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

March 9-10, 2018

Hosted by

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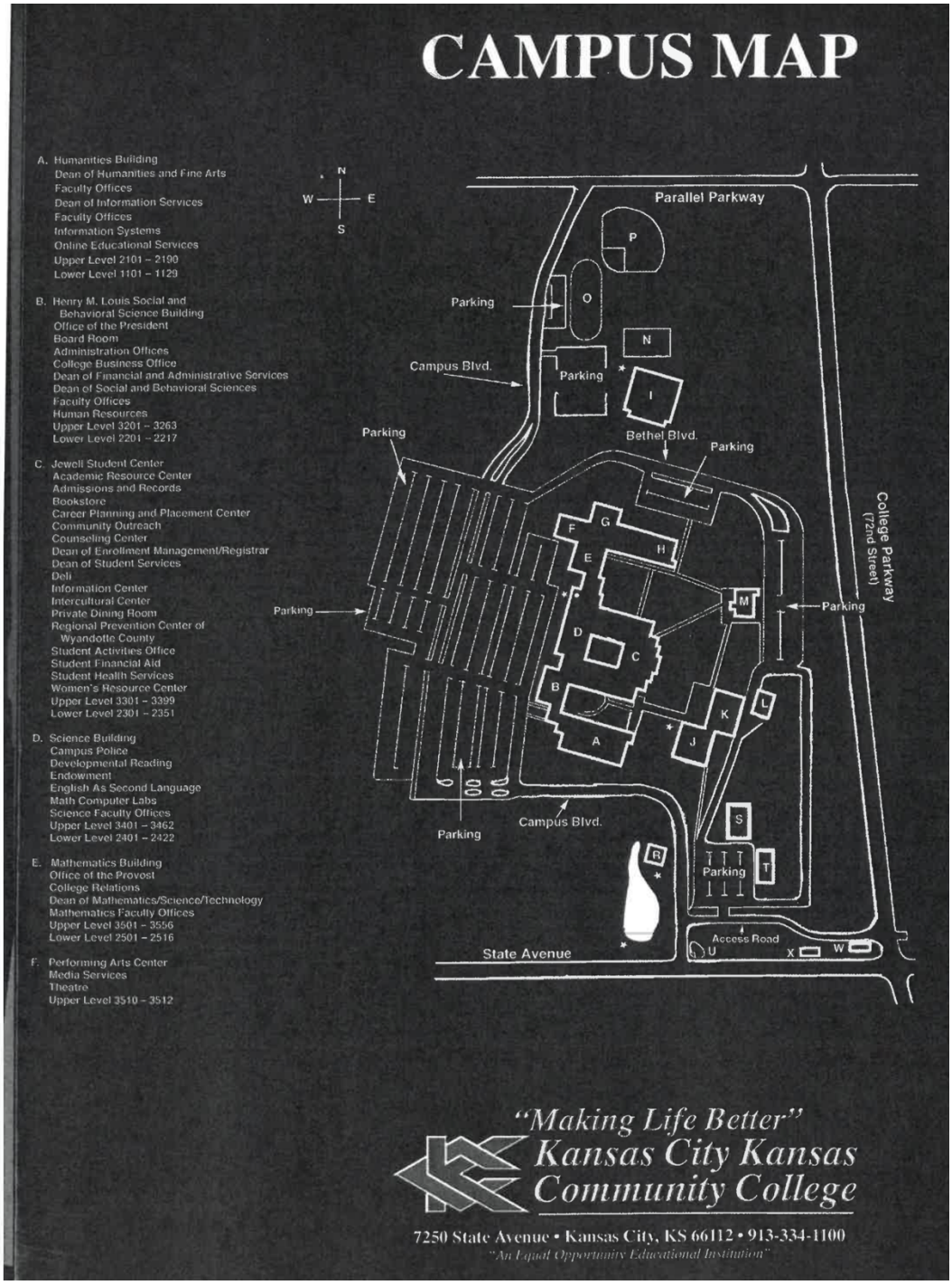

Take State Ave to Campus Blvd (just west of 72nd and State). Follow to Quindaro Ln. Parking between Argentine Ln and Armourdale Ln. Enter the Henry Louis Social Science Building. Go downstairs and to the back which takes you into the lower level of Jewell.

Keynote Speakers

Dr. Melanie R. Mormile, Associate Provost at Missouri University of Science and Technology, ASM Distinguished Lecturer

Dr. Mormile's specialty is with the metabolic capabilities of the members of the *Order Halanaerobiales* and their potential biotechnological applications. She has researched: 1) the ability of a haloalkaliphilic bacterium isolated from Soap Lake, Washington to generate electricity at pH 11.0 and 7% salinity: 2) A streamlined strategy for biohydrogen production with *Halanaerobium hydrogeniformans*, an alkaliphilic bacterium; 3) activity-based metagenomic screening and biochemical characterization of bovine rumen protozoan glycosyl hydrolases; 4) activity-based metagenomics screening and biochemical characterization of bovine ruminal protozoan glycoside hydrolases; and 5) characterization of a moderately halo-acidophilic bacterium isolated from Lake Brown, Western Australia; and 6) the impact of elevated CO2 concentrations on carbonate mineral precipitation ability of sulfate-reducing bacteria and implications for CO2 sequestration. She has 3 patents: 1) conversion of glycerol to 1,3 propanediol under haloalkaline conditions; 2) a combined fossil free process of lignocellulosis pretreatment with biological production; and 3) fossil fuel-free process of Lignocellulosic Pretreatment with Biological Hydrogen Production.

Dr. Karl Klose, PhD, Professor, Univeristy of Texas at San Antonio, ASM Distinguished Lecturer

Dr. Karl Klose received his Ph.D. in Microbiology at UC Berkeley, and performed postdoctoral studies at Harvard Medical School. He was then hired as a faculty member at the University of Texas Health Science Center in San Antonio (UTHSCSA), and later moved to the University of Texas at San Antonio (UTSA), and is the founder of the South Texas Center for Emerging Infectious Diseases. Klose's research focuses on understanding bacterial pathogenesis. His laboratory studies Vibrio cholerae and the potential bioweapon Francisella tularensis. Klose is an author on more than 90 publications, and has received funding from numerous sources. He has mentored many Ph.D., Masters, and undergraduate students. He served as the President of the Texas Branch of the American Society for Microbiology (2001-2003), and has been an organizer of multiple national and international meetings. He has twice been a recipient of ASM Visiting Professorships, in Kolkata, India and in Valparaiso, Chile. He received the 2002 Presidential Junior Research Scholar award at UTHSCSA and the 2009 President's Distinguished Research Achievement Award at UTSA. Klose has given a TEDx talk on antibiotic-resistant bacteria that is available on

YouTube and that has received over 40,000 views (https://www.youtube.com/results?search_query=karl+klose). Klose was recently elected a fellow of the American Academy of Microbiology.

Featured Branch Speakers

Dr. Heather Seitz, Ph.D. Associate Professor of Science, Johnson County Community College
PULSE Leadership Fellow-Midwest Great Plains Regional Network

Dr. Seitz is the first author and leader of the task force that created the Microbiology for Health Sciences Concept Inventory (MHSCI) and has worked closely to analyzed the data on student misconceptions. She has data to share from over 3,000 students at 15 institutions in the United States. Helping to uncover student misconceptions is incredibly important in improving understanding. Validated and reliable concept inventories are designed to uncover student misconceptions. Recently, the American Society of Microbiology supported the development of two concept inventories that will be discussed: the Microbiology Concept Inventory and the Microbiology for Health Sciences Concept Inventory. The concept inventories that have recently been published are aligned with the ASM Curriculum Guidelines. The data from the development of these concept inventories was used to help uncover the most common misconceptions in undergraduate microbiology. The process used to create the concept inventories and national trends in the data collected will be highlighted. Examples of how the concept inventories can be implemented into course design, assessment, and faculty development will also be shared.

Dr. Mark Hoffman, Ph.D., serves as the Chief Research Information officer for Children's Mercy and the Children's Research Institute. Dr. Hoffman's role is to accelerate and improve all types of research at the Children's Research Institute through such as data, applications and technology. In 2013, he joined the faculty at the University of Missouri at Kansas City (UMKC) in the Departments of Biomedical and Health Informatics and Pediatrics. At UMKC he launched he Center for Health Insights and brought new Informatics capabilities to the University, Including REDCap, i2b2 and the Cerner Health Facts data. During his 16 years at Cerner and 3 years at UMKC he had the opportunity to meet a wide variety of clinical research organizations around the world, learning about their successes and challenges. In 2016 Dr. Hoffman joined Children's Mercy where he is the primary investigator on a CDC grant to utilize clinical and laboratory data warehouses to inform quality improvement and he continues to serve on the faculty at UMKC. He has delivered a TED talk on the "Envrome" and won the

iThermometer category in the Google wearable devices in health care challenge in 2015. He is an inventor of 19 issued patents and a member of the American Academy of Inventors.

