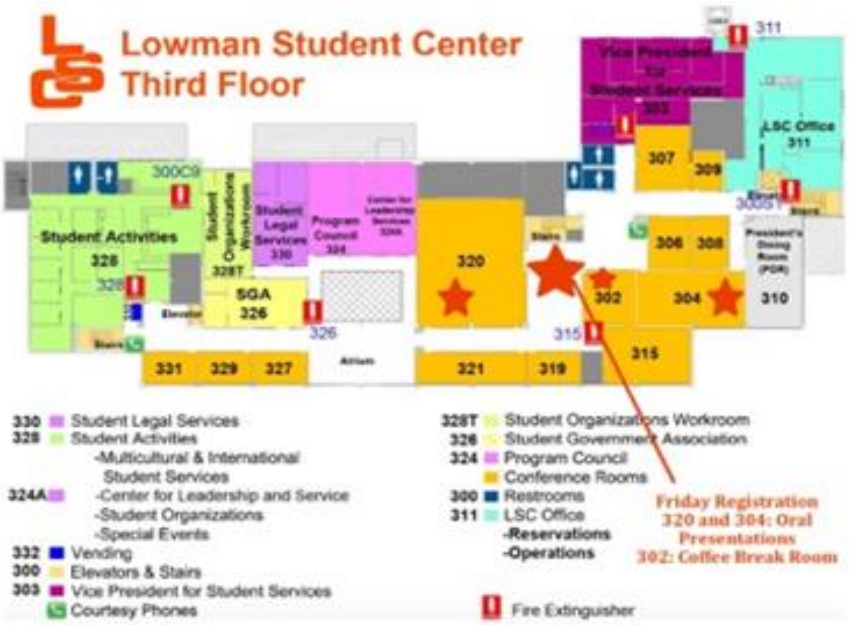

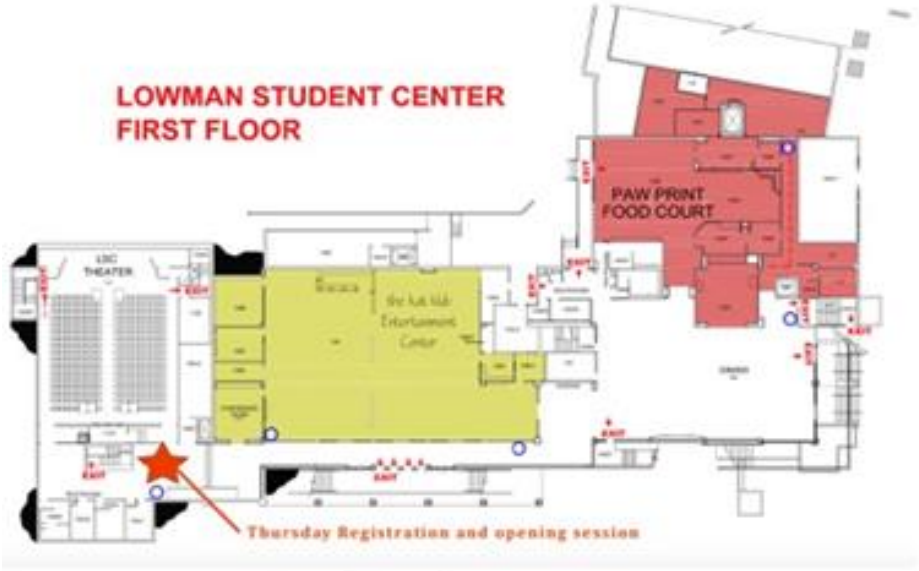

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