Joint Meeting of the Missouri and Missouri Valley Branches of the American Society of Microbiology

March 15-16, 2019

University of Nebraska Omaha

Creighton University

Sponsors:

Creighton University Department of Medical Microbiology and Immunology

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University of Nebraska Research Collaboration Initiative

On behalf of the University of Nebraska Omaha and Creighton University, we would like to welcome you to Omaha for the 2019 Joint Meeting of the Missouri and Missouri Valley Branches of the American Society of Microbiology. This meeting is the premier venue for undergraduate and graduate student researchers to present their work in the dynamic field of microbiology, network with other students and investigators from the region, as well as interact with ASM Distinguished Lecturers. This year we had 70 abstract submissions from undergraduate and graduate student researchers from each of the four states represented by our ASM branches. We hope this meeting will lead to new collaborations, friendships, and career opportunities for our members. Once again, welcome to Omaha!

Travis J. Bourret
President-Elect
ASM Missouri Valley Branch
Assistant Professor
Department of Medical Microbiology and Immunology
Creighton University

Schedule at a glance			
Friday, March 15, 2019			**Room**
4:00 - 5:00 PM	Mixer	Sponsored by the University of Nebraska Collaboration Initiative	Milo Bail Student Center
5:00 – 5:15 PM	Welcome		
5:15 – 6:30 PM	Dr. Elizabeth Rucks, UNMC	Mapping the *Chlamydia trachomatis* inclusion membrane with proximity labeling systems	
	Dr. Maureen Donlin, St. Louis University	Exploration of *Cryptococcus* resistance to echinocandins	
6:30 – 7:30 PM	Dinner		
7:30 – 8:45 PM	ASM Distinguished Lecturer		
	Dr. Cheryl Nickerson, Arizona State University	Spaceflight-induced Alterations in Microbial Virulence and Host-Pathogen Interactions: Novel Insight into Infectious Disease Mechanisms	
8:45 – 9:00 PM	Closing Announcements.		
Saturday, March 16, 2019			
8:00-9:45 AM	Coffee and Pastries	Sponsered by ThermoFisher	Harper Center Rm 3023
	Poster Session (Undergraduates & Graduate Students)		
10-11:00 AM	Oral Presentations I		Harper Center Rm 3027, 3028a, 3028b, & 3029
11:00 AM.-12:00 P	Oral Presentations II		Harper Center Rm 3027, 3028a, 3028b, & 3029
12:00-1:15 PM	Lunch and ASM Distinguished Lecturer: Dr. Aaron Best	Just Your Friendly, Neighborhood *E. coli* ? Population Diversity of *Escherichia* Isolated from Fresh Water Sources	Harper Center Ballroom West
1:15-2:00 PM	Selected Student Abstract Talks (15 min/ea)		
	Rob Todd, Creighton University	Genome Plasticity in *Candida albicans* is Driven by Long Repeat Sequences	Harper Center Ballroom West
	Radwa A. Hanafy, Oklahoma State University	Novel Anaerobic Gut Fungi Genera from Wild and Captive Herbivores Greatly Expand the Known Diversity within the Neocallimastigomycota	
	Brianna Bullard, University of Nebraska-Lincoln	A T-cell Biased Adenovirus Vectored Zika Virus Vaccine	
2:00-3:00 PM	Oral Presentations III		Harper Center Rm 3027, 3028a, 3028b, & 3029
3:00-3:30 PM	Dr. Matt Cabeen, Oklahoma State University	*B. subtilis* environmental stress sensors enable distinct response patterns and fitness benefits	Harper Center Ballroom West
3:30-4:00 PM	Dr. Kara De Leion, University of Missouri, Columbia	Genetic requirements for *Desulfovibrio vulgaris* Hildenborough biofilm formation	Harper Center Ballroom West
4:00-4:45 PM	Students meet with Keynote speakers		Harper Center Ballroom West
	Branch Business		Harper Center Rm 3028a & 3028b
4:45-5:00 PM	Awards and Closing remarks		Harper Center Ballroom West

ASM Distinguished Lecturers

Dr. Cheryl A. Nickerson, Ph.D
Center for Immunotherapy, Vaccines and Virotherapy
The Biodesign Institute
Arizona State University

Biographical Sketch

Dr. Cheryl A. Nickerson is a Professor in the School of Life Sciences, at the Biodesign Institute at Arizona State University. Her internationally recognized research takes a highly multidisciplinary and innovative approach that blends microbiology, tissue engineering, and physics to mimic the dynamic interactions between the host, its microenvironment, and the pathogens that lead to infection and disease. She focuses on characterizing the effects of biomechanical forces on bacterial pathogenesis mechanisms and host-pathogen interactions that regulate the transition between normal homeostasis and infectious disease. Her laboratory has developed several innovative model pathogenesis systems to study these processes, including 3- D organotypic tissue culture models as predictive platforms to study host-pathogen interactions, and characterizing pathogen responses to physiological fluid shear forces encountered in the infected host, as well as in the microgravity environment of spaceflight. Her research has flown on numerous NASA Shuttle missions, the International Space Station, and on SpaceX missions. She is a recipient of the Presidential Early Career Award for Scientists and Engineers and NASA's Exceptional Scientific Achievement Medal. She serves as founding Editor-in-Chief of the *Nature* journal *npj Microgravity*, and was selected as a NASA Astronaut candidate finalist.

Aaron A. Best, Ph.D.
Harrison C. and Mary. L. Visscher Professor of Genetics
Department of Biology
Hope College

Biographical Sketch

My first exposure to microbiology was during undergraduate training at William Jewell College (Liberty, MO), a small private liberal arts college (B.A. Biology, 1996). An undergraduate research experience led me to obtain a Ph.D. in Microbiology from the University of Illinois, Urbana-Champaign with Dr. Gary Olsen (2001) focused on evolution of transcription systems, followed by a post-doc with Dr. Carl Woese in molecular evolution. I pursued a career in academia that supported serious research and excellence in teaching, returning to a liberal arts environment where I am the Harrison C. and Mary L. Visscher Professor of Genetics at Hope College. I maintain an extramurally funded research program, incorporating undergraduates into all aspects of the research process; have combined research and teaching programs into a single endeavor; have taught and participated at a programmatic level in HHMI's SEA-PHAGES for nine years; incorporated research projects into a microbiology laboratory course; and started a laboratory course for first-year students on the microbial ecology of our watershed. My research centers on comparative genomics of environmentally derived *Escherichia* populations, molecular ecology of fresh water systems, integration of large-scale datasets into genome-scale metabolic models of bacteria, and assessment of integrating research into teaching on student education.

Missouri Branch Invited Speakers

Dr. Kara De Leon, Ph.D.
Post-doctoral Research Fellow
Department of Biochemistry
University of Missouri, Columbia

Biographical Sketch

My lab is focused on mechanistically understanding microbial community formation and function in a metal-contaminated subsurface. We do this by determining the genetic drivers of these activities. We are particularly interested in the activities of sulfate-reducing bacteria as they are capable of reducing numerous metals, changing their solubility, and thereby removing them from groundwater. The majority of sulfate-reducing activity in our subsurface site is found attached to sediment particles as a biofilm. Our current research is focused on the genetic requirements for biofilm formation in sulfate reducers. We use *Desulfovibrio vulgaris* Hildenborough as a model sulfate-reducer and the power of high-throughput mutant screenings followed by targeted mutagenesis and strain characterization to assess the importance of nearly every gene in the genome for biofilm formation. In determining the genes required for biofilm formation and environmental cues that initiate attachment or dispersion, we aim to reveal a mechanism to be targeted as a strategy to promote subsurface sulfate-reducing biofilms.

Dr. Maureen Donlin, Ph.D.
Research Professor
Director, Master's Program in Bioinformatics and Computational Biology
Department of Biochemistry and Molecular Biology
Saint Louis University School of Medicine

Biographical Sketch

My lab is focused on the study of the human fungal pathogen, *Cryptococcus neoformans*, an environmental pathogen that can cause disease in immunocompromised patients. One arm of our research is to understand the mechanisms by which *C. neoformans* regulates and remodels its cell wall in response to stress and antifungal therapies. In particular, we are exploring the role of a cell wall integrity pathway in this response. A second arm of our research is to identify novel small molecules that can inhibit growth of the fungus and potentially be developed into new anti-fungal therapies. We employ wet-bench molecular biology, genetic and biochemical approaches as well as computational analyses of high-throughput genomic and RNA sequence data.

Missouri Valley Branch Invited Speakers

Dr. Elizabeth Rucks, Ph.D.
Associate Professor
Department of Pathology and Microbiology
University of Nebraska Medical Center

Biographical Sketch

In the Rucks lab, we are interested in how the obligate intracellular Chlamydiae coordinate with their eukaryotic host cells to create their specialized developmental niche, called an inclusion. The entirety of the biphasic chlamydial developmental cycle occurs within the inclusion, and therefore, the organisms must acquire necessary nutrients by interacting with specific host pathways via the inclusion membrane. Our research focuses on understanding the function at the chlamydial inclusion of eukaryotic SNAREs (N-ethylmaleimide sensitive attachment protein receptors) and a family of chlamydial proteins known as Incs. SNARE proteins decrease the energy required to fuse a host vesicle with a target membrane, and we wonder if the SNARE proteins that are recruited to the chlamydial inclusion function in this capacity. Chlamydial Inc proteins are defined as proteins containing at least two large hydrophobic transmembrane domains flanked by termini that are exposed on the host cytosolic face of the chlamydial inclusion, and thus likely facilitate host-pathogen interactions. We are taking advantage of recently developed proximity labeling systems combined with new techniques in chlamydial genetics to help us understand how these classes of eukaryotic and prokaryotic proteins may be interacting with each other, which would provide clues as to how they are contributing towards establishing and maintaining the chlamydial inclusion.

Dr. Matthew Cabeen, Ph.D.
Assistant Professor
Department of Microbiology and Molecular Genetics
Oklahoma State University

Biographical Sketch

Work in the Cabeen lab is focused on understanding the fundamental biology of bacteria and bacterial "decision making"—how genetic and protein circuits and signaling pathways lead cells to enact a course of action. We employ two model organisms. In the opportunistic human pathogen *Pseudomonas aeruginosa*, we seek to identify and characterize new pathways that govern the formation of biofilms, which are notoriously hard to treat in human infections. In the soil bacterium *Bacillus subtilis*, we are dissecting the signaling functions of stressosomes, large cytoplasmic multi-protein complexes that sense and process environmental stress. In each case, our goal is to achieve a systems-level understanding of how bacteria enact different behaviors, from individual molecules and interactions to cell populations. Our research team is presently composed of four graduate students and twelve undergraduate scholars. Please visit cabeenlab.okstate.edu or follow us on Twitter @CabeenLab for more information.

General Microbiology Graduate Oral Presentations

Harper Center Rm 3027

Time	Session 1	Title
10:00 - 10:15 am	Rosalie C. Warner	Determining the Performance of a Novel *Toxoplasma gondii* DNA Vaccine
10:15 - 10:30 am	Amanda Brookhouser-Sisney	A Risk Assessment Study of *Staphylococcus aureus* and *Bacillus cereus* in Beans Based on the Potential for Product Accumulation During Food Processing.
10:30 - 10:45 am	Yingshan LI	Novel Small RNAs, with Distinct Biogenesis, in the Alga *Chlamydomonas*
10:45 - 11:00 am	Biraj Kayastha	A Novel Calcium (Ca^{2+}) Sensor, EfhP, Mediates Ca^{2+} Regulation of Virulence in *Pseudomonas aeruginosa*
	Session 2	
11:00 - 11:15 am	Bejan Mahmud	Secondary structure of the 5' untranslated region of coxsackievirus B3 genomic RNA
11:00 - 11:30 am	Ryan M. Singh	The Physiological Role of Nitric Oxide Synthase (NOS) in *Staphylococcus epidermidis*
11:30 - 11:45 am	Nicholas A. Wood	The ClpXP System of *Chlamydia trachomatis* Plays a Critical Role in Organism Physiology
11:45 - 12:00 pm	Zachary Scott	Characterization of the Role of PA5189 of *Pseudomonas aeruginosa* in Resistance to an Antimicrobial Peptide
	Session 3	
2:00 - 2:15 pm	M. Jane Morwitzer	Identification of RUVBL1 and RUVBL2 as Novel Cellular Interactors of the Ebola Virus Nucleoprotein
2:15 - 2:30 pm	Mackenzie E. Conrin	Characteristics of Novel *Podoviridae* Isolated from Freshwater Samples Against *Pseudomonas fluorescens*
2:30 - 2:45 pm	Christopher Hamm	Environmental Stress Sensors Maintain Characteristic Response Profiles across Diverse Stressors
2:45 - 3:00 pm	Abdulelah A. Alqarzaee	The ClpXP Protease Modulates the Chronological Lifespan of *Staphylococcus aureus*

Environmental Microbiology Graduate Oral Presentations

Harper Center Rm 3028a

Time	Session 1	Title
10:00 - 10:15 am	Archana Yadav	Enrichment and Genomic Characterization of a Novel Thermohaloanaerobic Bacterial Phylum from a Tertiary Oil Reservoir in Cushing, OK
10:15 - 10:30 am	Chelsea L. Murphy	The Rich Diversity of Delta Proteobacteria in Zodletone Spring, an Anoxic Sulfide-Rich Hydrocarbon Seep
10:30 - 10:45 am	William S. Marsh	Remediation of Produced Water by Halophilic Microorganisms
10:45 - 11:00 am	Christopher T. Garner	Enrichment and Isolation of Aerobic Methane-Oxidizing Bacteria from Extreme Environments

Medical Microbiology and Immunology Graduate Oral Presentations

Harper Center Rm 3028a

Time	Session 4	Title
11:00 - 11:15 am	Amanda K. Zalud	Redox Regulation of *Borrelia burgdorferi* Gene Expression
11:00 - 11:30 am	Tarosha B. Salpadoru	Mechanisms Contributing to Ca^{2+}-Induced Polymyxin B Resistance in *Pseudomonas aeruginosa*
11:30 - 11:45 am	Macy G. Olson	*In Vivo* Proximity Labeling to Identify a Novel Eukaryotic Protein Recruited to the Chlamydial Inclusion
11:45 - 12:00 pm		