Dr. Erika Lutter, PhD, Assistant Professor, Oklahoma State University

My lab is interested in mechanisms of host-cell exit, transmission and molecular pathogenesis of the obligate-intracellular pathogen *C. trachomatis*. *C. trachomatis* causes infections that have significant global medical and economic impact. Itis the leading cause of infectious blindness (trachoma) worldwide and the most reported bacterial sexually transmitted infection in the United States. As an obligate intracellular pathogen with a complex bi-phasic developmental life cycle, the ways in which *C. trachomatis* utilizes host signalling processes for survival and transmission are poorly understood. Until recently, *C. trachomatis* was genetically intractable but the latest advent of transformation methods has enabled the development of genetic tools to mutate, complement and express genetic constructs within *C. trachomatis*. Our research uses a combination of these newly developed genetic tools and cellular biology to address my goal of deciphering the interaction of *Chlamydial* proteins and myosin phosphatase and their role in host-cell exit and the transmission of *C. trachomatis*.

Dr. Anna Selmecki, PhD, Assistant Professor, Creighton University

My research is focused on understanding the mechanisms that control genome stability. My lab at Creighton University Medical School uses experimental evolution and comparative genomics to identify mutations that alter genome stability and gene expression in both pathogenic and non-pathogenic microbes. We use molecular biology, biochemistry and fluorescent microscopy to determine the mechanistic role of novel genome stabilizing mutations. Our experiments address the genetic and environmental causes of increased rates of chromosome aneuploidy in yeast. Finally, we develop mathematical models to understand how genome stability impacts the adaptation at the cellular and population level over different timescales. My vision is that these approaches will generate a more comprehensive and mechanistic understanding of genome stability and its role in adaptation than has been possible with traditional genetic approaches.

Schedule

March 9

5:00-5:15 p.m.	Welcome	Lower Level Jewell
5:15-5:45 p.m.	Heather Seitz, JCCC—evaluating national data on student misconceptions in microbiology	
5:45-6:15 p.m.	Mark Hoffman, —Medical data warehouses to inform quality improvements	
6:30-7:30 p.m.	Dinner	Adjacent dining room
7:30-8:45 p.m.	Keynote speaker: Melanie Mormile— Soap Lake: Microbial Studies from a Salty Alkaline Lake	
8:45-9:00 p.m.	Closing announcements	

March 10

8:00-9:30 a.m.	Poster Session I (Undergraduates)	Lower Level Jewell
9:30-11:00 a.m.	Oral Presentations I 3 rooms with presentations	General Session 1, Room 2325 Medical, Room 2326 Undergraduate Session 1, Room 2335
11:00 a.m.-12:00 p.m.	Oral Session II 3 rooms with presentations	General Session 2, Room 2325 Undergraduate Session 2, Room 2326 Undergraduate Session 3, Room 2335
12:00-1:30 p.m.	Lunch and Keynote speaker: Karl Klose—Killer Bunnies and Bioweapons: Tularemia and Biodefense	Lower Level Dining Room
1:30-2:00 p.m.	Students meet with Keynote speakers	Room 2325
2:00-3:00 p.m.	Poster session II (Graduate Students)	Lower Level Jewell
3:00-3:30 p.m.	Erika Lutter, Oklahoma State University--Chlamydia	
3:30-4:00 p.m.	Anna Selmecki, Creighton University Medical Center—yeast evolution	
4:00-4:15 p.m.	Break	
4:15-4:45 p.m.	Branch Business	Missouri—Room 2325 Missouri Valley—Room 2326
4:45-5:00 p.m.	Awards and Closing remarks	Lower Level Jewell

General Microbiology Graduate Student Oral Presentations

Session 1: Jewell 2325

9:30 a.m.	Nirakar Adhikari	D-motif Mutation in cAMP Phosphodiesterase, RegA, Leads to Developmental Phenotype Defect in *Dictyostelium discoideum*
9:45 a.m.	Biraj Kayastha	The Role of β-carbonic anhydrases in Calcium Deposition by *Pseudomonas aeruginosa*
10:00 a.m.	Jorge Lightfoot	Metabolically Engineering *Aspergillus nidulans* using RNA Interference
10:15 a.m.	Nick Kuburich	Investigating a Phosphodiesterase Involved in Amoeboid Multicellular Development and Signaling

Session 2: Jewell 2325

11:00 a.m.	Mariel Degado Cruz	Exploring the Potential Role of Vps1 as a Fusion Protein
11:15 a.m.	M.M. King	CarP is a Ca^{2+} Regulated Putative Phytase that is Unique to Pseudomonads and is Highly Conserved in Clinical Isolates
11:30 a.m.	Jared Smothers	Myosin mediates protein recycling toward the Golgi
11:45 a.m.	Wes Short	Searching for Recruitment Domains and Residues of Vps1 to Target Membrane

Medical Microbiology/Immunology Graduate Student Oral Presentations

Session 1: Jewell 2326

9:30 a.m.	Abby Rigsbee	Effect of the Outer Membrane Permeabilizer Compound 48/80 on Intrinsic Resistance to the Hydrophobic Biocide Triclosan in Opportunistic *Serratia* Species
9:45 a.m.	Jeff Shaw	Three Transketolases in *Salmonella enterica* Contribute to Defending Against Oxidative and Nitrosative Stresses
10:00 a.m.	Robert Todd	Segmental Aneuploidies Flanked by Inverted Repeats Cause Azole Resistance in the Fungal Pathogen *Candida albicans*
10:15 a.m.	Amanda Zalud	ROS/RNS Induced Changes in *Borrelia burgdorferi* Outer Surface Proteins
10:30 a.m.	Macy Olson	Determination of the Role of Chlamydial Inclusion Membrane Proteins in Inclusion Growth and Development by Proximity Labeling of Interacting Partners
10:45 a.m.	William Boyle	The *Borrelia burgdorferi bb0168*-Encoded DnaK Suppressor Enhances pH Dependent Lipoprotein Expression

Undergraduate or High School Oral Presentations

Session 1: Jewell 2335

9:30 a.m.	Lea Kafer	Utilizing *Galleria mellonella* to Determine the Role of Calcium in *Pseudomonas aeruginosa* Virulence
9:45 a.m.	Alison Guyer	Role of centromere heterogeneity in clinical isolates of *Candida albicans*
10:00 a.m.	Nathaniel Higdon	Analyzing Protein Interactions of The Herpes Simplex Type 1 UL34 Protein
10:15 a.m.	Keegan McGill	Determining the Localization of the Hypothetical Membrane Protein CBU_1651 from *Coxiella burnetii*
10:30 a.m.	Sam Koshy	Characterization of the role of redox-active cysteines in the regulatory function of DksA in the Lyme disease spirochete *Borrelia burgdorferi*

Session 2: Jewell 2326

11:00 a.m.	Ambur D. Robertson	Identifying Intracellular Tick-borne Illnesses in Bison*, Bison bison,* via Blood Cell Staining and PCR
11:15 a.m.	Daniel McLeod	Generating Deletion and Point Mutations to Study a Novel Phytase-like Protein, CarP, That Protects *Pseudomonas aeruginosa* from Elevated Calcium
11:30 a.m.	Georgie O. Tauber	Soil Microbe Isolation Surrounding Native and Invasive Grasses to Test for Antimicrobial Properties against E.S.K.A.P.E. relatives
11:45 a.m.	Makayla Nemecek	Longevity Analysis of Germ-free *Drosophila melanogaster*

Session 3: Jewell 2335

11:00 a.m.	Cullen Horstmann	Silver Nanoparticles on Yeast Viability with Bioinformatics Analysis
11:15 a.m.	Ryan Windish	Site Directed Mutagenesis of Known Vps1 Ubiquitination Sites
11:30 a.m.	Nicholas Wood	Initial Characterization of the Two ClpP Isoforms of *Chlamydia trachomatis* Suggests Independent Functionality for Each
11:45 a.m.	Christopher Johnson	

Abstracts

Poster Session 1 (Undergraduate Students)

1. **Impact of Fluconazole on Genome Copy Number Changes in *Candida albicans.*** Ann Braverman (Undergraduate)*, Robert Todd, Anna Selmecki. Creighton University Medical School, Omaha, Nebraska

Candida albicans is an opportunistic fungal pathogen that causes debilitating infections in immune-compromised individuals. Fluconazole, a fungistatic anti-fungal drug, is the most commonly prescribed treatment for Candidiasis due to its rapid bio-availability and low occurrence of side effects. However, due to the fungistatic nature and common, widespread use, fluconazole resistance has become a major threat to public health. One mechanism associated with antifungal drug resistance is the amplification of important drug-response genes. This increase in gene copy number can arise due to abnormal genome replication or segregation, and may cause changes in whole genome copy number (ploidy). In fungi, significant fitness increases can be attributed to ploidy increases (Selmecki et al. 2015; Gerstein et al., 2009; Adams et al. 1974). Here, we have optimized a flow cytometry based assay to rapidly detect ploidy changes in yeast-form fungi when exposed to fluconazole. Our ploidy assay coupled with an in-vitro evolution experimental system allows us to address how fluconazole exposure alters genome stability.

2. **Segmental Aneuploidies Flanked by Inverted Repeats Cause Azole Resistance in the Fungal Pathogen *Candida albicans***. Robert Todd, Tyler Wikoff (undergraduate)*, Shilpa Nair (undergraduate)*, Curtis Focht, Robert Thomas, Anna Selmecki. Creighton University School of Medicine, Omaha, Nebraska.

Candida albicans is the most prevalent fungal pathogen in immunocompromised individuals. Currently, treatment of *Candida* infections relies heavily on azoles, a family of fungistatic drugs that disrupt the biosynthesis of the fungal-specific sterol, ergosterol. Aneuploidy, amplification or loss of a chromosome, is a common genomic feature found in 50% of azole-resistant *C. albicans*. Previous work from the Selmecki Lab has shown that a specific segmental aneuploidy, an amplification of the left arm of chromosome 5, can confer azole resistance due to the amplification of two drug-response genes. Currently, little is known about how segmental aneuploidies form. Using next-generation sequencing technology, chromosome karyotyping, and long-range DNA sequencing we describe several novel segmental aneuploidies found in azole resistant *Candida* strains from diverse genetic backgrounds. These segmental aneuploidies consist of amplified regions of the genome, some of which can undergo copy number increases of greater than 12 copies. These amplifications contain important drug resistance genes and correlate with a significant increase in azole minimal inhibitory concentration. Here, we describe these novel segmental aneuploidies that confer azole-resistance and identify genetic features that elucidate a common mechanism of formation.

3. **The Effect of Garlic on *Escherichia coli* and *Staphylococcus aureus.*** Amber Mason (undergraduate)* Kansas City Kansas Community College, Kansas City, Kansas.

The active ingredient in garlic that inhibits bacteria has been shown to be allicin. The only way these substance work is in the raw uncooked form of garlic, or garlic extract as used in these experiments. Concentrations of 1 ml, 1.5 ml, and 2 ml were used to investigate the inhibitory effects on *E. coli* and *Staph aureus*. Both microbes were streaked on separate plates for confluent growth and inhibitory effects recorded after 48 hours incubation. The results demonstrated that *E. coli* was inhibited more effectively with increasing concentrations than *Staph aureus* where a few resistant colonies were evident.

4. **The Role of Apigenin in Treating Leukemia.** Remington Wilkerson (undergraduate)*, Kansas City Kansas Community College, Kansas City, Kansas.

Apigenin-4', 5, 6 trihydroxyflavone, an initiator of caspase-9, has been shown to induce leukemia cell apoptosis. It is found in relatively large quantities in celery, parsley, cilantro, oranges, onion, and chamomile tea. Chemically engineered supplements have been developed from the chamomile plant. Apigenin demonstrates antioxidant benefits that help metabolize cell damaging free radicals and disruption of metastasizing tumor cells in general. Apigenin has also been shown to have anti-inflammatory properties that help reduce inflammation and autoimmune diseases. This is a review of the literature poster that finds evidence from the National Cancer Institute and elsewhere showing that apigenin can prolong the life of leukemia patients.

5. **Genetic Knockdown System for *Chlamydia trachomatis.*** Emily Gietzen, Prakash Sah and Erika Lutter, Oklahoma State University, Stillwater OK

Chlamydia trachomatis is an obligate intracellular pathogen that is commonly sexually transmitted among humans. Until recently *Chlamydia* has been genetically intractable, thereby limiting genetic approaches. However, recent developments have allowed for the development of novel genetic tools which can be used to mutate specific genes. Unfortunately, gene inactivation by targetron or antibiotic cassette insertion can result in polar effects of neighboring genes making it difficult to study the genes within operons. This study focuses on developing a novel knockdown strategy by expressing the reverse complement specific *Chlamydia* genes on a shuttle plasmid. Once cloned, the plasmids are transformed back into *Chlamydia* and the genes expressed *in trans* will be transcribed and bind the RNA of the corresponding gene producing double stranded RNA which is degraded. This method will allow us to look at individual genes in an operon without the polar effects of mutations. This strategy is being used on an operon containing 6 genes. After the reverse complement of each gene is expressed, the decreased expression of the target gene will be assessed by reverse transcription PCR. These experiments will be the first to utilize a gene specific knockdown strategy in *Chlamydia*.

6. **Characterization *Elizabethkingia ursingii* Mutants Expressing Elevated Vancomycin Resistance.** Braden Lanier (undergraduate)*, William L. Johnson, and John E. Gustafson. Oklahoma State University Department of Biochemistry and Molecular Biology, Stillwater, Oklahoma.

The Gram-negative *Elizabethkingia* bacterial genus exhibit clinically-relevant intrinsic multiple antibiotic resistance. Clinical reports suggest that the peptidoglycan biosynthesis targeting antibiotic vancomycin which is commonly used to treat Gram-positive infections, can also be used to treat *Elizabethkingia* infections. In this study, we initially determined the vancomycin MICs of six genomically-characterized *Elizabethkingia* species following CLSI guidelines, which ranged from 2 to 64 mg/L. These MICs suggest that all *Elizabethkingia* species are vancomycin-resistant, and therefore, refractory to the bactericidal and growth inhibitory actions of this drug. *Elizabethkingia ursingii* demonstrated the lowest MIC (2 mg/L) while *Elizabethkingia meningoseptica* demonstrated the highest MIC (64 mg/L). We next isolated single *E. ursingii* colonies that survived on heart infusion agar supplemented with vancomycin (12, 14, 16, 18 and 20 mg/L) and noted that the number of surviving colonies was reduced as the vancomycin concentration was increased. The five isolates selected off the vancomycin containing HIA plates displayed MICs ranging from 32 to 256 mg/L, which are much higher MICs than that exhibited by the parent strain. These results suggest that *Elizabethkingia* can acquire elevated vancomycin resistance following brief vancomycin exposure. These findings do not support studies that suggest vancomycin therapy is effective against *Elizabethkingia* infections.

7. ***Drosophila melanogaster* Nora virus ORF1 Protein is Localized to the Nucleus.** Larissa K. Attema (undergraduate)*, Alexis M. Page, Brandon E. Luedtke, Brad L. Ericson and Kimberly A. Carlson. Department of Biology, University of Nebraska at Kearney, Kearney, Nebraska.

Nora virus is a picorna-like virus that is transmitted via the fecal-oral route. The genome of the virus contains four open reading frames (ORFs) known as ORF1, ORF2, ORF3, and ORF4. ORF1 was the focus in this study, and it is believed to encode an RNAi inhibitor. When we performed a sequence analysis of ORF1, we discovered a putative bipartite nuclear localization signal (NLS), which is a sequence of amino acids that directs the transport of proteins into the nuclei of cells. The goal of this project was to verify that this NLS was transporting ORF1 into the nucleus of the cell. We created an *ORF1-GFP* construct, as well as a mutant construct that deleted the NLS from ORF, transfected these into S2 cells, and observed using florescent microscopy. The results suggest nuclear localization, as the ORF1-GFP staining overlaps with DAPI staining in nuclei of the S2 cells, and the mutant construct showed GFP staining in the cytoplasm with no overlap with DAPI in the nuclei. To our knowledge, this is the first example of an RNA virus that specifies an RNAi inhibitor that translocates to the nucleus.