Medical Microbiology and Immunology Graduate Oral Presentations

Harper Center Rm 3029

Time	Session 1	Title
10:00 - 10:15 am	Alexis M. Page	Decreased Tricarboxylic Acid Cycle (TCA) in *Staphylococcus aureus* Increases Survival to Innate Immunity
10:15 - 10:30 am	Alyssa K. Whitney	Synergy Among Outer Membrane Porins and β-Lactamase Enzymes is Associated with Ceftolozane/Tazobactam Resistance in *Klebsiella pneumoniae*
10:30 - 10:45 am	Matthew C. Martens	Parasite-Specific Secreted Protein ROP1 Acts as a Novel Therapeutic Target Against *Toxoplasma gondii*
10:45 - 11:00 am		
	Session 2	
11:00 - 11:15 am	Maxwell A. Virus	CO-ADMINISTRATION OF FDA-APPROVED DRUGS ELICITS A SIGNIFICANT DECREASE IN CHRONIC *TOXOPLASMA GONDII* INFECTION CYST LOAD
11:00 - 11:30 am	John P. Mangold	Specific Iron Transporters are Required for Entry of the Novel Siderophore Cephalosporin, Cerdiferocol, in *Escherichia coli* and *Pseudomonas aeruginosa*
11:30 - 11:45 am	Ben Nelson	Antifungal Activity of Dendritic Cell Lysosomal Proteins against Cryptococcus neoformans
11:45 - 12:00 pm		
	Session 3	
2:00 - 2:15 pm	Austin Sanford	Preliminary Characterization of Pan-Parasitic Experimental Compounds against *Toxoplasma gondii* using *in vitro* and *in vivo* Models
2:15 - 2:30 pm	Corey S. Suelter	Interplay between CTX-M and OmpC Production in *Escherichia coli* are Important for Carbapenem Resistance
2:30 - 2:45 pm	Michelle King	A Putative Phytase, CarP, Required for Calcium Tolerance and Virulence in *P. aeruginosa,* is Differentially Regulated by Host Factors
2:45 - 3:00 pm		

Undergraduate Oral Presentations

Harper Center Rm 3028b

Time	Session 1	Title
10:00 - 10:15 am	Timothy E. Reznicek	A Structural Analysis of Human Herpesvirus 8 Polyadenylated Nuclear RNA through SHAPE-MaP Analysis
10:15 - 10:30 am	Justine M. Pitzer	Characterization of *Staphylococcus lugdunensis* Biofilms
10:30 - 10:45 am	Tiffani Jones	Do Dental Unit Waterline Maintenance Tablets Inhibit Bacterial Growth In Dental Line Water?
10:45 - 11:00 am	Kevin Figueroa	Does Calcium Regulate Rhamnolipid Production in *Pseudomonas aeruginosa?*
	Session 2	
11:00 - 11:15 am	Kyle Mellor, Gloria Hanrahan	The Effect of Peppermint Oil on Biofilm Development in *Staphylococcus epidermidis* Isolates
11:00 - 11:30 am	Casandra Salinas	Abnormal Ion Concentration in Cystic Fibrosis Lungs Impact Rhamnolipid Production in *Pseudomonas aeruginosa*
11:30 - 11:45 am	Dan L. Nabb	*Candida albicans* Induces Multidrug Tolerance in *Staphylococcus aureus* through Energy Depletion
11:45 - 12:00 pm	Samantha Sack	Innate Immune Cell Activation by Antischistosomal Compound SAS1
	Session 3	
2:00 - 2:15 pm	Matthew Froid	Targeted Therapy for the Future- The Use of Novel Antimicrobial Peptides against *P. aeruginosa*
2:15 - 2:30 pm	Andrew Pham	Utilizing Chemical Mutagenesis to Determine SW33 Mechanism of Action in *Toxoplasma Gondii*
2:30 - 2:45 pm	Kennedy Kluthe	Decreased Tricarboxylic Acid (TCA) Cycle Activity in *Staphylococcus aureus* Increases Survival to Innate Immunity

Selected Graduate Student Oral Presentations

Genome Plasticity in *Candida albicans* is Driven by Long Repeat Sequences

Robert T. Todd[1] (Doctoral Student)[*], Tyler Wikoff[1], Annette Beach[1], Anja Forche[2], Anna Selmecki[1]*
[1]Creighton University Medical School, Omaha, Nebraska, [2]Bowdoin College, Brunswick, Maine.

Genome rearrangements resulting in copy number variation (CNV) and loss of heterozygosity (LOH) are frequently observed during the somatic evolution of cancer and promote the rapid adaptation of fungi to novel environments. In the diploid, heterozygous fungal pathogen *Candida albicans*, CNV and LOH can confer increased virulence and antifungal drug resistance, yet the mechanisms driving these somatic genome rearrangements are not completely understood. Here, we examined the role of genome architecture during the formation of genetic variation in the diploid, heterozygous fungal pathogen, *C. albicans*. Our genome-wide analysis identified long repeat sequences that had prominent roles in generating genomic diversity across diverse strain backgrounds including clinical, environmental, and experimentally evolved isolates. These long repeat sequences included previously uncharacterized repeat sequences, centromeric repeats, repeats found within intergenic sequences, and repeats that span multiple ORFs and intergenic sequences. Importantly, long repeat sequences were found at every CNV and sequence inversion breakpoint observed, and frequently occurred at LOH breakpoints as well. Furthermore, these results were independent of genetic background or source of isolation. Thus, long repeat sequences found across the *C. albicans* genome underlie the formation of significant genome variation that can increase fitness and drive adaptation.

Novel Anaerobic Gut Fungi Genera from Wild and Captive Herbivores Greatly Expand the Known Diversity within the Neocallimastigomycota

Radwa A. Hanafy*(Doctoral), Mostafa S. Elshahed, Noha H. Youssef

Oklahoma State University, Department of Microbiology and Molecular Genetics

The Anaerobic gut fungi (AGF) (Neocallimastigomycota) are common inhabitants of the alimentary tracts of herbivores, where they play an important role in the degradation of ingested plant fibers. While the AGF diversity in domesticated hosts has been thoroughly investigated, very few studies have sampled AGF populations in wild herbivores. We hypothesized that the host animal's lifestyle greatly impacts the resident AGF community. A multiyear isolation effort from 27 fecal samples obtained from 11 domesticated, 7 captive, and 9 wild herbivorous species yielded 200 different AGF isolates that broadly captured both inter- and intra-genus levels diversities within the Neocallimastigomycota. We successfully isolated multiple novel representatives of 80% of the currently described genera. More importantly, 33 of the 200 isolates clustered into five distinct novel AGF genera, four of which were exclusively obtained from wild herbivores. Members of one of the novel genera isolated from Zebra represent the first cultured representatives of the previously uncultured ubiquitous lineage AL1.The isolated novel AGF genera displayed multiple interesting microscopic features, including multi-sporangiate thalli, papillated sporangia, pseudo-intercalary sporangia, and swollen sporangiophores. Our efforts greatly expand the known AGF phylogenetic diversity and highlight the value of sampling wild herbivores as a yet-untapped reservoir of novel AGF diversity.

A T-cell Biased Adenovirus Vectored Zika Virus Vaccine.

Brianna L. Bullard* (Doctoral), Brigette N. Corder, and Eric A. Weaver.

University of Nebraska – Lincoln, Lincoln, Nebraska

Development of a Zika virus (ZIKV) vaccine remains a global priority due to the risk of congenital Zika syndrome in developing fetuses and Guillain-Barre syndrome in adults. However, there are currently no licensed vaccines available to protect against ZIKV infection. Here, we developed two Adenovirus (Ad) vectored Zika virus vaccines by inserting a ZIKV prM-E gene expression cassette into human Ad types 4 (Ad4-prM-E) and 5 (Ad5-prM-E). Immune correlates in C57BL/6 mice indicate that Ad5-prM-E vaccination induces both strong anti-ZIKV antibody and T-cell responses. However, Ad4-prM-E vaccination only induces a strong T-cell response without development of detectable antibodies. In a challenge model utilizing immunocompetent C57BL/6 mice that were immunized and then treated with a blocking anti-IFNAR-1 antibody immediately before ZIKV challenge, 100% of Ad4-prM-E and Ad5-prM-E vaccinated mice survived while only 40% of sham vaccinated mice survived. This indicates that Ad4-prM-E vaccination is protective without the development of detectable anti-ZIKV antibodies. We will examine if this Ad4 vector has the potential to be a safer ZIKV vaccine platform because of the reduced risk of vaccine induced antibody dependent enhancement of Dengue virus. In addition, we will examine the mechanism by which the Ad4 vector causes the T-cell bias.

General Microbiology Session I:

Determining the Performance of a Novel *Toxoplasma gondii* DNA Vaccine.

Rosalie C. Warner (Masters)*, Paul H. Davis, Department of Biology, University of Nebraska at Omaha, Omaha, NE 68182.

Toxoplasma gondii is an obligate intracellular parasite that has the ability to infect all nucleated mammalian cells and is found worldwide. This parasite can cause severe ocular and neurological disease in immunocompromised persons as well as fetal abnormalities. There is currently no treatment available for the latent cyst forming stage of parasite infection and thus there is a need for an effective protective vaccine. This work highlights the design of a versatile DNA sequence that seeks to stimulate T cell- and antibody-mediated memory responses, as well as outlining the current and future experiments that must be completed to show the vaccine's efficacy against infection. As multi-epitope DNA vaccines have been shown to provide effective protection against other pathogens, the developed sequence consists of three full length *T. gondii* genes as well as three adjuvants that have been shown to give the desired immunity. Experiments to be conducted include vaccine transfection into mammalian cells to confirm the ability of the protein to be expressed, enzyme-linked immunosorbent assays to display the capability of the vaccine to elicit both a humoral and cell mediated immune response, and a demonstration of the vaccines capacity to provide effective protection against infection *in vivo*.

A Risk Assessment Study of *Staphylococcus aureus* and *Bacillus cereus* in Beans Based on the Potential for Product Accumulation During Food Processing.

Amanda Brookhouser-Sisney (Masters)*, Christopher Showalter, and Caleb Mayfield. University of Nebraska, Omaha, NE and Conagra Brands, Inc., Enterprise Microbiology, Research and Development, Omaha, Nebraska.

High moisture foods (A_w >0.91; pH > 4.5 and < 9.6) such as beans, may permit growth of *Staphylococcus aureus* and *Bacillus cereus*, and therefore, the potential formation of heat stable enterotoxin, given the processing conditions. Compliance with FSMA 21 CFR Part 117, Subpart B and Section 117.8 (c)(2) and (c)(3) requires a hazard analysis to be performed to determine if preventive controls are needed. An inoculated microbial challenge study was performed using pinto and navy bean samples collected following washing, soaking and blanching in the production process. A binomial linear model was fit to each and used to estimate *S. aureus* and *B. cereus* counts for a given time and temperature with a 95% confidence interval. Growth of the organisms in bean samples following washing did not exceed the Food Safety Limit (FSL; 10^5 CFU/g) (α <0.05) up to 120 hours. Bean samples following soaking and blanching both surpassed the FSL. The study findings highlight that preventive controls must be administered during bean processing to significantly minimize and prevent the growth of *S. aureus* and/or *B. cereus*. Preventive control management components must be in place in the facility's Food Safety Plan to ensure effectiveness of the preventive controls.

Novel Small RNAs, with Distinct Biogenesis, in the Alga *Chlamydomonas*

Yingshan Li (doctoral)*[1], Eun-Jeong Kim[2], Adam Voshall[1], Etsuko Moriyama[1], Heriberto Cerutti[1]

[1] School of Biological Sciences and Center for Plant Science Innovation, University of Nebraska-Lincoln, Lincoln, Nebraska, [2] Department of Life Science, Chung-Ang University, Seoul, Korea

RNA interference (RNAi) is a highly conserved process involved in gene regulation and defense responses in diverse eukaryotes. MicroRNAs (miRNAs) and small-interfering-RNAs (siRNAs), key components of the RNAi machinery, are derived from double-stranded RNAs by Dicer processing. The generated small RNAs (sRNAs), ~20-24 nucleotides long, are loaded into effector Argonaute (AGO) proteins and regulate complementary transcripts. The unicellular green alga *Chlamydomonas reinhardtii* carries three Dicer and three AGO homologs. However, the function(s) of AGO1, phylogenetically divergent from the other *Chlamydomonas* AGOs, remains unknown. We have recently identified AGO1-associated sRNAs by affinity-purification and deep sequencing. Interestingly, AGO1-bound sRNAs are distinctly longer than canonical miRNAs/siRNAs and prefer adenosine at their 5'-end instead of uridine, the predominant 5' termini nucleotide of miRNAs. Additionally, the expression of AGO1-bound sRNAs is not affected in null mutants of Dicer3 or by RNAi-mediated suppression of Dicer1, suggesting that their biogenesis is dicer-independent. Yet, putative precursor transcripts fold into a conserved secondary structure, albeit distinct from the hairpin structure of miRNA precursors. Our results suggest that *Chlamydomonas* harbors a novel type of sRNAs, with distinct biogenesis from canonical miRNAs/siRNAs. We hypothesize that AGO1-associated sRNAs plays a role in silencing selfish elements such as repetitive sequences, viruses and transposons.

A Novel Calcium (Ca^{2+}) Sensor, EfhP, Mediates Ca^{2+} Regulation of Virulence in *Pseudomonas aeruginosa*

B. B. Kayastha (Doctoral) [1*], R. Rogers[1], J. McCoy[1], S. Khanam[1], S. Peng[1], J. Deng[1], M. Barbier[2], M. Patrauchan[1]

[1]Oklahoma State Univ., Stillwater, OK, [2]West Virginia Univ., Morgantown, WV

Pseudomonas aeruginosa, an opportunistic pathogen, causes lethal chronic infections in patients with Cystic Fibrosis. Elevated calcium (Ca^{2+}) levels have been shown to enhance its virulence. Previously, we identified a putative Ca^{2+}-binding protein EfhP containing two EF-hand motifs and showed its mediatory role in Ca^{2+} regulation of virulence factors. Here, by using wax worm and murine macrophage infection models, we show the role of EfhP in virulence and intracellular survival. We purified the soluble portion of the protein and by using Isothermal Titration Calorimetry (ITC) confirmed that it specifically binds Ca^{2+}. We also confirmed that EfhP undergoes Ca^{2+}-induced hydrophobicity changes by using a fluorescent dye, 1-anilinonaphthalene-8-sulfonic acid. Initial pull down assay followed by LC-MS/MS showed that upon Ca^{2+} binding, EfhP interacts with FurA and Bfr, proteins involved in iron metabolism. To identify the residues involved in Ca^{2+}-binding, we are currently generating a series of point mutations. By using promoter activity assay, we showed that elevated levels of Ca^{2+} increase *efhP* transcription. Currently, we are testing the effect of other host factors on the promoter activity. In summary, we present evidences supporting that EfhP acts as a Ca^{2+}-sensor of *P. aeruginosa*, which mediates Ca^{2+} regulation of virulence and adaptations in the host.

General Microbiology Session II.

Secondary structure of the 5' untranslated region of coxsackievirus B3 genomic RNA.

Bejan Mahmud (Master's)*, Christopher M. Horn, William E. Tapprich.
University of Nebraska Omaha, Omaha, Nebraska.

Coxsackievirus B3 (CVB3) is an important human pathogen and has been determined as a causative agent of myocarditis and pancreatitis. The single-stranded, positive-sense genomic RNA of CVB3 serves as a template for the production of the viral structural and nonstructural proteins. In absence of an m7G cap, the viral RNA has to rely on cap-independent translation initiation mechanisms. Similar to other enteroviruses, the CVB3 genome has a long, highly structured 5' untranslated region (UTR), which has been identified as a determinant of viral virulence. The 5' UTR houses a cloverleaf-like structure and a type I internal ribosome entry site, the proper folding of which is necessary for the interactions of the 5' UTR with viral and host trans-acting factors and underlines the efficiency of viral replication. Here, we utilized a chemical probing method to characterize the secondary structure of the CVB3 5' UTR. We propose a model with novel structural features including realignment of major domains and newly identified long-range interactions. Taken together, our work brings us closer to bridging the gap between structure and function in processes essential for the replicative success of CVB3 and related enteroviruses.

The Physiological Role of Nitric Oxide Synthase (NOS) in *Staphylococcus epidermidis*

Ryan M. Singh (doctoral student) *[1], Elizabeth H. Hutfless[1], Sujata S. Chaudhari[1], Abdulelah A. Alqarzaee, Matthew C. Zimmerman[2] and Vinai C. Thomas[1]

[1]Center for Staphylococcal Research, Department of Pathology and Microbiology, University of Nebraska Medical Center, Omaha, Nebraska.
[2]Cellular and Integrative Physiology, University of Nebraska Medical Center, Omaha, Nebraska.

The free radical nitric oxide (NO) poisons bacterial respiration and is an efficient inhibitor of bacterial growth. Hence, it is unclear why some bacteria possess NOS, a metalloenzyme that catalyzes the formation of NO. Recent studies in *Staphylococcus aureus* and *S. epidermidis* have revealed a role for NOS in stimulating cytochrome *bo* activity through nitrite production. However, the mechanism by which NOS-derived nitrite activates respiration has remained unclear. We now demonstrate that the *S. epidermidis* 1457Δ*nos* mutant produces higher levels of endogenous superoxide anions ($O_2^{\cdot -}$) relative to the wild-type strain, resulting in respiratory inhibition and growth impairment of the Δ*nos* mutant. Furthermore, we have identified the heme-bound oxygen of the staphylococcal flavohemoglobin, Hmp, as the source of $O_2^{\cdot -}$ in the Δ*nos* mutant. Finally, we show that the increased levels of endogenous $O_2^{\cdot -}$ in the Δ*nos* mutant results from the inability of these cells to produce nitrite to control the expression of *hmp*. Our findings highlight a crucial role for bacterial NOS in limiting Hmp toxicity.

The ClpXP System of *Chlamydia trachomatis* Plays a Critical Role in Organism Physiology.

Nicholas A. Wood (Doctoral)*[1], Amanda M. Blocker[2], Martin Conda-Sheridan[1], Derek J. Fisher[2], and Scot P. Ouellette[1].

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Chlamydia trachomatis (Ctr), an obligate intracellular bacterium, undergoes a complex developmental cycle. The transition from the infectious, non-dividing elementary body (EB) to the non-infectious, replicative reticulate body (RB) and vice versa is not mediated by a division event that results in re-distribution of intracellular proteins. We hypothesize both primary (EB to RB) and secondary (RB to EB) differentiation are dependent on protein turnover. The Clp protease system is well characterized in other bacteria and conserved in Ctr. Our initial characterization of Ctr ClpP2 and X suggests they play key roles in growth and secondary differentiation. Overexpression of wild-type proteins had a minimal effect on Ctr growth whereas overexpression of inactive mutant proteins led to a significant decrease in growth. Further, we observed measurably smaller RBs upon mutant ClpP2X operon overexpression and significantly larger RBs with wild-type ClpP2X overexpression. Interaction and oligomerization studies show that ClpX forms a homo-hexamer, and we are currently investigating its interactions with ClpP2. Structurally, chlamydial ClpX retains the functional domains observed in other homologs but also encodes a poly-serine linker between its N-terminal zinc binding domain and C-terminal domain. Truncation and domain-specific mutants of ClpX have been constructed and are under investigation.

Characterization of the Role of PA5189 of *Pseudomonas aeruginosa*in Resistance to an Antimicrobial Peptide.

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Pseudomonas aeruginosa is a gram negative bacillus bacterium known for its high degree of antimicrobial resistance and pathogenicity. Antimicrobial peptides (AMPs) are peptides, usually between 12 and 50 amino acids in length that possess antimicrobial activity. The mechanism of action of only a few AMPs is known. I am working with the synthetic AMP DASamp2 which has been shown to be effective against *P. aeruginosa* in liquid cultures and in biofilms. Our lab has used transposon mutagenesis of *P. aeruginosa* to try to elucidate the mechanism of action of DASamp2. We isolated 12 mutants with altered susceptibility to DASamp2. Of those isolated, the F2-1 mutant was very promising because its MIC was increased 8 fold. We found that the F2-1 mutant had a transposon inserted into the promoter region of the gene PA5189. PA5189 is predicted to encode a transcription factor of unknown function. We hypothesized that the transposon was causing overexpression of PA5189. Using qRT-PCR, I have observed that the level of PA5189 mRNA is 7 fold higher in the F2-1 mutant than in wild type PCR. This suggests that overexpression of the predicted transcription factor PA5189 can affect sensitivity of *P. aeruginosa* cells to an AMP.

General Microbiology Session III.

Identification of RUVBL1 and RUVBL2 as Novel Cellular Interactors of the Ebola Virus Nucleoprotein

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Ebola virus (EBOV) is a filovirus that causes a severe, often fatal hemorrhagic fever. Productive viral infection relies on successful recruitment of host factors for various stages of the viral life cycle. To date, several investigations have discovered specific host-pathogen interactions for various EBOV proteins, however, relatively little is known about EBOV nucleoprotein (NP) with regard to host interactions. In the present study, we aimed to elucidate NP-host protein-protein interactions (PPIs). Affinity purification-mass spectrometry (AP-MS) was used to identify candidate NP cellular interactors. Candidate interactors RUVBL1 and RUVBL2 were confirmed to interact with NP in co-immunoprecipitation (co-IP) and immunofluorescence (IF) experiments. Functional studies using a minigenome system revealed that siRNA-mediated knockdown of RUVBL1, but not RUVBL2, moderately decreased EBOV minigenome activity. Super resolution structured illumination microscopy (SIM) was used to identify an association between NP and components of the R2TP complex, which includes RUVBL1, RUVBL2, RPAP3, and PIH1D1, suggesting a potential role for the R2TP complex in capsid formation. Overall, we identify RUVBL1 and RUVBL2 as novel interactors of EBOV NP and for the first time report EBOV NP recruitment of the R2TP complex, which may provide novel targets for broad-acting anti-EBOV therapeutics.

Characteristics of Novel *Podoviridae* Isolated from Freshwater Samples Against *Pseudomonas fluorescens*.

Mackenzie E. Conrin (Masters)* and Avery Misfeldt

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Bacteria are responsible for many Healthcare-Associated infections each year and are evolving to become multidrug resistant (MDR). *Pseudomonads* are a class of bacteria that infect a variety of plants and animals and are known to gain resistance to multiple antibiotics. Bacteriophages, also called phages, are naturally occurring bacterial viruses. Isolating and characterizing these bacterial killers offers the potential for strain specific treatment of MDR infections. In screening freshwater samples from across Nebraska against a *Pseudomonas fluorescens* host, we have discovered a horde of nearly identical, novel phages of the *Podoviridae* family. These T7-like phages have stubby tails that allow them to attach to their host and deliver their genomic material. Using an array of microbiology and bioinformatic tools we have characterized these *Podoviridae* to determine plaque and virion morphology, genomic sequence and protein annotation. The genome was accepted for publication as a newly discovered phage sequence with the name, UNO-SLW (Accession KX431888). Our overall goal is to gain a deeper understanding of these phages and how they interact with their *P. fluorescens* host. This understanding will lead us closer to utilizing phages for the therapeutic treatment of *Pseudomonad* infections, including those that have become MDR.

Environmental Stress Sensors Maintain Characteristic Response Profiles across Diverse Stressors.

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The model organism *Bacillus subtilis* contains large cytoplasmic protein complexes known as stressosomes that are responsible for sensing stress through four RsbR (R) protein paralogs and then activating a general stress response. Each of these R proteins mediates a distinct, characteristic response pattern when exposed to ethanol as a stressor. We thus asked whether these response patterns would differ during exposure to other stressors. We engineered strains containing each R protein individually. Using a fluorescent reporter in conjunction with microfluidics, we visualized the stress response in single cells growing under uniform conditions over hundreds of generations. We discovered that each R protein responded with the same characteristic pattern irrespective of the stressor used, including ethanol, salt, oxidative stress, acid stress, and antibiotics. This result prompted us to ask which region of the protein is responsible for its characteristic response. Each R protein contains a conserved core region and a variable sensor region. Engineering hybrid proteins with a mix of these conserved and variable gene sequences allows us to ascertain which region of the protein truly manages the stress response by comparing the hybrid R protein response profiles with the previously observed individual R protein responses.

The ClpXP Protease Modulates the Chronological Lifespan of *Staphylococcus aureus*

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Bacterial pathogens like *Staphylococcus aureus* are generally well-adapted to counter starvation and exhibit extended chronological lifespans (CLS) wherein their reproductive potential is maintained long after stasis. However, cells that enter stationary phase following aerobic growth in media containing excess glucose rapidly lose their viability. The latter phenomenon was previously shown to be dependent on acetate, a byproduct of glucose catabolism that caused intracellular acidification and an increase in endogenous ROS. The mechanism/s that govern this rapid decline in CLS are poorly understood. Here, we reveal an unexpected role for the ClpXP protease in modulating CLS following intracellular acidification. Typically, the Clp protease is involved in the maintenance of protein homeostasis. However, inactivation of either *clpP* or its ATPase, *clpX*, increased CLS relative to the wild-type strain despite an increase in the levels of acid catalyzed toxic intracellular protein aggregates in these mutants. Furthermore, we demonstrate that the increased *clpXP* expression and activity in the wild-type strain following intracellular acidification results in the deterioration of the cellular anti-oxidant capacity, the consequence of which is a reduced CLS. Based on these findings, we propose that ClpXP may function to maintain fitness by reducing the CLS of damaged cells within a non-dividing population.

Environmental Microbiology Session I.

Enrichment and Genomic Characterization of a Novel Thermohaloanaerobic Bacterial Phylum from a Tertiary Oil Reservoir in Cushing, OK.

Archana Yadav (Doctoral)*, Ibrahim F. Farag, Javier Vilcaez-Perez, Noha H. Youssef, and Mostafa S. Elshahed.

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To characterize the response of subsurface microbial community to nutrient stimulation in oil reservoirs, microcosms were established using formation waters, isolated soy protein and crude oil from a hypersaline (17%), high temperature (50˚C) tertiary oil reservoir in Cushing, OK. After 100 days, 3 distinct microbial taxa were enriched i.e. *Deferribacter*, *Kosmotoga* and an OTU unaffiliated with all recognized bacterial phyla. Survey of global 16S rRNA datasets identified this lineage as rare member of microbial community in very few studies, predominantly anaerobic saline and hypersaline habitats. Using genome-resolved metagenomics, a near complete genome was recovered. Phylogenetic analysis using 120 protein coding genes confirmed its position as a novel bacterial phylum. Genomics analysis predicts a slow-growing, non-spore forming, and non-motile gram-negative rod. Metabolically, the organism is predicted to be a strict anaerobic, fermentative heterotroph, with chiefly aminolytic substrate preference along with sugars (glucose and mannose). Further, the identification of propionate degrading capacity, presence of hydrogenases, and lack of respiratory capacities suggests the possibility of syntrophic growth on propionate. Adaptation to high salinity and high temperature is mediated by the synthesis and uptake of osmoprotectants like glycine betaine, cDPG and glutamate. Efforts to isolate and visualize this novel lineage is currently underway.

The Rich Diversity of Delta Proteobacteria in Zodletone Spring, an Anoxic Sulfide-Rich Hydrocarbon Seep.

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The class Delta Proteobacteria encompasses microorganisms with a wide range of metabolic capacities, notably in the sulfur cycle. Improvements in sequencing technologies and computational methodologies enable access to the genomes of uncultured microorganisms and highlighted the unprecedented scope of diversity within the uncultured Delta Proteobacteria. The class has recently been split into 13 phyla, with the majority of the cultured representatives falling within 5 phyla. We seek to explore the identities, capabilities, and ecological roles of organisms from the remaining uncultured Delta Proteobacteria taxa in Zodletone Spring, an anoxic environment laden with sulfide and methane in southwestern Oklahoma. Metagenomic sequencing produced 44 genomic bins belonging to phyla formerly grouped in the Delta Proteobacteria. Here, we are focusing on genomes belonging to the phyla Desulfobacterota (class BSN033), UBA10199, MBNT15, and RBG-13-61-14 to characterize their role in elemental cycling in the spring sediment. Preliminary analysis suggests the ability of members of the BSN033 to perform dual nitrogen and sulfur cycling roles and of members of UBA10199 to perform dissimilatory nitrate reduction and denitrification.

Remediation of Produced water by Halophilic Microorganisms

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One of the largest waste streams in the oil and gas industry is produced water (PW). Estimates show that roughly 21 billion barrels per year in the US alone. PW is highly saline and contains heavy metals, dissolved and dispersed hydrocarbons, and naturally occurring radioactive materials. Hence, PW handling represents one of the more significant environmental challenges to the oil industry, not only from an environmental point of view, but also from a technical and cost view. The use of a microorganism, to at least remove the hydrocarbon portion of this water, could be highly cost effective and energy efficient and can be reused for fracking. We have developed microbial enrichment cultures from a crude oil-impacted brine in Kuwait. This culture is capable of degrading hydrocarbons at salinity ranging from 3 -32% NaCl. Currently we are evaluating this enrichment's ability to survive toxic PW and also degrade hydrocarbons in PW. Our preliminary studies with PW from Grant County, OK showed high toxicity towards our enrichment, consequently no degradation of hydrocarbons occurred even after incubation for > 3 weeks. However, upon dilution of the PW to 50% with tap water, the culture was able to completely degrade hydrocarbons in just 5 days.

Enrichment and Isolation of Aerobic Methane-Oxidizing Bacteria from Extreme Environments.