8. **THE MASS EMERGENCE OF 17-YEAR CICADA ACCELERATES LITTER DECOMPOSITION.** Ismert, K., Reazin, C., Morris, S., Jumpponen, A., Sikes, B., Zeglin, L. 1 Kansas State University, Manhattan, KS (KPBS) 2 University of Kansas, Lawrence, KS (KU)

Magicicada septendecim accumulate nutrients during their feeding and provide a nutrient pulse as they emerge to mate. To understand how the mating and subsequent massive deposit of cicada impact litter decomposition in soil, we conducted a litter bag experiment in two field sites in the state of Kansas. We targeted bur oak (*Quercus macrocarpa* Michx.) and hackberry (*Celtis occidentalis* L.) leaf litter, with and without cicada carcasses. At both sites, we compared litter bags with 10g of hackberry or bur oak litter with or without an additional 2g of cicada. The 30µm mesh litter bags were incubated *in situ* and sampled at the time of deployment (T0), one month (T1), two months (T2), four months (T3), or six (KPBS) and twelve (KU) months later (T4). Our data show that (i) both litter types decomposed faster with cicada and (ii) although the recalcitrant litter decomposed faster in the cicada-amended treatment, it still retained greater proportion of the original mass at T4 than the easily decomposable litter. These results highlight nutrient pulses from insect population dynamics may alter nutrient cycling and decomposition. Analyses of these experiments continue and additional efforts aim to dissect bacterial and fungal communities and their dynamics over time.

9. **Using Inverse PCR and Sequencing to Identify Genes in Pseudomonas aeruginosa that Contribute to Susceptibility to the Antimicrobial Peptide DASamp2.** Matthew M. Froid (Undergraduate)*, Donald Rowen, PhD. University of Nebraska-Omaha, Department of Biology, Omaha Nebraska

Increased resistance to conventional antibiotics is making the treatment of bacterial infections more difficult. Due to this dilemma, new alternatives to traditional antibiotics are currently being sought. DASamp2 is a promising new antimicrobial peptide that has been experimentally shown to be effective against both Gram positive and negative bacteria. In an effort to determine the target of DASamp2 and to determine the ease at which bacteria can develop resistance to this compound, we previously isolated mutant *Psuedomonas aeruginosa* strains with showed increased resistance to DASamp2. I determined that mutation in one mutant, (RMB1) was located in the gene *mexT* by using inverse PCR and sequencing. The gene, *mexT*, is believed to encode a transcriptional regulator involved in the production of extracellular efflux pumps. However, the regulation of the production of efflux pumps in *P. aeruginosa* is complex and poorly understood. Further characterization of this gene regulatory network is valuable due to the large increase in resistance to DASamp2 (8 fold or higher) seen in the RMB1 strain. Such a significant increase in resistance is highly suggestive that the efflux pumps regulated by *mexT* play a role in determining the sensitivity of *P. aeruginosa* cells to DASamp2.

10. **Isolation and Characterization of Halo-Acidophilic Microorganisms from Lake Gneiss, Western Australia.** Ashley Segobiano (Undergraduate)*, Melanie R. Mormile, and Sarah Stewart Johnson. Missouri University of Science and Technology, Rolla, Mo.

Western Australia lakes, located in the Yilgron Craton, may be the best terrestrial analogue to previous Martian conditions as there are many geochemical similarities. We hypothesize that haloacidophilic bacteria reside in these lakes. Lake Gneiss is among the most extreme of these lakes, with a pH of around 1.4 and saturated salt conditions. The purpose of the present study was to develop enrichments for the isolation and characterization of haloacidophiles to gain a better understanding of the organisms that reside in these harsh environments. Medium designed to simulate lake conditions, microscopic observation, and molecular analyses were employed to achieve the aforementioned goals. After six months of growth, the first enrichment of Lake Gneiss had turbidity and developed a pH of 0.5. DNA extraction was performed on the first enrichment of Lake Gneiss and a genomic library was generated. Our results support our hypothesis that bacteria can exist at extreme low pHs and salt molarities and provides hints of the kind of life that could have previously existed on Mars.

11. **Detection of the Nora virus Regulated Proteins, Vir-1 and Vago, in *Drosophila melanogaster* Hemolymph.** Isaac J. Lee (Undergraduate)*, Amanda Macke, Darby J. Carlson, and Kimberly A. Carlson. Department of Biology, University of Nebraska at Kearney, Kearney NE 68849

Research into the innate immune response of *Drosophila melanogaster* against viruses may help identify their functions in humans. Two viral regulated proteins, Virus induced RNA-1 (Vir-1) and Vago, are candidates for analysis because they are biosynthetic products of the innate immune system. As of yet, the function of these proteins is uncharacterized in Nora virus infection. The pathology of Nora virus is unknown, but a cognitive locomotor defect has been identified in our lab. Our hypothesis is that if Nora virus infection is causing the locomotor defect, then the most likely route of transmission from the gut to the brain would be through the hemolymph. Western blot analysis of Nora virus infected flies demonstrates the presence of Nora virus, Vir-1, and Vago within the hemolymph. Since Nora virus was previously thought to only be located within the gut of *D. melanogaster,* this is a new finding that may indicate infection in other tissues. More research must be conduction on Vir-1 and Vago, but it is now possibly identified as part of the proteome comprising the hemolymph of Nora virus positive flies.

12. **Isolation of Soil Microbes to Test against E.S.K.A.P.E. Relatives for Antimicrobial Properties.** Sara Nansel (Undergraduate)* and Claudia Carvalho. Fort Hays State University, Hays, Kansas.

The E.S.K.A.P.E. pathogens, (*Enterococcus faecium, Staphylococcus aureus, Klebsiella pneumoniae, Acinetobacter baumannii, Pseudomonas aeruginosa,* and *Enterobacter* species) are a group of bacteria that have developed multiple resistances to antibiotics (Boucher et al.). Antibiotic resistance occurs when bacteria develop the capability to adapt and multiply in the presence of antibiotics. This presents a world-wide health concern as antibiotics that were commonly used to treat these infections are no longer effective. By testing bacteria obtained from soil samples collected around the Hays, KS area against relatives of the E.S.K.A.P.E. pathogens, the goal is to find new antimicrobial properties that will act against the relatives and then, potentially, the E.S.K.A.P.E. pathogens. From each soil sample, 16 isolates were selected based on morphological differences to test against the E.S.K.A.P.E. relatives to observe inhibitory effects. After selecting six pure isolates exhibiting this property, a combination of staining techniques, biochemical testing, as well as genetic analysis was performed for bacterial identification and characterization. The isolates were found to be both Gram negative and Gram positive and of the *Bacillus* and *Pseudomonas* genera. Organic extraction is currently being performed to isolate and purify the inhibitory component of each isolate.

13. ***Chlamydia trachomatis* Manipulation of Host Kinases.** Nick Nelson (undergraduate)*, Prakash Sah, and Erika Lutter, Oklahoma State University, Stillwater OK

Chlamydia trachomatis is an intracellular pathogen that causes an estimated 3 million infections each year in the United States. Infections can lead to further complications such as infertility and ectopic pregnancy. During infection, *C. trachomatis* lives in a parasitophorous vacuole termed an inclusion. From within the inclusion the bacteria manipulate host-cell functions in order to aid in its survival. Many proteins, including kinases, are recruited to the inclusion membrane. Within this study we focused on host kinases and phosphorylated kinase substrates. Recruitment of kinases and kinase substrates to the inclusion during infection was monitored by immunofluorescence using kinase specific antibodies. The overall protein levels and levels of phosphorylated protein substrates were analyzed by SDS-PAGE and western blot. The analysis of phosphorylated substrates for MAPK, CDK and PKA showed altered phosphorylation as the *C. trachomatis* infection progressed over 48 hours. Both the MAPK-CDK and PKA pathways control and play a significant role in cell proliferation and apoptosis which would aid *C. trachomatis* in its survivability. Manipulation of host kinase activity manipulation by *C. trachomatis* is essential to deciphering host-pathogen interactions and may lead to future novel therapeutic targets.

14. **Relationship Between Locomotor Function and Nora Virus Infection in *Drosophila melanogaster.*** Amanda McCown (undergraduate)*, Abigail Benz, & Kimberly A. Carlson, Department of Biology, University of Nebraska at Kearney, Kearney, Nebraska.

Nora virus is a member of the picornavirus family that infects *Drosophila melanogaster* with no published pathogenic effects. A previously unstudied pathogenic effect of Nora virus is locomotor ability. In this study, the effect Nora virus has on longevity and locomotor ability is being examined. Locomotion is examined using geotaxis, where flies have one minute to cross a threshold one inch from the top of the cage. Treatment groups include Nora virus infected, uninfected and Drosophila C Virus (DCV) infected flies. Longevity curves created using a Student's t-test demonstrate that Nora virus infected flies are significantly slower in their climbing abilities compared to uninfected flies, which supports a relationship between geotaxis and locomotor dysfunction in infected flies. No significant difference was seen in locomotor ability between DCV infected and Nora virus infected flies. Nora virus viral load was determined utilizing qRT-PCR with results that demonstrate a bimodal curve for Nora virus infection. This data suggests that Nora virus does effect locomotor function and can be classified as a pathogenic effect of the virus.