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Methane (CH_4) is a potent greenhouse gas that plays an important role in atmospheric chemistry and exerts a strong influence on global climate change. The predominant sink for CH_4 is biological oxidation by a group of phylogenetically-diverse, aerobic and anaerobic microorganisms known collectively as methanotrophs. Currently, not much is known about methanotrophy in extreme environments, where evidence of active CH_4 cycling exists. To fill this gap in our knowledge, we have investigated aerobic methanotrophy at Zodletone Spring, a sulfide- and CH_4-rich spring in southwestern Oklahoma. CH_4-oxidizing enrichment cultures were established from Zodletone Spring sediments. Enrichments were monitored for CH_4 oxidation over a period of several weeks using gas chromatography. Methanotrophs from these enrichments isolated on chemically defined agar media were then identified by amplification and sequencing of a fragment of the 16S rRNA gene. Enrichment cultures from Zodletone Spring were highly active, with some samples consuming as much as 400 µmoles of CH_4 over a period of 20 days. The cultivable fraction of the methanotrophic community within these enrichments was dominated by Type I methanotrophs most closely related to species of *Methylobacter* and *Methylomonas*. These results demonstrate that extreme environments have the potential to contribute to the global methane budget.

Medical Microbiology Session I.

Decreased Tricarboxylic Acid Cycle (TCA) in *Staphylococcus aureus* Increases Survival to Innate Immunity.

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The tricarboxylic acid (TCA) cycle is a key component in producing ATP. Within *Staphylococcus aureus*, resistance and virulence are easily transferred from one strain to another, making antibiotic resistance a major public health problem. While resistance is heavily studied, antibiotic tolerance is not. Persister cells are a subpopulation of dormant cells tolerant to antibiotic killing. This subpopulation is thought to be the underlying cause of many chronic and relapsing infection. Recent work has revealed that persister formation in *S. aureus* is dependent on lowered ATP levels. However, the underlying mechanism of persister formation is still unknown, as is the implications of persisters in terms of antibiotic tolerance. An organism's first line of defense is the innate immune system, including antimicrobial peptides (AMPs). AMPs are a key component of both the human and *Drosophila* innate immune system. Challenging *S. aureus* with the AMPs, LL-37 and hBD-3 revealed several logs of killing. Deletion of TCA cycle genes resulted in 100-fold more survival compared to wild type. Currently, experiments are being performed using a *Drosophila* model for infection. Preliminary data suggests that deletion of TCA cycle genes induces persister formation, and persisters pose a challenge for the innate immune response.

Synergy Among Outer Membrane Porins and β-Lactamase Enzymes is Associated with Ceftolozane/Tazobactam Resistance in *Klebsiella pneumoniae*

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K. pneumoniae can become resistant to β-lactams through loss of outer membrane porins: OmpK35, OmpK36 and/or PhoE. The purpose of this study was to evaluate the relationship between porin and β-lactamase production in clinical isolates of *K. pneumoniae* resistant to ceftolozane/tazobactam (C/T). We hypothesize that resistance to C/T by *K. pneumoniae* is due to loss of the outer membrane porins- OmpK35, OmpK36 and/or PhoE- in the presence of an ESBL and/or plasmid-encoded AmpC (pAmpC). E-tests and disc diffusion were used to determine susceptibility. Outer membranes and whole cell protein were assessed by western blot. 2 strains had all three porins, 18 were missing one porin, and 11 were missing two porins. Susceptibility varied across the porin phenotypes, with strains lacking in OmpK36 and/or PhoE more likely to be resistant to C/T. 7 isolates lacked all three porins, of which 5 were resistant. 2/7 susceptible isolates produced no ESBL or pAmpC. These data indicate that porin profile or β-lactamase type cannot predict C/T susceptibility in *K. pneumoniae*.

Parasite-Specific Secreted Protein ROP1 Acts as a Novel Therapeutic Target Against *Toxoplasma gondii*.

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The intracellular parasite *Toxoplasma gondii* infects 25-30% of the global population, with most exposed individuals experiencing only mild symptoms; however, severe clinical presentation occurs in patients with compromised immune systems and in the fetuses of pregnant women. Benzoquinone hydrazone (KG8) was previously demonstrated by our laboratory to reduce acute parasite burden *in vitro* and *in vivo*; therefore, we aimed to elucidate a potential target of KG8 in *T. gondii*. Our method implemented a random chemical mutagenesis with ethyl methanesulfonate and a subsequent increase in compound pressure to select resistant parasite populations. Isolates were subjected to whole-genome sequencing and analyzed for mutations conferring resistance with an in-house bioinformatics pipeline. Results showed that a single nonsynonymous C→T (P207S) mutation in the secreted protein ROP1 generated a 6.3-fold increase in the IC_{50} of KG8 in mutant populations relative to the unmutated controls. PCR hypermutagenesis of this gene enhanced the observed resistance to 12.6-fold that of the parent strain. Subsequent *in silico* analyses provided evidence for putative low-energy binding interactions between ROP1 and KG8 and predicted a previously-unreported phosphotransferase domain within this highly-disordered protein. These data support the use of ROP1 as a novel and parasite-specific pharmaceutical target against *T. gondii*.

Medical Microbiology Session II.

CO-ADMINISTRATION OF FDA-APPROVED DRUGS ELICITS A SIGNIFICANT DECREASE IN CHRONIC *TOXOPLASMA GONDII* INFECTION CYST LOAD

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The apicomplexan parasite *Toxoplasma gondii* is able to infect virtually all warm-blooded animals and is estimated to infect up to 30% of the human world population. The chronic stage of infection, in which the parasite resides as intracellular cysts in host muscles, organs, and the brain, is lifelong and currently has no treatments available to alleviate infection. Chronic infection is thought to be asymptomatic in immunocompetent individuals, although more studies have begun to look at associations between chronic *T. gondii* infection and neurobehavioral disorders such as schizophrenia, bipolar disorder I, and epilepsy. Several FDA-approved drugs have been shown to significantly decrease *T. gondii* cysts in hosts, but none of the drugs have completely eliminated infection when administered alone. The goal of this study was to evaluate combinations of FDA-approved drugs against chronic *T. gondii* infection in order to completely eradicate the chronic cyst stage of infection or decrease cyst burden more than previously described. We hypothesized that we could significantly reduce cyst burden in *T. gondii* chronically infected mice by combining FDA-approved drug treatments that have shown efficacy against *T. gondii* infection.

Specific Iron Transporters are Required for Entry of the Novel Siderophore Cephalosporin, Cerdiferocol, in *Escherichia coli* and *Pseudomonas aeruginosa*.

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The novel siderophore cephalosporin, Cerdiferocol, has shown potent antibacterial activity in Gram-negative bacteria. Three iron transporters have been linked to the effectiveness of the drug: CirA and Fiu in *Escherichia coli* and PiuA in *Pseudomonas aeruginosa*. Upon deletion of the iron transporter genes, the minimal inhibitory concentrations of Cerdiferocol were increased 16-fold. Indicating an important role in the process of transporting the drug across the outer membrane. The long-term goal of this study is to characterize these iron transporters in clinical isolates of *E. coli* and *P. aeruginosa*. The purpose of this study was to obtain preliminary data on the sequences of these iron transporters to identify wild types strains that could be used as controls when comparing the iron transporters among clinical isolates. Primers for PCR analysis were created and optimized to produce single bands. The PCR product was purified using Amicon® Ultra Centrifugal Filters and sent for sequence analysis. Sequencing results have been obtained and are currently being analyzed by comparing the sequence obtained to the sequences of *E. coli* BW25113 and *P. aeruginosa* PAO1. Analysis will determine if the strains sequenced from our laboratory can be used as control strains for further studies.

Antifungal Activity of Dendritic Cell Lysosomal Proteins against *Cryptococcus neoformans*.

Benjamin Nelson (Doctoral)*, Savannah Beakley, Brittney Conn, Emma Maritz, Sierra Posey, and Karen L. Wozniak.

Oklahoma State University, Stillwater, OK.

Cryptococcus neoformans is an opportunistic fungal pathogen that causes over 225,000 yearly cases of cryptococcal meningitis, leading to 180,000 annual deaths in HIV/AIDS patients. Innate phagocytes, such as macrophages and dendritic cells (DCs) are the first cells to interact with the pathogen. DCs kill *C. neoformans* by degradation within the protein rich lysosome. It has been shown that purified DC lysosomal extracts as well as fractionated lysosomal extracts can kill the pathogen *in vitro*. Furthermore, purified cathepsin B, a lysosomal protein, has also been shown to kill *C. neoformans*, and because many fractions can kill the fungus, we hypothesize that other lysosomal proteins can do the same. Mass spectrometry of the lysosomal extract identified >3000 different proteins. Purified lysosomal proteins were incubated with *C. neoformans* to screen for killing. Antifungal proteins were further tested with dose dependent assays to show if titration of the protein had any effect. Finally, these proteins were tested for cytotoxicity using J774 macrophages. Results showed that several proteins were able to kill the fungus and some were effective over a wide range of concentrations. Also, all tested proteins had low cytotoxic effects. Future studies will examine mechanism(s) of fungal killing by lysosomal proteins.

Medical Microbiology Session III.

Preliminary Characterization of Pan-Parasitic Experimental Compounds against *Toxoplasma gondii* using *in vitro* and *in vivo* Models.

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University of Nebraska Medical Center, Omaha, Nebraska[a]; University of Nebraska at Omaha, Omaha, Nebraska[b].

Toxoplasma gondii is an obligate intracellular parasite that has infected nearly 60 million individuals in the United States alone. Acute infection causes ill and potentially lethal effects to immunocompromised individuals, and developing fetuses when mothers become infected during pregnancy. The aim of this research was to screen a family of experimental compounds that had previously been shown to be active against a panel of apicomplexan and helminth parasites for activity against acute *T. gondii* infection. Both an *in vitro* and murine *in vivo* models were utilized to determine overall efficacy of these compounds, potential host cell toxicity, and to drive further design and synthesize of derivative compounds. Three highly potent and selective compounds were evaluated for success in clearing a lethal acute infection of a murine model. One compound was successful at permitting 100% survival with no apparent toxicity or subsequent ill effects, indicating successful clearance of a lethal acute infection. Further classification of the compound will be performed to determine possible mode of action as well as new compound derivatives to be evaluated.

Interplay between CTX-M and OmpC Production in *Escherichia coli* are Important for Carbapenem Resistance

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OmpC porin downregulation with extended-spectrum β-lactamase (ESBL) production is associated with carbapenem resistance. This study evaluated the interplay between porin and CTX-M production in the emergence of carbapenem resistance. CTX-M transformants and a transconjugant were generated in BW and *ΔompC* strains. Conjugation was performed by filter mating. Vectors overexpressing CTX-M-14/CTX-M-15 were transformed by electroporation. Expression was analyzed by qRT-PCR. Cefotaxime and carbapenem minimum inhibitory concentrations were measured by Etest®. BW and *ΔompC* were susceptible to all evaluated antibiotics (0.003-0.19 μg/mL). The clinical isolate donor was cefotaxime-resistant (>256 μg/mL) and carbapenem-susceptible (≤0.38 μg/mL). The CTX-M-14 transconjugant was carbapenem-susceptible (≤0.25 μg/mL). BW CTX-M-14/CTX-M-15 transformants were cefotaxime-resistant (>256 μg/mL) and carbapenem-susceptible (≤0.25 μg/mL). The *ΔompC* CTX-M-14/CTX-M-15 transformants were carbapenem-resistant (1->32 μg/mL). $bla_{\text{CTX-M}}$ expression in all transformants was 8-12-fold higher than the clinical isolate. The transconjugant and donor had no difference in expression. These data suggest a threshold of ESBL expression is required for carbapenem resistance in the absence of OmpC. An 8-fold increase in ESBL expression was sufficient for carbapenem resistance in the absence of OmpC but lower expression levels were not. Carbapenem resistance in the presence of an ESBL but not a carbapenemase may not always predict the mechanism of carbapenem resistance.

A Putative Phytase, CarP, Required for Calcium Tolerance and Virulence in *P. aeruginosa,* is Differentially Regulated by Host Factors.

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Pseudomonas aeruginosa is an opportunistic pathogen causing life-threatening infections. Previously, we showed that elevated calcium (Ca^{2+}) increases production of virulence factors and infectivity. In the effort to characterize Ca^{2+} regulatory network, we have identified a **Ca**$^{2+}$–**r**egulated β-**p**ropeller protein, named CarP. CarP is required for Ca^{2+} tolerance, maintenance of intracellular Ca^{2+} homeostasis, and Ca^{2+}-regulated production of pyocyanin. Here we present that CarP plays a major role in Ca^{2+}-induced induction of pyoverdine and virulence in *Galleria mellonella*. To better understand the role of CarP in *P. aeruginosa* virulence and interactions with a host, we studied transcriptional regulation of *carP*. After an *in silico* analysis of *carP* intergenic region, we cloned the predicted regulatory elements upstream of promoterless *lux* operon and monitored the luminescence. Only the fragments containing 36 bp of *carP* 5' showed a response to Ca^{2+}, which required CarR for induction. These fragments also responded to = iron and oxidative stress. The transcriptional profiles and challenging with cell supernatants suggested a regulatory role of an unknown QS molecule in a CarR- and Ca^{2+}-dependent manner. Overall, the complex transcriptional regulation of *carP* in response to multiple factors associated with a host, suggests importance of this protein in *P. aeruginosa* infections.

Medical Microbiology Session IV.

Redox Regulation of *Borrelia burgdorferi* Gene Expression.

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Borrelia burgdorferi, the causative agent of Lyme disease, is naturally maintained in various mammalian hosts and its tick vector *Ixodes scapularis*. The ability of *B. burgdorferi* to successfully complete its infectious cycle depends on the regulation of a repertoire of lipoproteins. Lipoprotein expression is controlled by a limited number of transcription factors (including the alternative sigma factor RpoS) in response to environmental challenges encountered in hosts during infection, including changes in pH, temperature, osmolarity, and nutrient availability. Due to *B. burgdorferi*'s reliance on host signals to regulate lipoprotein expression, metabolic changes in hosts have widespread implications for *B. burgdorferi* gene expression. While within *I. scapularis*, *B. burgdorferi* encounters host-derived reactive oxygen species (ROS) and reactive nitrogen species (RNS)—both of which are byproducts of host metabolism and the roles of which have yet to be described in *B. burgdorferi* infectivity. In the following study, we tested the hypothesis that ROS and RNS serve as regulatory signals for *B. burgdorferi*. Surprisingly, sublethal concentrations of H_2O_2 or sper/NO triggered widespread changes in gene expression, particularly in genes regulated by RpoS. These data suggest ROS and RNS contribute to the regulation of genes required for *B. burgdorferi* to complete its natural infectious cycle.

Mechanisms Contributing to Ca^{2+}-Induced Polymyxin B Resistance in *Pseudomonas aeruginosa.*

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Pseudomonas aeruginosa is responsible for fatal chronic lung infections in cystic fibrosis patients. It is also the third leading cause of nosocomial infections. Treatment of *P. aeruginosa* infections is becoming increasingly challenging with the occurrence of multidrug resistant isolates. Therefore, cationic peptides such as polymyxin B have made a comeback as the "last hope" therapeutic agents. Here we show that the presence of elevated levels of calcium (Ca^{2+}) plays an important role in the development of resistance in *P. aeruginosa* to polymyxin B. Through chemical mutagenesis, we identified three genes: PA2803, PA3237, and PA5317 that contribute to Ca^{2+}-enhanced resistance to polymyxin B. This was confirmed by susceptibility assays of the corresponding transposon mutants and in-trans complemented strains. Genome-wide RNA-Seq analyses revealed that the transcription of PA2803 and PA3237 is significantly upregulated in the presence of 5 mM Ca^{2+}. However, the transcription of the two component systems PhoPQ, PmrAB and ParRS, earlier shown to control resistance to polymyxin B in *P. aeruginosa,* are down-regulated by Ca^{2+}. These observations suggest involvement of unique Ca^{2+}-dependent mechanisms. We aim to elucidate the role of the identified genes in Ca^{2+}-driven resistance to polymyxin B, which will provide insights into antibiotic resistance mechanisms in *P. aeruginosa.*

***In Vivo* Proximity Labeling to Identify a Novel Eukaryotic Protein Recruited to the Chlamydial Inclusion.**

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Chlamydia trachomatis (Ctr) is the leading cause of bacterial sexually transmitted infections. Ctr, an obligate intracellular bacterium, resides within a membrane-bound vacuole termed an inclusion. *Chlamydia* modifies the inclusion with inclusion membrane proteins (Incs), the functions of which are unknown. Bacterial two-hybrid analysis found some Incs (e.g. IncF) interacted with numerous Incs while others (e.g. IncA) did not. We hypothesize that some Incs organize the inclusion through Inc-Inc interactions whereas other Incs promote chlamydial-host interactions by binding eukaryotic proteins. To test our hypothesis, we implemented an ascorbate peroxidase proximity labeling system (APEX2), which labels proximal proteins with biotin *in vivo*. We transformed Ctr with plasmids encoding IncF-APEX2, $IncA_{TM(transmembrane\ domain)}$-APEX2, IncA-APEX2, or APEX2. Affinity purification-mass spectrometry was used to identify biotinylated human and chlamydial proteins from five biological replicates. The interaction data were analyzed and revealed all Inc-APEX2 constructs labeled eukaryotic proteins in a proximity-dependent manner. Consistent with our hypothesis, IncF-APEX2 biotinylated more Inc proteins whereas IncA-APEX2 biotinylated more unique eukaryotic proteins. One eukaryotic protein, LRRF1, was biotinylated by all Inc-APEX2 constructs, indicating a high affinity for the inclusion membrane that was validated by immunofluorescence. Our data highlight the utility of APEX2 to map protein-protein interactions at the Ctr inclusion membrane.

Undergraduate/High School Session I.

A Structural Analysis of Human Herpesvirus 8 Polyadenylated Nuclear RNA through SHAPE-MaP Analysis.

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Kaposi's sarcoma-associated herpesvirus (KSHV also called Human Herpesvirus 8 or HHV8) is a herpesvirus linked to Kaposi's sarcoma and two forms of lymph node cancer. HHV8 infects cells and replicates its viral genetic material, creating an RNA transcript with no known protein coding function. The lncRNA encoded by HHV8 is known as polyadenylated nuclear RNA (PAN RNA). PAN RNA has been shown to promote important functions of HHV8, such as gene expression, replication, and immune modulation, despite PAN RNA's lack of direct protein translation. PAN has been shown to associate with multiple transcription related complexes that promote lytic replication including k-Rta, the driving transcription factor of the HHV8 lytic cycle. Analysis of the structure of the PAN RNA molecule gives perspective on how the molecule performs its role in the lytic cycle. The SHAPE-MaP method chemically modifies the molecule, converts it into cDNA wherein modified nucleotides generate mutations. By sequencing the molecule, the shape of the molecule can be inferred through algorithmic analysis. Using the exceptionally accurate SHAPE-MaP method to analyze PAN structure will refine the current understanding of the secondary structure of the molecule.

Characterization of *Staphylococcus lugdunensis* Biofilms.

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Like *Staphylococcus aureus* and *Staphylococcus epidermidis*, *Staphylococcus lugdunensis* can be found on the skin as normal flora. While *S. aureus* has been the primary focus of the medical community, there are new concerns that a related bacterium, *S. lugdunensis,* has been responsible for biofilm-induced infections, similar to those caused by *S. aureus* and *S. epidermidis*. Contributing to the pathogenic nature of this organism is its ability to form a biofilm, the culprit behind cases of endocarditis and severe prosthetic joint infections. To further characterize, we tested antibiotic susceptibility of *S. lugdunensis* biofilms, as well as whether the biofilms were protein mediated. To identify genetic factors essential for biofilm formation in *S. lugdunensis*, we mutagenized *S. lugdunensis* by treating with ethyl methanesulfonate (EMS). Individual mutagenized cells were separated using a cell sorter and examined for biofilm formation at different timepoints. Mutations resulting in high biofilm and low biofilm formers were sequenced to identify genes responsible for the phenotypes. A mutation within the *S. lugdunensis* surface protein A (*slsA)* gene was common among all the low biofilm formers suggesting high expression of this protein is important in biofilm formation. Currently, a genetic knockout is being constructed to confirm these results.

Do Dental Unit Waterline Maintenance Tablets Inhibit Bacterial Growth In Dental Line Water?.

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Dental offices must maintain dental unit waterlines to prevent infections in patients. *Mycobacterium abscessus* infected over 72 children in the United States from inadequate water quality maintenance procedures. This experiment will determine if two FDA-approved maintenance tablet brands, BluTab® and Atdec ICX®, are effective at inhibiting specific microbes from dental unit waterlines. The hypothesis was that air syringes would harbor more bacteria than bottles. Office A utilized BluTab® diluted in municipal water, and Office B used Atdec ICX® diluted in distilled water. Water samples were taken in duplicate from 5 operatories at each dental office, with one sample taken directly from treated, 1 L bottles and one from air-water syringes at the end of the line. Water samples were plated onto EMB, Cetrimide, and Salivarius/mitis agar incubated at 25°C for 24-72 hours and colonies were counted. No *Pseudomonas* was recovered. Overall significance (p = 0.0003) revealed Office A harbored more bacteria (CFU/ml) in operatories 2 and 4 in the air syringe. Office A grew more bacteria (p = 0.02) on Salivarius/mitis agar than EMB agar. Gram positive microbes appear viable in the municipal water or water lines. This research can help dental offices determine effective disinfection methods for dental units.

Does Calcium Regulate Rhamnolipid Production in *Pseudomonas aeruginosa?*

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Pseudomonas aeruginosa is a Gram-negative, opportunistic pathogen that causes severe infections, both acute and chronic. *P. aeruginosa* can swim, twitch and swarm, which contribute to its ability to colonize and spread infection. Previously, we have shown that elevated calcium (Ca^{2+}) increases the pathogen's motility and virulence. Bacterial swarming relies on the production of rhamnolipid, a biosurfactant lowering the surface tension and facilitating swarming. In order to identify the mechanism of Ca^{2+} regulation of swarming, we have tested the effect of Ca^{2+} on rhamnolipid production by monitoring the expression of P*rhlAB*-gfp fusion. In agreement with the RNAseq analyses, P*rhlAB*-gfp activity increased with the increase of Ca^{2+}. To identify the components of Ca^{2+} signaling network mediating Ca^{2+} regulation of *rhlAB*, we monitored P*rhlAB*-gfp activity in the mutants lacking such components. So far, the mutant lacking *calC*, a Ca^{2+} leak channel, showed abolished Ca^{2+} induction of *rhlAB* expression, suggesting the importance of the intracellular Ca^{2+} in this regulation. We are currently validating the promoter data by testing rhamnolipid production using plate-assay. This project will elucidate the role of the *P. aeruginosa* Ca^{2+} signaling network in regulating rhamnolipid biosynthesis and swarming, that both contribute to virulence of the pathogen.

Undergraduate/High School Session II.

The Effect of Peppermint Oil on Biofilm Development in *Staphylococcus epidermidis* Isolates.

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As part of the human epidermal microbiota, *Staphylococcus epidermidis* can cause nosocomial infections, particularly if capable of producing a biofilm. This project investigates the ability of peppermint oil to decrease biofilm formation in *S. epidermidi*s. Following an initial trial with five essential oils, peppermint oil was shown to decrease biofilm production and was selected for further study. Fourteen isolates which had been previously characterized as moderate or high biofilm producers were chosen. Strains were grown overnight on tryptic soy agar, transferred to tubes of phosphate buffered saline (PBS), and standardized. Aliquots of *S. epidermidis* were transferred to 96-well plates containing tryptic soy broth supplemented with 1% glucose and then topped with aliquots of peppermint oil of varying concentrations. Four replicates of 12 wells were conducted for each strain at each oil concentration. Growth was determined using spectrophotometry. Wells were then emptied, washed with PBS, and dried. The biofilm was stained with crystal violet which was then solubilized with acetic acid before quantifying by spectrophotometry. The total growth and biofilm development for each concentration of oil was compared to the control. Biofilm production was also compared between moderate and high biofilm producing isolates at various oil concentrations.

Abnormal Ion Concentration in Cystic Fibrosis Lungs Impact Rhamnolipid Production in *Pseudomonas aeruginosa*.

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Pseudomonas aeruginosa can cause severe acute and chronic infections in immunocompromised patients, most notably infecting the lungs of cystic fibrosis (CF) patients. One of the mechanisms of virulence in the pathogen is its ability to swarm. Swarming motility contributes to the formation of biofilms and relies on the production of glycolipid biosurfactant rhamnolipid. In order to combat *P. aeruginosa* infections in CF patients, there is a need for a better understanding of how the abnormal chemical conditions in the lungs affect the virulence of the pathogen. We first aimed to determine the impact of several conditions commonly associated with CF lungs on the expression of *rhlA,* required for rhamnolipid biosynthesis. For this, we used a genetic construct (P*rhlA-gfp* fusion), containing *rhlA* promoter upstream of *gfp,* encoding green fluorescent protein. Thus far, we have determined that elevated to 5% CO_2 abolished *rhlA* transcription and that the presence of 10 mM Ca^{2+} increased *rhlA* promoter activity. We are currently validating these results by testing rhamnolipid production using methylene blue/cetyl trimethylammonium bromide plate assay. These results will generate an insight about the specific conditions associated with the host that regulate rhamnolipid production and therefore virulence of *P. aeruginosa*.

Candida albicans Induces Multidrug Tolerance in _Staphylococcus aureus_ through Energy Depletion.

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Many relapsing *Staphylococcus aureus* infections are mediated by biofilm formation. Polymicrobial biofilm and planktonic cultures formed by *S. aureus* and the fungal organism *Candida albicans* are 10- to 100-fold more tolerant to a variety of antibiotics when compared to cultures containing *S. aureus* alone. No suitable explanations for increased multidrug tolerance have been proposed. Most antibiotics freely diffused through polymicrobial biofilms. The ability of vancomycin to bind to *S. aureus* did not appear to be affected by the presence of *C. albicans.* The possibility of increased tolerance through secreted products was ruled out after concentrated *C. albicans* supernatant was unable to increase tolerance. Growth in either spent *C. albicans* or *S. aureus* media increased tolerance 100-fold compared to fresh media. Based on findings that *S. aureus* forms drug tolerant cells through ATP depletion, we examined nutrient exhaustion and subsequently decreased ATP production. Extracellular glucose assays confirmed that polymicrobial cultures depleted available glucose faster than monocultures. *S. aureus* exhibited lower levels of TCA cycle activity when grown in the presence of *C. albicans*. Furthermore, *S. aureus* cells in polymicrobial cultures displayed lower membrane potential than those in monocultures. Polymicrobial cultures also displayed lower intracellular ATP levels. Collectively, these data demonstrate lowered metabolic activity through nutrient deprivation is a mechanism for increased multidrug tolerance within polymicrobial cultures.

Innate Immune Cell Activation by Antischistosomal Compound SAS1.

Samantha Sack (Undergraduate)*, Caelyn Armshaw, and Paul Davis

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Left untreated, the neglected tropical disease schistosomiasis can persist for years and cause vital organ damage. Praziquantel, the currently prescribed treatment, is effective against mature parasites but fails to eradicate juvenile worm infection. The antischistosomal compound SAS1 has shown greater efficacy *in vivo* against juvenile and mature parasites in both murine and primate models. However, this anthelminthic activity does not translate *in vitro*, indicative of a host-dependent mechanism of action. It is hypothesized that SAS1 facilitates the activation of the host immune system, specifically priming murine eosinophils to undergo increased levels of degranulation following stimulation. To test this, male mice were orally administered SAS1 or vehicle control. Splenocyte suspensions were obtained and stimulated with secretagogue. In order to quantify degranulation, the release of granulocytic proteins eosinophil peroxidase and superoxide anions were measured by enzyme-linked immunosorbent assay and spectrophotometric analysis of the reduction of cytochrome c, respectively. Preliminary results reveal that the experimental drug increased innate immune cell degranulation following low levels of stimulation compared to controls. Confirmation of the mode of action may progress the release of SAS1 as a clinical treatment for schistosomiasis in order to combat resistance to Praziquantel and introduce treatment early in infection.

Undergraduate/High School Session III.

Targeted Therapy for the Future- The Use of Novel Antimicrobial Peptides against *P. aeruginosa*

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Pathogenic bacteria, such as the gram-negative bacterium *Pseudomonas aeruginosa,* are becoming resistant to our current arsenal of antibiotics at an alarming rate. *P. aeruginosa* is a leading cause of nosocomial acquired infections and is a primary co-morbidity in patients with compromised immune systems. One potential source of new antibiotic agents is antimicrobial peptides. Antimicrobial peptides (AMPs) are small proteins, and some have shown a high degree of efficacy and broad-spectrum activity against both Gram-positive and Gram-negative bacteria. An experimental AMP has been developed at UNMC, DASamp2, has shown to be effective against virulent bacteria, including *P. aeruginosa*. To help assess the usefulness of DASamp2, we have isolated a mutant strain with 8-fold resistance to the compound. Previous work established that the gene *mexT* might play a role in the increased resistance to DASamp2 observed in the mutant strain. This gene is responsible for encoding a transcription activator that affects the expression of the *MexEF-OprN* efflux pump and that the target of DASamp2 must lay beyond the cell wall of *P. aeruginosa*. Our goal was to confirm that *mexT* was responsible for the increased resistance by restoring wild-type sensitivity to the experimental compound in the mutant strains.