15. **Investigating the role of inflammatory cytokines during influenza A virus and *Aspergillus fumigatus* coinfections *in vivo*.** Meagan D. Rippee-Brooks (Undergraduate)*, Christopher R. Lupfer. Missouri State University Department of Biology, Springfield, Missouri.

Bacterial coinfections with influenza A virus (IAV) are extremely serious and life-threatening. However, there exists limited data gathered about the importance of fungal infections with IAV. Clinical case reports indicate that fungal coinfections do happen and suggest the pandemic 2009 IAV has a propensity to predispose patients to secondary fungal infections more than previous IAV strains. However, the factors involved remain to be determined. We have developed an *in vitro* model using primary mouse bone marrow derived macrophages infected with IAV and coinfected with the opportunistic fungal pathogen *Aspergillus fumigatus*. Our results indicate that IAV and fungal coinfections synergistically enhance cell signaling and cytokine production. We propose this exacerbated immune response during IAV and *A. fumigatus* predisposes clinical patients to more severe pneumonia, and we seek to identify the pathways responsible for the heightened cytokine responses and their significance *in vivo*.

16. **Prevalence of Culturable Endophytic Bacteria in a Variety of Seed Species.** Faith Higgins* (Undergraduate), Gianna Morrie* (Undergraduate), Ashten Grabill* (Undergraduate), Heather M. Seitz. Johnson County Community College, Overland Park, Kansas.

Antibiotic resistance is a serious threat to the world, and untreatable infections are no longer a prediction for the future, they are happening right now. Unfortunately, the pace of antibiotic discovery is not keeping up with the rapid evolution of resistance to microbes. Few new antibiotics have been discovered in the past 30 years (McIntosh, 2016). Endophytic bacteria can be defined as those bacteria that colonize the internal tissue of the plant showing no external sign of infection or negative effect on their host (Holliday, 1989 & Boyle, 2006). Endophytic bacteria are able to lessen or prevent the deleterious effect of certain pathogenic organisms in plants (Ryan, 2008). The investigation of endophytic strains for novel antimicrobial metabolites is an untapped source with minimal previous research. We are focusing on finding and classifying endophytic bacteria that produce novel antibiotics that can treat pathogens. In this research we will demonstrate our approach to identifying endophytic bacteria and share the prevalence of culturable strains of bacteria found on a variety of species. Further we have documented the antimicrobial properties of the endophytic bacteria from multiple species.

17. **Translesion Synthesis Protein Abundance in Proliferating Cells.** Jazmine Snow (Undergraduate)*, Sebastian Wendel, Nicholas Wallace. Division of Biology, Kansas State University, Manhattan, KS

Human Papillomavirus (HPV) causes nearly every cervical cancer, with approximately 14 million infections annually in the United States. These cancers are the result of HPV oncogenes (HPV E6 and E7) manipulating the host cells. These changes were characterized by transcriptome analysis of tumor and control cervical samples and found increased expression of genes needed for translesion synthesis (TLS). This led to the hypothesis that highly proliferating cells encounter more DNA damage resulting in an induction of TLS, a DNA damage tolerance pathway. To test this hypothesis, we determined whether the proliferation of human foreskin fibroblasts (HFFs) could be modulated. We grew HFFs in a gradient of fetal bovine serum (FBS) concentrations. When the cells neared confluence, they were harvested and counted on a hemocytometer. This data showed an increase in cell proliferation correlating with increasing FBS concentration (R^2=0.87). Harvesting proteins from parallel treated cells determined that TLS protein levels mirrored proliferation rates.
Our *in vitro* data show that HPV oncogenes prevent cervical cancer cells from inducing expression of Polη, a TLS polymerase. I will determine if HPV oncoproteins prevent proliferation-induced increases in TLS protein levels by repeating these experiments in HFFs expressing HPV oncogenes.

18. **Identification of Candidate Cell Division Proteins in *Planctomyces limnophilus* Using the BACTH Two-Hybrid System.** Brianna Steiert (Undergraduate)*, Dr. Lilah Rahn-Lee. William Jewell College, Liberty, Missouri.

Understanding cell division mechanisms in bacteria is important because cell division is fundamental for bacterial reproduction and survival. Most bacteria divide by binary fission and are assisted by proteins such as FtsZ that assembles into a ring at mid-cell during division. This research uses planctomycetes, a group of bacteria that are unusual because they do not use FtsZ for division. Planctomycetes have many distinctive phenotypic features including the compartmentalization of cells with internal membranes and a membrane-bound nucleoid. The purpose of this research is to study the division mechanism by screening a *Planctomyces limnophilus* genomic DNA prey library against several cell division protein baits using the BACTH two-hybrid system. Through bioinformatics work, planctomycetes genes were compared to genes in other bacterial genomes known to be involved in cell division to identify the baits of FtsK and FtsI. Bait and prey interactions will be screened through co-transformed into DHM1 cells, and prey that interact with bait will be characterized to determine the *Planctomyces limnophilus* sequence in the prey. These genes will be candidates for members of the planctomycete unique cell division mechanism.

19. **The construction of a genetic device to investigate the regulation of ploidy in cyanobacteria.** Seki K. Anderson (Undergraduate)* and Dr. Lilah Rahn-Lee. William Jewell College, Liberty, Missouri.

Cyanobacteria is a bacterial phylum known to exhibit polyploidy; different cyanobacteria species have different copy numbers, and depending on the strain, ploidy ranges from as low as three copies to as high as 218 genome copies. Bacterial strains are defined by their differences in genetic makeup, so we hypothesize there is a genetic factor that plays a role in determining the number of chromosome copies within a cyanobacteria species. We are interested in studying two strains within the same genus that are different in genome copy number; *Anabaena cylindrica* has an average of 25 genome copies and *Anabaena variabilis* has an average of 8 genome copies. To look for genetic determinants of copy number, we are constructing a bistable sensor that detects the number of chromosome copies a strain of cyanobacteria has. Once the genetic device is made and has been tuned, we plan to transform a genomic library from one species to the other and use our bistable genetic device to screen for individuals with changed copy number. This research will expand upon our understanding of cell division and bacterial diversity, as we aim to discover the mechanisms cyanobacteria use to count and regulate their chromosome copy number.

20. **Prevalence of antibiotic resistant strains of *Enterococcus* spp. and *Acinetobacter* spp. in community household environment.** Rebekah Elliott (Undergraduate)*, Jada Caplinger, and Anuradha Ghosh. Department of Biology, Pittsburg State University (Pittsburg, KS)

With increasing prevalence of antibiotic resistance threats, there is an upsurge in the occurrence of community-acquired infections. The purpose of this study is to assess the ecology and prevalence of *Enterococcus* spp. and *Acinetobacter* spp. (that are well-known antibiotic resistant nosocomial pathogens) in the household environment. Each household sampling kit contained 5 swabs for each of shoe bottom, restroom, cleaning supply, kitchen top, and door step/handle as well as a demographic data sheet to be filled up. A total of 30 such kits (n=150) have been processed. The swabs were subjected to enrichment using selective media for test bacterial species. Total number kits positive for growth of suspected enterococci and *Acinetobacter* spp. were determined. Only few cleaning supplies showed growth for enterococci whereas the kitchen top showed more frequent enterococcal contamination. Although majority of the locations swabbed were contaminated with suspected *Acinetobacter* spp., door step/handles were free of any selected microbe. Overall, most of the swabbed locations were contaminated with biochemically confirmed *Acinetobacter* spp. in contrast to fewer with enterococci. A panel of antibiotics were tested for their susceptibility. Further PCR amplification of selective genes will be carried out to confirm at the species level. The antibiotic-resistant isolates will be genotyped and compared to their relative nosocomial strains. The community will be outreached with recommended cleaning protocol and stewardship in antibiotic consumption and resistance. The outcome of this study may help facilitate effective and appropriate antibiotic treatment of community-acquired infections.

General Microbiology Graduate Student Oral Presentations

1. **D-motif Mutation in cAMP Phosphodiesterase, RegA, Leads to Developmental Phenotype Defect in *Dictyostelium discoideum*.** Nirakar Adhikari (doctoral student)*, Nick Kuburich, Jeff Hadwiger. Oklahoma State University, Stillwater, Oklahoma.