Utilizing Chemical Mutagenesis to Determine SW33 Mechanism of Action in *Toxoplasma Gondii.*

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The intracellular parasite *Toxoplasma gondii* (*T. gondii*) infects up to one third of the world's population and is the causative agent of toxoplasmosis. In immunocompromised hosts ocular, pulmonary, and central nervous system diseases can occur. Current treatment against *T. gondii* consists of pyrimethamine, a dihydrofolate reductase inhibitor that shows toxicity in humans. Due to the limitations in effective toxoplasmosis treatments, much work has been done to develop new drugs. An experimental compound, SW33, has shown promising results *in vivo* and *in vitro* while exhibiting low toxicity in the host. Before SW33 can undergo further development, a known or suspected mechanism of action (MoA) is required. One method to determine the MoA is chemical mutagenesis. Often, a MoA involves the binding of the drug to a specific protein. Randomly dispersed single nucleotide variances can be introduced into the genome with a chemical mutagen and populations can then be selected for under various drug concentrations. When a mutation is introduced at the drug binding site, resistance develops. A resistant population can then be clonally isolated and sequenced to find the drug target. Therefore, a chemical mutagenesis approach can be used to characterize experimental compounds such as SW33 to determine its likely MoA.

Decreased Tricarboxylic Acid (TCA) Cycle Activity in *Staphylococcus aureus* Increases Survival to Innate Immunity.

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Staphylococcus aureus is a bacterial pathogen that is responsible for over one million infections a year. A major concern regarding *S. aureus* infections stems from failure to respond to antibiotic therapy. One hypothesis for this phenomenon is persister cell formation. Persister cells are a subtype of dormant cells that have a high tolerance to antibiotics making them harder to treat in a medical setting. Recent work demonstrated that persisters form when low intracellular ATP is present. Specifically, when the tricarboxylic acid (TCA) cycle is disrupted an increase in antibiotic tolerance is observed. We hypothesized that persisters not only were problematic for antibiotic treatment but also to components of innate immunity that resemble antibiotics in function (antimicrobial peptides). Previous experiments showed that when the TCA cycle gene, *fumC*, was disrupted, a higher tolerance to antimicrobial peptides was observed. Preliminary studies also revealed the *fumC* mutant had higher survival than wild type *S. aureus* within macrophages. To discern the underlying mechanism for this phenotype, reactive oxygen species, reactive nitrogen species, and pH were examined more closely. Early results suggest the *fumC* knockout is better able to withstand the reactive oxygen stress at an acidic pH compared to wild type *S. aureus*.

Poster Number	Graduate Student - Poster Presentation	
A1	Brigette N. Corder	Efficacy of Novel Universal Influenza Vaccine Immunogens
A2	Charlotte E. Key	Recruitment and Characterization of Immune Cell Populations in a *Chlamydia trachomatis* Murine Infection Model
A3	Itzela A. Cruz-Solanilla	Characterization of a Novel Glycerol-3-Phosphate Dehydrogenase (GPD2) in the Alga *Chlamydomonas Reinhardtii*
A4	Lisa M. Jorgenson	Determining Protein-Protein Interactions between Eukaryotic SNARE Proteins and Chlamydial Inclusion Membrane Proteins
A5	Chelsea L. Murphy	Oribtrap-based Shotgun Proteomics for the Identification of Extraradical Proteins from *Rhizophagus irregularis*
A6	Nathan Hatch	Investigating Amino Acid Starvation Responses in *Chlamydia trachomatis*
A7	Deepali Luthra	Effect of high Ca^{2+} in Enhancing Adherence of *Pseudomonas aeruginosa* to Lung Epithelial Cells
A8	William K. Boyle	Development an *in vitro* transcription assay for *Borrelia burgdorferi* transcription factor DksA
A9	Madeline Toews	How do *Bacillus subtilis* RsbR Paralogs Affect Fitness and Sense Stress?
A10	Benjamin Nelson	Interactions of *Cryptococcus neoformans* with Human Airway Phagocytes
A11	Colleen La Force	Cloning and Expression of *Chlamydia trachomatis* Inclusion Membrane Proteins
A12	Justin Bowen	Characterization of the Nitrogen Assimilation Regulator (*gln* G) Role in *Escherichia coli* Colonization of the Mammalian Intestines
A13	Brian A. Dillard	Revisiting the Oil Fly Bacteria from the La Brea Tarpits
A14	Joshua Wiggins	Blocking HIV Infection Using Engineered *Lactobacillus*
A15	Joel Frandsen	Characterization of Morin Derivatives for Enhancement of Neural Glyoxalase Pathway
A16	Ashlee Hawkins	Analysis of the Interactions Between *C. neoformans* and Pulmonary Macrophages
A17	Sean Carr	Redirecting Carbon and Energy Towards Isoprene as an Anaerobic Respiration/Fermentation Product
Poster Number	**Undergraduate/High School Poster Presentation**	
B1	Seth Ostdiek	Persister Formation in *Staphylococcus epidermidis* Clinical Isolates
B2	Trevor Daubert	Characterization of the Survival and Immunogenicity of *Staphylococcus aureus* Persisters within Macrophages
B3	Ty Derouen	Protein kinase A manipulation by *Chlamydia trachomatis* during infection
B4	Rohan Das	Rapid Qualitative Colorimetric Viability Analysis of *Planctomycete* Bacterium
B5	Caitlin Ingram	Determining the Prevalence of Vectored Diseases in Elkhorn Valley *Dermacentor variabilis* in Nebraska
B6	Tyler Rollman	Buggy Creek virus Phenotype Dynamics in Swallow Bugs (*Oeciacus vicarius*)
B7	Dominique Poncelet	Community Analysis of Methane-Oxidizing Bacteria
B8	Steven R. Bibly	The Development of a Reverse Genetic System for Influenza A
B9	Mary Kate Morrissey	Transcriptional Regulation of Calcium Sensor, EfhP, in the Human Pathogen *Pseudomonas aeruginosa*
B10	Patricia Harte-Maxwell	Analysis of RNAseq Data Reveals Media State Dependent Transcript Profile in *Candida albicans*
B11	Tyler D. Wikoff	Identification and Characterization of Large Repeat Sequences Throughout the *Candida albicans G* enome
B12	Danielle Sedgwick	*Manduca Sexta* as a Model of Intestinal Colonization
B13	Michael Backer	Overexpression and Purification of the *Borrelia burgdorferi* Gene Regulatory Protein BosR

Graduate Student Poster Session.

Efficacy of Novel Universal Influenza Vaccine Immunogens.

Brigette N. Corder (Doctoral)*, Brianna L. Bullard, & Eric A. Weaver

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Every year Influenza infects 10-50 million people in the United States. The current vaccination methods only protect against certain strains and provide limited protection against infection. Therefore, creating universal vaccine immunogens which induce broad protection against influenza is imperative. Hemagglutinin (HA), the viral surface glycoprotein, is a target for many influenza vaccines including our current study. Previous studies show that a centralized consensus HA vaccine provides high levels of broadly-protective immunity against several H1N1 influenza challenge strains. To improve the vaccine efficacy, we constructed two novel universal influenza vaccine immunogens based solely on unique sequences. First, a consensus of unique H1 HA sequences (CoUS) was designed using the most common amino acid at each location in the HA sequence. Second, a mosaic HA immunogen was constructed by repeating *in silico* recombination events and favoring repetitive 9-mers to include the most common potential B and T-cell epitopes. Preliminary data shows that, when mismatched, our novel vaccine immunogens provide higher T-cell responses as compared to wild-type comparator genes. In addition, both the Mosaic and CoUS strategies are able to recognize more epitopes from across the H1 serotype. This project will reveal the most promising HA immunogens for a universal influenza vaccine.

Recruitment and Characterization of Immune Cell Populations in a *Chlamydia trachomatis* Murine Infection Model

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Chlamydia trachomatis is an obligate intracellular organism that is the leading cause of preventable blindness and sexually transmitted bacterial infections. *C. trachomatis* exhibits a biphasic developmental cycle involving infectious elementary bodies (EB) and non-infectious, replicative reticulate bodies (RBs). At the end of its developmental cycle, EBs disseminate to neighboring cells either via host cell lysis or a novel mechanism of exit, extrusion. It has been hypothesized that extrusions provide a means to evade recognition by the immune system due to their enclosure within host membrane Herein, female mice were intra-vaginally infected with either a *C. trachomatis* serovar L2 wild type strain or a mutant strain containing a silenced CT228 gene, which produces significantly more extrusions *in vitro*, relative to the wild type. For this study, whole reproductive tracts were digested, and immune cells were collected for flow cytometry to quantitate and characterize immune cell populations recruited to the reproductive tracts of mice infected with wild type versus the mutant strain. From the data that was obtained, it was determined that there were no significant differences amongst the concentrations of immune cell populations between the L2 wild type infected mice versus the mutant infected mice.

Characterization of a Novel Glycerol-3-Phosphate Dehydrogenase (GPD2) in the Alga *Chlamydomonas Reinhardtii.*

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The green alga *Chlamydomonas reinhardtii*, like many eukaryotic microalgae, accumulates triacylglycerol (TAG) under certain environmental stresses, such as nitrogen deprivation. TAG is of interest because it is an essential precursor for biofuel production. Canonical glycerol-3-phosphate dehydrogenases catalyze the synthesis of glycerol-3-phosphate (G3P), a key precursor for glycerolipid and TAG synthesis in eukaryotes. The *C. reinhardtii* genome encodes five GPD homologs. Interestingly, GPD2 is a novel multidomain enzyme, consisting of a phosphatase motif fused to a G3P dehydrogenase domain. GPD2 expression is significantly up-regulated under nutrient deprivation or high salinity, coincidental with the accumulation of TAG or glycerol. Conversely, RNA-mediated silencing of GPD2 reduced TAG and glycerol production under the same stresses. Based on these observations, we hypothesize that GPD2 contributes to the synthesis of both glycerol and TAG, depending on the environmental conditions. Thus, one of our goals is to decipher how the enzymatic activities (i.e., dehydrogenase and phosphatase) of GPD2 are regulated under different environmental conditions. Finding how cells regulate GPD2 enzymatic activities, and what other components GPD2 may interact with, may contribute to broadening our biochemical and cytological understanding of algal TAG and glycerol metabolic pathways, with possible implications for biotechnological biofuel/biomaterial production.

Determining Protein-Protein Interactions between Eukaryotic SNARE Proteins and Chlamydial Inclusion Membrane Proteins.

Lisa M. Jorgenson (Doctoral)*, Scot P. Ouellette, Elizabeth A. Rucks.

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Chlamydia trachomatis (Ct) is the leading cause of bacterial sexually transmitted infections. Throughout the chlamydial developmental cycle, organisms grow within a membrane-bound vacuole termed an inclusion. The inclusion membrane (IM) interacts with the host and is comprised of host and chlamydial lipids and proteins. *Ct* modifies the IM with a family of proteins called Incs. Incs are thought to recruit host proteins for the benefit of *Ct.* We hypothesize that Incs recruit eukaryotic proteins by functionally mimicking the host proteins or the binding partners for these proteins. To test our hypothesis, we focused on the chlamydial recruitment of 2 eukaryotic proteins VAMP4 and syntaxin 10, which belong to the SNARE protein family and have been shown to be important for chlamydial development. SNARE proteins are required for membrane fusion events. Some Inc proteins are predicted to contain coiled-coil or SNARE domains, but it is unknown if Incs play a role in recruitment of SNARE proteins or fusion with host vesicles. Preliminary studies using the bacterial two-hybrid system have identified Incs containing either SNARE-like or coiled-coil domains as potential binding partners for VAMP4 and syntaxin 10, which we propose to validate interactions and determine the function of those interactions *in vivo.*

Orbitrap-based Shotgun Proteomics for the Identification of Extraradical Proteins from *Rhizophagus irregularis*

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Arbuscular mycorrhizal fungi (AMF) are obligate symbiont of plant roots. Due to their close association with plant roots and slow growth rate, large-scale proteomic studies were previously hampered. Advances in culturing AMF in root organ cultures has facilitated obtaining root-free fungal biomass for proteomic studies. However, multiple plates (~50-100) were required to obtain enough total protein (75-100 µg) for proteomic analysis. The recent availability of genomic sequences from AMF, and advances in LC/MS/MS as well as search algorithms for peptide detection provide an opportunity for positive identification of AMF peptides with much less requirement for total proteins. As a proof of principal, extraradical mycelium from *Rhizophagus irregularis* cultivated on a single chicory root-organ plate was used for total protein extraction followed by gel LC/MS/MS using LTQ Orbitrap XL mass spectroscopy. Both MaxQuant and Byonic™ MS/MS search engines were used for peptide identification against the proteome of *Rhizophagus irregularis*. From only 4 µg total protein, 162 peptides were confidently mapped to *Rhizophagus irregularis* proteins. This number is comparable to previous studies that used >70 µg of total proteins. This methodology represents a great improvement that will allow future high throughput identification of peptides in AMF during various growth settings.

Investigating Amino Acid Starvation Responses in *Chlamydia trachomatis.*

Nathan Hatch (Doctoral)* and Scot Ouellette.

University of Nebraska Medical Center, Omaha, Nebraska.

Chlamydia trachomatis is the leading cause of bacterial sexually transmitted infections in the world. Being an obligate intracellular pathogen, *C. trachomatis* relies on the host cell for various nutrients used for growth and development. *In vivo*, the host immune system will release interferon-gamma (IFNγ) to combat infection. Importantly, *C. trachomatis* is a tryptophan (trp) auxotroph and is starved for this essential nutrient when its human host cell is exposed to IFNγ. To survive this, chlamydiae enter an alternative growth state referred to as persistence. When chlamydial persistence is induced by IFNγ, transcriptional changes occur based on trp codon-content. We hypothesize that these changes in transcription are dependent on the particular amino acid starvation state. To better characterize the persistent state induced by IFNγ and to investigate the chlamydial response mechanisms acting when other amino acids become unavailable, we tested the efficacy of tRNA synthetase inhibitors, indolmycin and AN3365, to mimic starvation of trp and leucine (leu), respectively. We show that blocking trp and leu tRNA charging induces (i) aberrant morphology and (ii) changes in transcription indicative of persistence. With these data, we find that indolmycin and AN3365 are valid tools that can be used to model the persistent state.

Effect of high Ca^{2+} in Enhancing Adherence of *Pseudomonas aeruginosa* to Lung Epithelial Cells.

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Pseudomonas aeruginosa being an opportunistic human pathogen forms biofilms in airway mucosal epithelium in the lung cells of cystic fibrosis (CF) patients. Ca^{2+} accumulates in pulmonary fluids of CF patients and regulates hyperinflammatory host response to bacterial infections. Studies show that Ca^{2+} binds directly to the Ca^{2+}-binding protein EfhP of *P. aeruginosa* and elevated Ca^{2+} leads to increased virulence in *P. aeruginosa*, but little is known about how Ca^{2+} regulates *P. aeruginosa* virulence during infection of human cell lines. The goal of this study is to determine how Ca^{2+} affects adherence of *P. aeruginosa*. The adherence of *P. aeruginosa* was determined with the wild-type strain PAO1, PAO1043 (*efhp* deletion mutant) and PAO1043.pMF (complemented strain expressing EfhP) utilizing human lung epithelial cell lines (A549) in low and high Ca^{2+} conditions. RPMI was determined to contain approximately 0.5-0.67 mM Ca^{2+}, which is spiked to 5mM to obtain the high Ca^{2+} condition for the assays. Adherence studies show that at a multiplicity of infection (MOI) of 10 bacteria/cell, there is no significant difference in between these strains when compared in low and high Ca^{2+} conditions, but at a MOI of 50, PAO1 shows a significant difference of $P<0.001$ in adherence between Ca^{2+} conditions.