Cyclic nucleotide phosphodiesterase molecules have clinical significance because they down regulate second messenger cAMP or cGMP in cell. In *Dictyostelium discoideum* the MAP kinase (MAPK), ERK2, down-regulates the phosphodiesterase, RegA. RegA lowers the level of cAMP, an important regulator of cell aggregation and differentiation. RegA has a MAPK docking site (D-motif) that might be important in interactions with MAPKs. Characterizing the function of this D-motif in RegA will likely provide insights on the interactions between RegA and MAPKs during the developmental life cycle of *Dictyostelium*. These findings can lead to a better understanding in other eukaryotes including mammals. In the absence of D-motif, little or no interaction is expected to occur between RegA^{D-} and Erk2. This might prevent the down-regulation of RegA^{D-} and cause reduced levels of cAMP. Low levels of cAMP are expected to delay the aggregation and differentiation of *Dictyostelium* cells. RegA D-motif was mutagenized and the corresponding mutant gene (*regA*$^{D-}$) has been expressed into regA^{-} mutant cells. The clones with stable phenotypes showed that these *regA*$^{D-}$ clones were slower in overall rate of development and have defect in their developmental stage. Western blot analysis will be used to determine if any interactions occur between RegA^{D-} and Erk2.

2. **The Role of β-carbonic anhydrases in Calcium Deposition by *Pseudomonas aeruginosa.*** Biraj B. Kayastha (Doctoral)*[1], Shalaka Lotlikar[1], Erin Gallaway[1], Claudiu T. Supuran[2], and Marianna Patrauchan[1], [1]Oklahoma State University, Stillwater, OK, [2]Univeristy of Florence, Florence, Italy

Pseudomonas aeruginosa is an opportunistic human pathogen causing life-threatening infections in patients with cystic fibrosis and infective endocarditis, which can be associated with calcification at later stages. Earlier results showed that *P. aeruginosa* produces extracellular deposits of calcium (Ca^{2+}) when grown at elevated Ca^{2+} levels. We identified and characterized three β-carbonic anhydrases (CAs): psCA1, psCA2, and psCA3. While the deletion of all three encoding genes delayed *P. aeruginosa* growth at ambient air by 4 hours, it caused no impact on static growth at 5% CO_2. Measuring both deposited and free Ca^{2+} in liquid medium for the wildtype showed that static growth conditions and 5% CO_2 favor Ca^{2+} deposition. Analyses of CA mutants determined that psCA1 is the major contributor to calcium deposition. Deletion of psCA1 alone caused ~2 fold decrease in Ca^{2+} deposition and almost abolished free Ca^{2+} removal from the medium at both 5 and 10 mM Ca^{2+}. The results show that psCA2 also contribute to Ca^{2+} deposition at 10 mM Ca^{2+}, but psCA3 plays no role in this process. Currently we are testing the effect of CA inhibitors on the β-CA-induced Ca^{2+} deposition and the role of β-CA-dependent calcification in biofilm formation and virulence of *P. aeruginosa.*

3. **Metabolically Engineering *Aspergillus nidulans* using RNA Interference.** Jorge Lightfoot (Doctoral)*, Rolf Prade. Oklahoma State University, Stillwater, Oklahoma

The filamentous fungi, *A. nidulans*, can produce nearly 100 grams per liter of industrially relevant proteins under optimal conditions. However, many of these proteins are degraded or produced alongside other proteins, which drastically reduce their efficacy in a cellulose fermentation reaction. We propose a novel mechanism, utilizing RNA interference, to combinatorially silence genes, which degrade or contaminate client proteins. Using dual promoters, we will flank a sequence containing 30 or 40bp complementary sequences for multiple client genes. This will induce double stranded RNA production, in turn loading these individual complementary sequences into the Argonaute complex, silencing the messenger RNA for each target gene. We have also utilized LC-MS/MS to examine changes in the proteome of our silenced strains. We have seen marked decreases in our target gene sequences as well as the induction of new proteins, acting as a compensation mechanism for the fungus. Our silenced strains, when transformed to produce client proteins, have also had a marked change in the amount of protein produced, as well as how long it lasts in the media during production. We have continued this work by silencing genes responsible for unwanted amylolytic activity in client protein production.

4. **Investigating a Phosphodiesterase Involved in Amoeboid Multicellular Development and Signaling.** Nick Kuburich (Doctoral)*, Nirakar Adhikari, Jeff Hadwiger. Oklahoma State University, Stillwater, Oklahoma

Many eukaryotic signaling pathways use cAMP as a secondary messenger to evoke specific responses to different external stimuli. Here, localized levels of cAMP can be controlled by phosphodiesterases, which are sometimes regulated by phosphorylation. *Dictyostelium discoideum* offers an excellent model system to study the regulation of phosphodiesterases as it contains relatively few cAMP-specific phosphodiesterases compared to mammals. The cAMP-specific phosphodiesterase, RegA, regulates important steps in *Dictyostelium* development and is negatively regulated by the MAP kinase, ERK2. This inactivation occurs periodically by external cAMP pulse where a cell-signaling pathway activates ERK2. Mammalian studies have suggested that the cAMP-dependent protein kinase, PKA, can also regulate the phosphodiesterase activity. This putative regulation of PKA on the activity of RegA has not been fully investigated in *Dictyostelium*. Mass spectrometry was used to detect potential phosphorylation sites on RegA. Two sites of interest have been identified, including a PKA phosphorylation site. Phosphomimic and phosphoablative mutations for the three sites have been constructed. The phenotypes of cells carrying these mutations have been analyzed for their impact on development and cell fate. These data support the hypothesis that RegA is regulated by multiple phosphorylation to regulate signaling during multicellular development.

5. **Exploring the Potential Role of Vps1 as a Fusion Protein.** Mariel Delgado Cruz (Masters)*, Dr. Kim, Kyoungtae. Missouri State University, Springfield, MO

Protein trafficking within the cell is more than just the series of steps necessary to get cargo from one end of the cell to another location, it also involves, the easily overlooked, fusion step. Fusion is the very last step in trafficking when the transported cargo is passed on from the carrier vesicle on to the target membrane. This step in intracellular trafficking has often been characterized by the use of SNARE proteins, however, there are a multitude of proteins involved in this step. Among them are Rabs, Golgins, and Multitethering proteins. Vps1 was suspected to play a role in homotypic Golgi fusion after noticing that a depletion in this GTPase resulted in an increase of Late Golgi numbers. This led to the hypothesis that Vps1 is functioning as a fusion protein acting to bring two late Golgi compartments together in order to form a lesser number of larger Golgi compartments. This question was explored using yeast two hybrid where it has been found that Vps1 interacts with Golgi localized SNAREs: *Tlg1, Tlg2, Vti1*, and *Snc1*. To further investigate this question a GST pulldown assay and an *in vivo* yeast mating experiments are all underway.

6. **CarP is a Ca^{2+} Regulated Putative Phytase that is Unique to Pseudomonads and is Highly Conserved in Clinical Isolates.** M. M. King (Doctoral)*, S. Mares, and M. Patrauchan. Oklahoma State University Stillwater, OK

Pseudomonas aeruginosa is a versatile pathogen causing various infections in a human body. It is important to identify the host factors that signal to *P. aeruginosa* the patient's immunocompromised status, triggering the virulence of the pathogen. Our earlier studies suggested that elevated calcium (Ca^{2+}) is one of such signals, as it increases virulence in *P. aeruginosa*. We have identified a protein CarP that is important for Ca^{2+}-regulated infectivity in *P. aeruginosa*. Sequence analysis predicted phytase activity and showed that *carP* is unique to Pseudomonads. High-resolution RNAseq and sequence analyses suggested complex regulation of *carP* by multiple host factors. Promoter activity assays confirmed the regulatory role of elevated Ca^{2+}, H_2O_2, and CO_2. To characterize the role of *carP* during infections we aim to determine whether the encoding and regulatory sequences of *carP* are conserved in clinical isolates (CI). The *in silico* analysis revealed these sequences are more than 98% identical in ~2,000 CI collected from patients with burn wounds, upper respiratory and urinary tract infections, and keratitis. The identified mutations do not alter protein sequences. This work shows that *carP* and its regulatory elements are preserved during infections and highly responsive to host environment, suggesting their importance in *P. aeruginosa* pathogenicity.

7. **Myosin mediates protein recycling toward the Golgi.** Jared Smothers (Master's)*, Paul Ballhorn*, Vy Nguyen*, Dr. Kyoungtae Kim* Missouri State University, Springfield, Missouri

Myosin family proteins are ATP-dependent motors that share the same basic properties of actin binding. The chemical energy provided by ATP-hydrolysis in the head domain generates the "power stroke" necessary to "walk" along actin filaments. In this study, using confocal microscopy we assessed the potential roles of all five myosin's in *Saccharomyces cerevisiae*, and their effect on the recycling of Snc1 and Vps10. *MYO*1 knockout strains had no significant defect in Snc1 recycling, but displayed severe defects in the trafficking of Vps10 toward the Golgi, manifested by abnormal localization to the lumen of the vacuole. Myo3 and Myo5 are paralogs that have little to no significant traffic defect when knocked out individually, but the double deletion of both *MYO*3 and 5 led to severe defects in Snc1 and Vps10 trafficking. Two temperature sensitive strains of Myo2, *myo2-16* and *myo2-66*, demonstrated trafficking defects at restricted temperature conditions. Among all five members, only *MYO*4 deletion did not affect protein recycling pathways. Together, our data provides novel insights into the function of Myo-family proteins in protein recycling traffics destined towards the *trans*-Golgi Network.

8. **Searching for Recruitment Domains and Residues of Vps1 to Target Membrane.** John C. (Wes) Short (Masters)*, Shiva Kumar Goud Gadila, Kyoungtae Kim. Missouri State University, Springfield, Missouri.

Intracellular membrane trafficking requires classical dynamins and dynamin-like proteins (DLPs), ubiquitous throughout the eukaryotic domain. Dysfunction of classical dynamins in humans is linked to Alzheimer's and other neuropathies. Loss of ortholog yeast DLP Vps1 disrupts pathways of vesicle trafficking, which we use to investigate gene function. *In vitro* assays have shown that Vps1 interacts directly with membrane to exert its membrane-remodeling power. However, the molecular signal for the recruitment of Vps1 to target membranes has not been described. To investigate, genetic variants of Vps1 were N-terminally fused with mRFP and overexpressed in wild type (WT) and *vps1*Δ background with genomically-tagged GFP membrane-associated protein markers, and examined by confocal microscopy for colocalization. The Vps1 variants were sets of domain fragments alone and in combinations, serially shortened C-terminal truncations, and selected point mutations at suggested binding sites, which would reestablish or abolish Vps1 recruitment and function. Results showed that all tested variants abolished the normal phenotype, and no tested point mutations caused abnormality, suggesting that the fully-formed 3D shape with its intact catalytic and polymerization domains is essential to its ability to target membranes. Further structural analysis will narrow down possible binding patches for further testing.

Medical Microbiology/Immunology Graduate Student Oral Presentations

1. **Effect of the Outer Membrane Permeabilizer Compound 48/80 on Intrinsic Resistance to the Hydrophobic Biocide Triclosan in Opportunistic *Serratia* Species.** [1]A. Rigsbee (Masters)*, [2]S. Katz Amburn, [3]B. King, [4]K. Boyina, [4]J. Yang, [1]W. Sprinkles, [3]A.A. McDonald, [5]V.M. Patel, [1]F.R. Champlin. [1]Oklahoma State University Center for Health Sciences, Tulsa, OK; [2]Rogers State University, Claremore, OK; [3]Northeastern State University, Broken Arrow, OK; [4]Oklahoma State University, Stillwater, OK; [5]Union High School, Tulsa, OK.

Unlike most hydrophobic molecules, the biocide triclosan is able to penetrate the gram-negative bacterial outer membrane. Atypical resistance to triclosan in the nosocomial opportunists *Pseudomonas aeruginosa* and *Serratia marcescens* is largely due to outer membrane properties that result in impermeability for hydrophobic compounds. The present study was undertaken to determine if these cell envelope impermeability properties are conserved and underlie triclosan resistance in other opportunistic *Serratia* species. General intrinsic resistance to hydrophobic compounds was assessed using three disparate bioassays and one chemical assay. Batch culture kinetics in the presence of combinations of triclosan or the hydrophobic antibiotic novobiocin and the outer membrane permeabilizer compound 48/80 allowed analysis of outer membrane involvement in intrinsic resistance. Ten individual species ranged from generally refractory to sensitization, as seen with *Serratia liquefaciens*, to extremely susceptive, as seen with *Serratia odorifera*. Moreover, those species which exhibited intrinsic resistance to both novobiocin and triclosan were readily sensitized to different degrees by chemical disruption of their outer membrane exclusionary properties**.** These data suggest that disparate opportunistic pathogens within the genus *Serratia* differ phenotypically with regard to the degree to which outer membrane exclusion contributes to intrinsic resistance to hydrophobic molecules in general, and triclosan specifically.

2. **Three Transketolases in *Salmonella enterica* Contribute to Defending Against Oxidative and Nitrosative Stresses.** Jeff A. Shaw (Doctoral)* and Travis J. Bourret. Creighton University School of Medicine, Omaha, Nebraska.

As a facultative intracellular pathogen, *Salmonella enterica* serovar Typhimurium must combat host-derived reactive oxygen species (ROS) and reactive nitrogen species (RNS) that can damage bacterial DNA, modify proteins and enzymes, and disrupt the redox state of invading intracellular bacteria. To power antioxidant defenses and overcome these cytotoxic effects, *S*. Typhimurium utilizes the oxidative branch of the pentose phosphate pathway (oxPPP) to generate reducing power in the form of NADPH. In the non-oxidative branch (non-oxPPP), enzymes generate metabolic intermediates that can be channeled to glycolysis, recycled back to the oxPPP, or utilized as precursors or biosynthetic reactions. This study investigated the contribution of transketolases in the non-oxPPP to resisting ROS and RNS. The *S*. Typhimurium genome contains genes for three transketolases (TktA, TktB, and STM2340-41), each of which catalyzed the transketolase enzymatic reaction. When exposed to ROS and RNS, mutant strains lacking all three transketolases demonstrated the greatest sensitivity *in vitro*, most of which was attributable to loss of TktA. Furthermore, a Δ*tktA* Δ*tktB* mutant had attenuated virulence following intraperitoneal infection of C57BL/6 mice, but virulence was restored in iNOS-deficient mice. This study reveals an essential role for transketolases in resisting ROS and RNS, and suggests that they may contribute to the intracellular survival of *S*. Typhimurium.

3. **Segmental Aneuploidies Flanked by Inverted Repeats Cause Azole Resistance in the Fungal Pathogen *Candida albicans.*** Robert Todd (graduate)*, Tyler Wikoff, Shilpa Nair, Curtis Focht, Robert Thomas, Anna Selmecki. Creighton University School of Medicine, Omaha, Nebraska.

Candida albicans is the most prevalent fungal pathogen in immunocompromised individuals. Currently, treatment of *Candida* infections relies heavily on azoles, a family of fungistatic drugs that disrupt the biosynthesis of the fungal-specific sterol, ergosterol. Aneuploidy, amplification or loss of a chromosome, is a common genomic feature found in 50% of azole-resistant *C. albicans*. Previous work from the Selmecki Lab has shown that a specific segmental aneuploidy, an amplification of the left arm of chromosome 5, can confer azole resistance due to the amplification of two drug-response genes. Currently, little is known about how segmental aneuploidies form. Using next-generation sequencing technology, chromosome karyotyping, and long-range DNA sequencing we describe several novel segmental aneuploidies found in azole resistant *Candida* strains from diverse genetic backgrounds. These segmental aneuploidies consist of amplified regions of the genome, some of which can undergo copy number increases of greater than 12 copies. These amplifications contain important drug resistance genes and correlate with a significant increase in azole minimal inhibitory concentration. Here, we describe these novel segmental aneuploidies that confer azoleresistance and identify genetic features that elucidate a common mechanism of formation.

4. **ROS/RNS Induced Changes in *Borrelia burgdorferi* Outer Surface Proteins**. Amanda K. Zalud (doctoral)*, Travis J. Bourret. Creighton University, Omaha, Nebraska.

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 large repertoire of lipoproteins. Lipoprotein expression is controlled by a limited number of transcription factors in response to diverse environmental challenges encountered during infection, including changes in pH, temperature, osmolarity, nutrient availability, and host-derived reactive oxygen species (ROS) and reactive nitrogen species (RNS). In the following study, we tested the hypothesis that ROS and RNS serve as regulatory signals for lipoprotein expression in *B. burgdorferi*. To test this hypothesis, we compared the expression of immunogenic lipoproteins in *B. burgdorferi* grown in BSK II and exposed to hydrogen peroxide or spermine NONOate. A shift in pH from 7.6 to 6.8 lead to widespread changes in lipoprotein expression, which has been shown previously. Surprisingly, sublethal concentrations of hydrogen peroxide or the nitric oxide donor spermine NONOate inhibited the pH-dependent changes in lipoprotein expression. These data suggest ROS and RNS contribute to the regulation of lipoprotein expression in *B. burgdorferi* and may impact its ability to complete its natural infectious cycle.