Development an *in vitro* transcription assay for *Borrelia burgdorferi* transcription factor DksA

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The Lyme disease causing spirochete *Borrelia burgdorferi* restructures the transcription of transmission and infection enabling genes in response to its environment. DnaK Suppressor Protein (DksA) is global transcriptional regulator essential for response to the environment in many pathogens and in *B. burgdorferi* DksA is required for expression of mammalian infection enabling lipoproteins during growth in culture. The *B. burgdorferi* DksA, when expressed in *Escherichia coli*, contain structural features such as α-helices and metal coordination that are conserved in DksA proteins. DksA is known to function by controlling the activity of the RNA polymerase at σ70 targeted promoters. To test the hypothesis that the *B. burgdoferi* DksA affect RNA polymerase σ70 dependent expression, we are currently developing an *in vitro* transcription assay based on RNA polymerase extracted from *B. burgdorferi*. The β' subunit of the *B. burgdorferi* RNA polymerase core was genetically modified with a C-terminal histidine tag. Western blot and LC-MS protein identification determined that the RNA polymerase purified by Ni-metal affinity column contains all the subunits of RNA polymerase core. Current *in vitro* transcription assays indicate *B. burgdorferi* RNA polymerase transcript elongation at the *flgB* promoter is inhibited by DksA.

How do *Bacillus subtilis* RsbR Paralogs Affect Fitness and Sense Stress?

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Bacillus subtilis uses cytoplasmic multiprotein complexes called stressosomes for sensing environmental stressors. The sensing function of the stressosome is evidently performed by a mixture of 5 different paralogous sensing proteins, termed RsbRA, RsbRB, RsbRC, RsbRD and YtvA. To examine how these proteins affect fitness under salt stress, we studied intra-specific competition between strains containing single RsbR paralogs. When single RsbR strains were competed in 1M NaCl in exponential phase, we found that RsbRD outcompeted RsbRA, RsbRB and RsbRC. More surprisingly, we found that; the RsbRD strain helped the cell to grow more efficiently than the wild-type in exponential phase under 1M NaCl stress. Collectively our results suggest that different RsbR paralogs confer different protections for cells against environmental stressors. However, little is known about how the sensory RsbR proteins in the stressosome complex actually sense stress to initiate and and activate the stress response signal transduction pathway. We assume that stress sensing activity is initiated at the variable N-terminal region of an RsbR protein, which is exposed to the cytoplasm. In future experiments we will identify functional and non-functional amino acid residues in the N-terminal region of RsbRA that eventually drive the response to environmental stressors.

Interactions of *Cryptococcus neoformans* with Human Airway Phagocytes.

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Cryptococcus neoformans is an opportunistic fungal pathogen that causes over 225,000 yearly cases of cryptococcal meningitis, leading to 180,000 annual deaths in HIV/AIDS patients. Innate phagocytes, such as macrophages and dendritic cells (DCs) are the first cells to interact with the pathogen. Destruction of *C. neoformans* occurs within DCs, but survival can occur inside some macrophages. Six different subsets of airway phagocytes have been characterized in a healthy human lung. However, the phagocyte subsets responsible for the killing or survival of *C. neoformans* are unknown. We hypothesize that there are differences with uptake and survival of *C. neoformans* among the subsets. Healthy human bronchoalveolar lavage (BAL) containing these six phagocytic subsets was incubated with mCherry-expressing *C. neoformans* for two hours. Cells were examined by flow cytometry to determine association of the fungus with each subset and by fluorescence microscopy to determine intracellular cryptococcal morphology, indicating killing or replication. Results showed that all phagocyte subsets interacted with *C. neoformans*, and different fungal morphologies were observed in two subsets. These findings suggest the outcome depends on the specific phagocyte subset *C. neoformans* encounters. Future single-cell RNA sequencing studies will identify differently regulated genes in phagocyte subsets following interaction with *C. neoformans*.

Cloning and Expression of *Chlamydia trachomatis* Inclusion Membrane Proteins.

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Chlamydia trachomatis is an obligate, intracellular pathogen that resides inside host cells within an inclusion. To replicate and grow, *Chlamydia* usurps host cell proteins from within this vacuole. To do this, *Chlamydia* produce and secrete proteins, termed inclusion membrane proteins (Incs), that insert in the inclusion membrane with the N- and C- terminus facing the host cytosol. *C. trachomatis* is predicted to have 50 Incs, however few have known functions. Little knowledge about their function is gained via bioinformatics as they lack similarity to any proteins outside of *Chlamydia,* therefore characterizing Incs and possible functions difficult. The goal is to clone and express the C-terminus of certain Incs which will be used to produce purified protein for future crystallography studies. This study focuses on the *CT229-CT224* operon, only found in human pathogens. Here, the cloning strategy of an Inc into pET28a, generating a C-terminus Inc fusion to a 6X His tag. To date CT226, CT227 & CT228 have been successfully cloned, verified by sequencing and transformed into BL21 for expression studies. Once Inc proteins are produced, they will be prepared for crystallography. By assessing structures, insights may be gained as to possible functions based on similarity to other characterized proteins.

Characterization of the Nitrogen Assimilation Regulator (*gln*G) Role in *Escherichia coli* Colonization of the Mammalian Intestines.

Justin Bowen (Doctoral)*, Jerreme Jackson, and Tyrrell Conway.

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Nitrogen, often in the form of ammonia, is used by bacteria to generate amino acids. The *glnG* gene in *Escherichia coli* codes for the nitrogen assimilation regulator transcription factor, NtrC, which activates the regulon responsible for survival during ammonia limitation. Under nitrogen limited growth conditions NtrC upregulates transcription of glutamine synthetase which, along with additional proteins, facilitates transport and degradation of nitrogen containing compounds ultimately leading to an increase in intracellular glutamine. While the *E. coli* transcriptional response to nitrogen starvation *in vitro* has been studied extensively, nitrogen starvation during *E. coli* colonization of the mammalian intestine has received little attention. In this study, we used a *glnG* knockout (Δ*gln*G) mutant to study the role of nitrogen metabolism in MG1655 colonization of the mouse intestine. Total RNA from MG1655 recovered from the mucosal lining of the mouse intestine was prepared and sequenced by differential RNA-seq analysis, which allows mapping of all *glnG*-dependent promoters. This allows us to target and generate mutants of genes regulated by *glnG* to further study nitrogen metabolism in colonized *E. coli*. Additionally, metabolites available to *E. coli* in the intestines were analyzed by Mass-spec. The results indicate that *E. coli* is nitrogen-limited in the mouse intestine.

Revisiting the Oil Fly Bacteria from the La Brea Tarpits

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Helaeomyia petrolei (oil fly) larvae mature in the asphaltene and polyaromatic hydrocarbon rich asphalt seeps of Rancho La Brea, Los Angeles, Calif. These larvae are able to pass high amounts of viscous asphalt through their digestive system with no discernible negative effects. While they do not derive nutrients from the asphalt, they can survive and grow in this harsh environment. Similar to all life, these oil fly larvae have a complex gut flora that are also in contact with the asphalt and thus should possess some form of solvent tolerance. Previously, we characterized the presence ($2x10^5$ B/larva) and antibiotic resistance of these bacteria [Kadavy et al. AEM 65:1477-1482 (1999) and 66:4615-4619 (2000)]. Of 14 isolates derived from this gut flora, *Alcaligenes sp.* OF2 was picked for whole genome sequencing. Based on phylogenetic analysis of molecular markers like 16s rRNA and RecA, OF2 should be classified as a new species. The differences between OF2 and its closest neighbors such as *Alcaligenes faecalis* should give insights into how OF2 survives in such an extreme environment. Of particular interest are solvent tolerant enzymes for industry and efflux pumps that confer resistance to both antibiotics and polyaromatic hydrocarbons.

Blocking HIV Infection Using Engineered *Lactobacillus.*

Joshua Wiggins (Doctoral)*[1], Wenzhong Wei[1], Duoyi Hu[1], Vladimir Verbanac[2], Dane Bowder[1], Michael Mellon[1], Andrew Tager[2], Joseph Sodriski[2], and Shi-Hua Xiang[1]

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Lactobacillus bacteria are a prominent member of the human microbiome and typically colonize mucosal surfaces within the body. They are generally recognized as safe bacteria and are therefore important in the production of various foods and probiotics. They can also be used as a potential delivery vehicle for therapeutics and vaccines. In this study, we utilize *Lactobacillus* for blocking HIV infection and transmission since HIV typically enters the body through mucosal surfaces. To do this, we inserted the human CD4 gene into the chromosome of *Lactobacillus* for surface display of the CD4 molecules that can capture HIV particles. We characterized these engineered bacteria *in vitro* to show that they are capable of adsorbing viruses. We also used a humanized mouse model to test the *in vivo* efficacy of protection from HIV infection, demonstrating the potential of this approach to prevent sexual transmission of HIV.

Characterization of Morin Derivatives for Enhancement of Neural Glyoxalase Pathway.

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High metabolic requirements render the brain susceptible to oxidative stress (OS). The glyoxalase pathway (GP) is a neuroprotective antioxidant system that functions through sequential reactions to detoxify metabolic byproducts, including the highly reactive methylglyoxal (MG). MG induces structural modifications to cellular constituents forming inflammatory advanced glycation end products (AGEs). The accumulation of MG and AGEs contributes to progression of neurodegenerative diseases, including Alzheimer's Disease (AD). Flavonoids are polyphenol secondary plant metabolites that have potent antioxidant function. We previously determined that flavonoid morin possesses the ability to enhance the GP. We hypothesized structural modifications to morin would increase its ability to enhance glyoxalase pathway function. The purpose of this study was to determine the effect of bromination and nanoparticle encapsulation on morin's ability to protect neurons against MG mediated OS. Cultured mouse cerebellar neurons were insulted with MG, and treated with morin, dibromo-morin, and morin nanoparticle. The protective effects of morin derivatives was determined through cell viability, glyoxalase pathway function, antioxidant protein expression, and cellular signaling pathways. In agreement with our hypothesis, the derivatives exhibited elevated detoxification of MG, increased expression of glyoxalase pathway proteins, activated Nrf2 signaling, and decreased apoptosis compared to morin.

Analysis of the Interactions Between *C. neoformans* and Pulmonary Macrophages.

Ashlee Hawkins (Masters)*, Ben Nelson and Karen Wozniak.

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Cryptococcus neoformans, an intracellular fungal pathogen, causes cryptococcal meningitis. The pathogen has the ability to evade the immune response by surviving within select pulmonary macrophages. With over 220,000 cases and 180,000 deaths annually*, C. neoformans* is the most common cause of adult meningitis and a leading cause of death in HIV/AIDS patients in Sub-Saharan Africa. This study is being conducted to understand the mechanism of interaction between *C. neoformans* and pulmonary macrophages using a murine model. We hypothesize that there is differential clearance of *C. neoformans* by alveolar and pulmonary tissue macrophages. For these studies, we used killing assays and flow cytometry. Results showed that both alveolar and tissue macrophages inhibited *C. neoformans* growth. Flow cytometric analysis showed distinct macrophage subset populations, as detailed in previous murine lung research, within the tissue macrophage group that included Ly6C-, Ly6C+, monocytes and interstitial macrophages. Further analysis of the tissue macrophage subsets suggests a correlation between the quantitative presence of particular subsets and the amount of cryptococcal inhibition. Future studies will use fluorescence microscopy to determine morphological changes within macrophage populations and single-cell RNA sequencing to determine which genes are up- or down-regulated within the subsets, following interaction with *C. neoformans*.

Redirecting Carbon and Energy Towards Isoprene as an Anaerobic Respiration/Fermentation Product

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A defining feature of archaea is the use of isoprene lipids for cell membranes rather than the fatty acid esters synthesized by bacteria and eukarya. Isoprene monomer is a useful building block for a wide variety of chemicals including rubber, gasoline, adhesives, and flavors, which are typically refined from petroleum. Yeast and E. coli have been engineered to produce isoprene monomer, but overcoming low efficiency and yield do to inherent metabolic bottlenecks in these systems requires intensive engineering efforts. We hypothesize archaea may be coaxed to produce isoprene monomer at yields that surpass bacterial and eukaryotic systems by taking advantage of the naturally high metabolic flux through the archaeal isoprenoid lipid pathway. However, it is possible that archaea may not tolerate synthesis of volatile isoprene monomer because it could deprive the cell of the ability to synthesize sufficient quanitities of cell membrane. Surprisingly, expression of isoprene synthase in Methanosarcina was not lethal and cells grew at a comparable rate to a vector only negative control strain ($p<0.012$). Mass balance results demonstrate that carbon for isoprene comes at the expense of carbon dioxide production, which was reduced by 20% compared to the control strain ($p<0.007$). These observations suggest that in engineered strains, isoprene monomer may have become a fermentation/respiration product, which would require reconfiguring carbon and electron fluxes through the central methanogenesis pathway. We further demonstrate the ability to produce isoprene monomer from anaerobic digestate, which suggests engineered cells can compete for substrate with wild methanogen populations in a complex microbial community. Our results show archaeal isoprene production can exceed yields from bacterial and eukaryal systems without the need for extensive metabolic optimization.

<u>Undergraduate Student Poster Session.</u>

Persister Formation in *Staphylococcus epidermidis* Clinical Isolates.

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Staphylococcus epidermidis is a commensal organism, often found within the exterior microbiota of mammals. Robust *S. epidermidis* biofilms often form in healthcare settings causing chronic infection mediated through indwelling of medical devices which is further exaggerated due to the target population consisting of immunocompromised individuals. These chronic conditions can be explained by a subpopulation of dormant cells, known as persisters, growing within a biofilm. Recent work has determined that screening *S. epidermidis* clinical isolates following vancomycin challenge can provide information as to whether an isolate is a relatively high persister former or low persister former. To identify a specific mechanism for persister formation in *S. epidermidis*, bacterial cells were mutagenized with ethyl methylsulfonate (EMS). Following EMS treatment, enrichment for high persister isolates was performed and candidates were sent for whole genome sequencing.

Characterization of the Survival and Immunogenicity of *Staphylococcus aureus* Persisters within Macrophages.

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Staphylococcus aureus is known for its ability to cause chronic reoccurring infections in clinical settings. The organism's ability to thrive despite antibiotic treatment has led to the severity of disease that is often exhibited with these infections. It has been hypothesized that energy-dependent persister formation leads to antibiotic tolerance in *S. aureus*. Persister cells are a dormant-like phenotypic variant of *S. aureus* that resemble cells growing in stationary phase. Previously, it was shown that a knockout in the tricarboxylic acid (TCA) cycle gene, *fumC*, which reduced cellular ATP, led to an increase in the number of persisters. While persisters have been examined in relationship to antibiotic tolerance, it is currently unknown how persisters interact with the innate immune system. To examine this further, we are currently testing to see if the *fumC* knockout is less immunogenic within the macrophage. Using flow cytometry, we will monitor the amount of reactive oxygen species (ROS) and reactive nitrogen species (RNS) produced by the macrophage in response to the wild type *S. aureus* and the *fumC* knockout. Specifically, we will use the fluorescent dyes 4-amino-5-methylamino-2',7'-difluoroflurescein (RNS), CM-H_2DCFDA (ROS), as well as pHrodo to determine the pH.

Protein kinase A manipulation by *Chlamydia trachomatis* during infection

Ty Derouen (undergraduate)*, Prakash Sah and Erika Lutter

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The most commonly reported STD in the United States is *Chlamydia trachomatis* which can lead to pelvic inflammatory disease, tubal infertility and even increased risk of cervical cancer. After infection, manipulation of different protein kinases aid in its replication processes for further development of the pathogen. Protein Kinase A (PKA) is an enzyme in the host cell that phosphorylates other proteins for activation. PKA's activity is regulated by the changing levels of cyclic AMP within cells. Not much is known about the intracellular Chlamydial manipulation of host cellular kinases post infection. *Coxiella burnetii* is another bacterial obligate intracellular pathogen where the inhibition of host cell PKA negatively impacts the parasitophorous vacuole required for the growth and survival. We hypothesize that *C. trachomatis* operates in a similar manner by manipulating PKA pathways to regulate intracellular development inside the host. The use of western blots helped determine if *C. trachomatis* manipulated host-cell PKAs by measuring the growth of the protein in the cell at different time points post infection. PKA and PKA substrates were confirmed to be more active as the post-infection time increased. These findings conclude that PKAs are significant for the intracellular growth and development *C. trachomatis.*

Rapid Qualitative Colorimetric Viability Analysis of *Planctomycete* Bacterium.

Rohan Das (Undergraduate)*, Andrew Dulek, and Dr. Josef D. Franke.

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The phylum *Planctomycetes* of the domain bacteria consists of several species with atypical membrane organization for Gram-negative bacteria. As such, *Planctomycetes* have increasingly drawn interest in areas of evolutionary cellular biology research. *Planctomycete* species exhibit slow growth rates, susceptibility to contamination, and variable viability under different growth conditions, which present challenges for ongoing studies. Prior to this study, colorimetric vital dyes have not been commonly utilized to determine viability in bacteria. However, given the atypical membrane features of *Planctomycete* species, our study hypothesized that such dye techniques might be effective. This study implemented live-dead dye incubation assays to determine if qualitative colorimetric analysis is feasible for different species within the *Planctomycete* phylum. The study employed three colorimetric staining dyes, trypan blue, methylene blue, and erythrosin B, for numerous *Planctomycete* species. Cell counts and imaging of the assays were used to determine if a particular dye could serve as a generalized indicator of viability across the *Planctomycete* phylum. The findings of this study provide a rapid, and cost-effective methodology for determining and maintaining viability in *Planctomycete* cultures. Additionally, this study provides further insight for future experiments that employ fluorescent indicators such as propidium iodide to determine viability among *Planctomycete* species.

Determining the Prevalence of Vectored Diseases in Elkhorn Valley *Dermacentor variabilis* in Nebraska.

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American Dog Ticks *(Dermacentor variabilis)* are the most prevalent hard bodied tick in Nebraska. *D. variabilis* are known to vector numerous infectious bacteria. The different pathogenic bacteria that may be present in Elkhorn River Valley *D. variabilis* ticks have yet to be determined and compared to prevalence rates in other parts of Nebraska. In this study, *D. variabilis* ticks from various sites near the Elkhorn River in Nebraska were collected. Total DNA was extracted and polymerase chain reaction (PCR) using species specific primers and gel electrophoresis was used to identify DNA of spotted fever group *Rickettsia, R. rickettsii, E. chaffeensis, E. ewingii, C. burnetii, A. phagocytophilum,* and *F. tularensis. E. ewingii* was found in 23% of the Elkhorn River Valley ticks, but all infectious bacteria were identified in ticks from the Elkhorn Valley. Further analysis must be completed on both Elkhorn Valley and Platte River ticks to confirm positive samples through PCR with a single primer and DNA sequencing.

Buggy Creek virus Phenotype Dynamics in Swallow Bugs (*Oeciacus vicarius*).

Tyler Rollman (Undergraduate)*, Troy Rowan, Ben Ryan, and Carol Fassbinder-Orth.

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Alphaviruses are arthropod-borne viruses known to cause millions of cases human disease each year. Among alphaviruses, Buggy Creek virus (BCRV) is unique as it is transmitted by a cimicid insect (the swallow bug) closely related to the human bed bug (*Cimex lectularius & Cimex hemipterus*). While human bed bugs are not known to transmit human disease, they are considered potentially competent vectors, making studies of alphavirus-cimicid vector systems pertinent to prevent possible future epidemics. Previous research has indicated that BCRV undergoes seasonal changes in virulence in swallow bugs, with virally-induced cytopathic effect being highest in the summer, but low the rest of the year. However, specific details of these seasonal changes have not been elucidated. We compared the cytopathic effect of swallow bug homogenates collected monthly with apoptotic activity in Vero cells, and determined that while cytopathic effect may be undetectable throughout much of the year, apoptotic activity is a more sensitive measurement for alphavirus phenotype determination in these insect vectors. Levels of virally-induced apoptosis were detectable and differentiable among bug ages and months, especially May through July. These results have the potential to improve our ability to predict the timing and seasonality risk of arboviruses in other disease systems.

Community Analysis of Methane-Oxidizing Bacteria.

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University of Oklahoma Department of Microbiology and Plant Biology.

Methane is a major heat trapping gas, accounting for nearly 10% of the greenhouse effect. Some organisms are capable of metabolizing this reduced carbon gas to its least potent form, carbon dioxide. These methane oxidizing organisms, methanotrophs, could have a significant global impact in removing methane from the atmosphere and overall reducing the greenhouse gas effect; however, relatively little is known about the microbial communities methanotrophic organisms thrive in. The purpose of this project is to identify methanotrophs and gain a better understanding of the bacterial communities they habitat. This was done by analyzing 16s rDNA sequences retrieved from anaerobic organic rich methanotrophic environments using QIIME2 software. Six groups of supporting bacteria were detected across all samples containing methane-oxidizing bacteria including: *Bacteroidales, Anaerolineae OPB11, Clostridiales, Myxococcales, Treponema, and TSCOR003-O20 of the Fibrobacteres* phyla. Perhaps most notably, *Chloroflexi Anaerolineae,* a known photosynthetic bacterium, displayed a close phylogenetic relationship and comparable relative abundance with methanotrophic organisms, suggesting a potential symbiotic co-evolution of the two organisms. Ultimately, the understanding of methanotrophic symbiotic relationships could have a significant impact in the global carbon cycle, and carbon emissions.

The Development of a Reverse Genetic System for Influenza A.

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Most of the molecular studies of influenza A virus that use a reverse genetics approach are based on laboratory-adapted strains of the virus, such as A/Udorn/1972 (A/Udorn). Growth curves show that a clinical isolate of influenza A virus grows much slower than a laboratory strain but ultimately reaches a similar titer. This raises the possibility that some of the molecular details of influenza A virus replication may be different in clinical and laboratory strains of virus. To examine that possibility, a reverse genetics system that codes for a clinical isolate of virus is being generated. So far, cDNA copies of two of the 8 influenza A genome segments have been cloned into Pol I reverse genetics plasmids and sequenced, and preliminary comparisons between the sequence of the clinical isolate and lab strain have been made. As expected, the sequence of the clinical isolate is significantly different than the laboratory strain. Further studies are required to identify functional differences between the two virus strains.

Transcriptional Regulation of Calcium Sensor, EfhP, in the Human Pathogen *Pseudomonas aeruginosa*

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Pseudomonas aeruginosa is an opportunistic human pathogen that causes lethal infections in immunocompromised and hospitalized patients. It is a prevalent pathogen in diseases like pneumonia and cystic fibrosis (CF). Certain diseases, including CF, are associated with abnormally high levels of calcium (Ca^{2+}). Previously, we reported that elevated levels of Ca^{2+} enhance the production of virulence factors of *P. aeruginosa*. We also identified a putative Ca^{2+} sensor, EfhP containing two EF-hand Ca^{2+}-binding motifs and showed that it mediates Ca^{2+} regulation of virulence. We hypothesized that elevated levels of Ca^{2+} up-regulate the transcription of *efhP*. RNA-seq data showed that addition of 5mM Ca^{2+} increases the *efhP* transcription by four fold. Here, by using promoter activity assay, we validate the inducing effect of Ca^{2+} on *efhP* transcription. We also show that the two-component system, BfmRS, negatively regulates *efhP* transcription at elevated Ca^{2+}. Currently, we are testing the effect of other host factors, including limited iron, oxidative stress and elevated carbon dioxide on *efhP* promoter activity. In conclusion, the results of our current and future studies will determine the host factors and molecular mechanisms that regulate *efhP* expression. Ultimately, these results will help devise strategies to control *P. aeruginosa* infections by blocking Ca^{2+}-enhanced virulence.

Analysis of RNAseq Data Reveals Media State Dependent Transcript Profile in *Candida albicans*

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Candida albicans is an opportunistic fungal pathogen and the leading cause of candidiasis infections. The pathogenesis of *C. albicans'* relies on alternating yeast and hyphal states via the process of filamentation, and characteristics of filamentation are major qualifications when clinically assessing the severity of infection. In a research setting, media type and state impact the filamentation of *C. albicans,* leading to observed phenotypes which do not necessarily reflect *in vivo* traits. To examine the effect various media have on filamentation a time course analysis of *C. albicans* strain SC5314 was performed. Cells from an overnight sample were plated on one of the following media: YPD, Lee's, Spider, FBS, and RPMI, and grown at intervals 30, 60, 90, and 120 minutes at 30℃ or 37℃. Plates were imaged before cells were collected for RNA extraction. Evaluations of transcript levels at later stages of filamentation have been poor predictors of genes involved in filamentation; therefore, it was decided that RNA would be extracted from cells collected at 30 minutes post induction, a time point that precedes the development of filaments in inducing conditions based on imaging. RNAseq data has been analysed to reveal the activity of genes at the initiation of filamentation.

Identification and Characterization of Large Repeat Sequences Throughout the *Candida albicans* *G*enome

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Candida albicans is the most prevalent opportunistic fungal pathogen amongst humans. It relies on mutational changes at both the chromosomal and sub-chromosomal level to acquire drug resistance and confer pathogenicity. Genome rearrangements resulting in copy number variation (CNV) and loss of heterozygosity (LOH) frequently occur during the rapid adaptation of these fungi to novel environments, especially during antifungal drug treatment. However, the mechanism(s) that lead to CNV and LOH are not completely understood. Here, we investigate the involvement of long, previously undescribed repetitive sequences in the *C. albicans* genome, and their association with CNV and allele ratio breakpoints. Though repetitive sequences such as MRS, tRNA, and LTR regions have been studied previously in the context of the *C. albicans* genome, we expand this list to include coding and intergenic sequence in regions of the genome previously unknown to contain repetitive sequences. Using a combination of next- generation sequencing technology, whole genome global alignment algorithms, and genome visualization, ubiquitous repetitive sequences were compiled, individually validated and characterized, and grouped to create a robust list of sequences that can quickly provide useful information.

Manduca Sexta as a Model of Intestinal Colonization

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The intestinal microbiome is a natural reservoir for many microorganisms including Enterobacteriaceae, *Enterococcus*, *Candida*, and *Clostridia* species, which reside as commensals with the potential to cause nosocomial infections after gaining access to sterile sites. Because nosocomial infections affect 7% of hospital patients living in developed countries and may lead to a decreased quality of life, morbidity, and even mortality, identifying and reducing pathogen reservoirs has become a top priority. However, the signaling pathways essential for acquiring nutrients that mediate replication within the intestinal mucosal layer remain poorly understood. To address this knowledge gap, we developed a streptomycin-treated *Manduca sexta* model for characterizing the physiology of *Escherichia coli* stably colonized in the midgut epithelium. *M. sexta*, an agricultural pest of solanaceous plants, has been used extensively to characterize the mechanisms of *Bacillus thuringiensis* insecticidal crystal proteins and as a model of invertebrate gut physiology and innate immunity. Relative to vertebrate models, the *M. sexta* GI tract is morphologically simple, colonized by a smaller and less diverse microbiota, and an underexplored resource for characterizing host-microbe interactions. The data presented here supports the potential of the streptomycin-treated *M. sexta* model as a valid complement to vertebrate models for studying mechanisms of intestinal colonization.

Overexpression and Purification of the *Borrelia burgdorferi* Gene Regulatory Protein BosR

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Borrelia burgdorferi, the bacterium that causes Lyme disease, is the most common vector-borne pathogen in both the United States and Europe. Throughout its pathogenic life cycle, *B. burgdorferi* encounters oxidative stress in the form of reactive oxygen species (ROS). The DNA-binding protein BosR (Borrelia oxidative stress regulator) regulates gene expression necessary for *B. burgdorferi* to be able to resist oxidative stress. We set out to determine whether BosR is a redox-active gene regulatory protein. First, we generated an arabinose-inducible plasmid encoding *bosR* with a C-terminal FLAG epitope tag (pBAD/HisA:: $bosR_{FLAG}$) that was transformed into a TOP10 strain of *Escherichia coli*. Subsequently, the optimal conditions for overexpressing His-tagged BosR via L-arabinose induction were then experimentally determined by treating several cultures with a gradient of L-arabinose. Proteins derived from whole cell lysates of the bacterial cultures were separated by SDS-PAGE. Subsequently, a western blot using an anti-His primary antibody was performed to determine the production of BosR. After determining the optimal L-arabinose concentration to overexpress BosR, TOP10 *E. coli* pBAD/HisA:: $bosR_{FLAG}$ cultures were grown at this concentration. Currently, we are attempting purification of the N-terminal 6xHis-tagged BosR proteins using a cobalt resin. As BosR is DNA-binding and a transcriptional regulator, electrophoretic mobility shift assays (EMSA) will be performed to determine the ability of wild-type BosR to bind to cognate promoters.