5. **Determination of the Role of Chlamydial Inclusion Membrane Proteins in Inclusion Growth and Development by Proximity Labeling of Interacting Partners.** Macy G. Olson (doctoral student)*, Elizabeth A. Rucks and Scot P. Ouellette. University of Nebraska Medical Center, Pathology and Microbiology, Omaha, Nebraska.

Chlamydia trachomatis (Ctr), a developmentally-regulated, obligate intracellular pathogen, is the leading cause of bacterial sexually transmitted infections. Chlamydiae grow within a pathogen-specified organelle, termed the inclusion. The inclusion membrane mediates interactions between the host and pathogen. To support chlamydial-host interactions, the inclusion membrane is heavily modified throughout the developmental cycle by chlamydial proteins called Incs which encode two or more transmembrane motifs. We hypothesize that Ctr uses temporal control of Inc expression to facilitate inclusion development, whereby Incs expressed early (e.g. IncF) play an essential role in organization/establishment of the inclusion and Incs expressed later (e.g. IncA) are involved in nutrient acquisition. Here, we compare IncF and IncA over-expression to determine their roles in inclusion development, including how these Incs are involved in protein-protein interactions at the inclusion membrane. To examine our hypothesis, we created Ctr L2 transformants with IncF-APEX2, $IncA_{TM\ (transmembrane\ domain)}$-APEX2, IncA-APEX2, or APEX2 only. APEX2 is an ascorbate peroxidase that biotinylates proximal and interacting proteins *in vivo*. Preliminary studies show that biotinylated proteins identified by mass spectrometry analysis support currently known data for Incs and their eukaryotic protein binding partners. These studies are important for understanding molecular mechanisms involved in establishment of this intracellular pathogenic niche.

6. **The *Borrelia burgdorferi bb0168*-Encoded DnaK Suppressor Enhances pH Dependent Lipoprotein Expression.**
William K. Boyle (doctoral)[1]*, Jeff A. Shaw[1], Ashley Groshong[2], Jon S. Blevins[3], Frank C. Gherardini[4] and Travis J. Bourret[1]. Creighton University School of Medicine, Omaha, Nebraska[1], UConn Health, Farmington, Connecticut[2], University of Arkansas for Medical Sciences, Little Rock, Arkansas[3], National Institute of Allergy and Infectious Diseases, Hamilton, Montana[4].

Borrelia burgdorferi, the causative agent of Lyme disease, must sense transmission favoring conditions within tick vectors and increase the expression of infectivity associated lipoproteins. We hypothesized that a putative transcriptional regulator, the DnaK suppressor protein (DksA), plays a role in coordinating the transcriptional response of *B. burgdorferi* to environmental signals encountered during infection. To test this hypothesis, wild-type and *dksA*-deficient *B. burgdorferi* strains were subjected to growth conditions mimicking the tick midgut during bloodmeal acquisition by shifting mid-log cultures (5×10^7 spirochetes / ml) grown in BSKII (pH 7.6) to BSKII (pH 6.8), and allowing them to reach stationary phase (2×10^8 spirochetes / ml). Immunoblots of spirochete lysates with serum from *B. burgdorferi*-infected mice indicate that wild-type spirochetes increased the expression of immunogenic proteins in response to pH shift, while *dksA*-deficient strains exhibited a constitutively low expression of these pH-inducible proteins. Further, we observed that *dksA* is required for the expression of the key infectivity associated lipoproteins DbpA and OspC, along with the alternative sigma factor RpoS in response to pH shift. Together, these data indicate DksA is a regulator that, directly or indirectly, controls the RpoS-dependent expression of immunogenic proteins in response to changes in pH.

Undergraduate or High School Oral Presentations

1. **Utilizing *Galleria mellonella* to Determine the Role of Calcium in *Pseudomonas aeruginosa* Virulence.**
Leah Kafer(Undergraduate)*[1], Michelle King[1], Mariette Barbier[2], Marianna Patrauchan[1]
Oklahoma State University, Stillwater, OK[1], West Virginia University, Morgantown, WV[2]

Pseudomonas aeruginosa is an opportunistic pathogen infecting the lungs of Cystic Fibrosis patients, known to have abnormally high levels of calcium (Ca^{2+}). Our lab has shown that elevated Ca^{2+} increases plant infectivity and the production of several virulence factors including pyocyanin, pyoverdine, and rhamnolipid in *P. aeruginosa.* Based on these observations, we hypothesized that elevated Ca^{2+} enhances *P. aeruginosa* virulence in an animal host as well. To test this hypothesis, I have optimized a virulence model using wax worms, *Galleria mellonella*. First, we aimed to determine the half lethal dose (LD_{50}) of *P. aeruginosa*. For this, we injected the worms with PAO1 grown in 0 mM or 5 mM Ca^{2+}. The latter died in greater quantities and faster than those infected with PAO1 grown without Ca^{2+}. The LD_{50} of PAO1 in 0 mM Ca^{2+} is two-fold higher than that of PAO1 grown in 5 mM Ca^{2+}. Currently, we are determining the role in virulence of three proteins, EfhP, CarP, and CalC, previously shown to mediate Ca^{2+} regulation of virulence factors production. This knowledge will enable identification of the components of Ca^{2+} regulatory network controlling *P. aeruginosa* virulence, which is a step towards learning novel ways to fight *P. aeruginosa* infections.

2. **Role of centromere heterogeneity in clinical isolates of *Candida albicans*.** Alison Guyer (undergraduate)*, Robert Todd, Anna Selmecki

Candida albicans is a fungus naturally found within the human microbiome. When exposed to stress, *C. albicans* can rapidly evolve through effective means such as gain or loss of an entire chromosome (aneuploidy). Specifically, when *C. albicans* is exposed to the antifungal drug fluconazole it may acquire a segmental aneuploidy called isochromosome 5L [i(5L)]. i(5L) arises from a breakpoint in the chromosome 5 centromere (CEN5), generating two copies of the left arm of chr5. Previous research has demonstrated i(5L) to be a key component in fluconazole drug resistance, but the mechanism driving i(5L) formation is unknown. An interesting feature found within CEN5 is a heterozygous sequence encoding a long terminal repeat (LTR). The purpose of this study is to explore the potential role of chr5 heterogeneity in the formation of i(5L) and acquisition of fluconazole drug resistance. We conducted an in vitro evolution experiment in which *C. albicans* were exposed to fluconazole and screened for changes in heterozygosity of the LTR and another heterozygous locus on chr5L, the mating type locus (MTL). We found that most *C. albicans* clinical isolates maintained bothheterozygous loci after 100 generations in fluconazole.

3. **Analyzing Protein Interactions Of The Herpes Simplex Type 1 UL34 Protein.** Nathaniel B. A. Higdon (Undergraduate)*, Susan L. Bjerke. Washburn University, Topeka, Kansas.

Herpes Simplex Virus Type-1 (HSV-1) is easily communicable and infections can occur in different locations. HSV-1 proliferates within the host cell nucleus. Once replication is complete the virus exits the nucleus. Viral protein U_L34 is essential for departure from the nucleus. It's unknown which nuclear proteins U_L34 is interacting with during this phase. U_L34 is a highly conserved protein in all human herpesviruses and an ideal candidate for future drug treatments. Blocking UL34 function in HSV-1 would prevent exit of the nucleus and spread of infection. To determine interaction partners for U_L34, pulldown assays were performed. In our assay, purified U_L34 protein was mixed with HEp-2 cell lysate; U_L34 and any binding partners were then removed from the mixture. Our past results showed some potential U_L34 binding partners, however, upon experimental replications, inconsistent results were obtained. To obtain similar results from our assays, we expressed a new GST control protein in E. coli. We will use this control to compare to the proteins that are being pulled down by a GST-tagged U_L34 protein. We hope our results will again show potential U_L34 binding partners. If more convincing protein interactions occur, isolation experiments will be performed to identify the binding protein(s).

4. **Determining the Localization of the Hypothetical Membrane Protein CBU_1651 from *Coxiella burnetii*** Keegan McGill (Undergraduate)* Brandon Luedkte, University of Nebraska at Kearney Department of Biology, Kearney, Nebraska

Coxiella burnetii is an obligate intracellular pathogen and the etiological agent of Query Fever. To cause disease, *C. burnetii* uses the Type IVB secretion system (T4BSS) to establish a replicative compartment, termed the parasitophorous vacuole (PV), and from here manipulate host cell functions via the release of effector proteins. A potential effector protein encoded by the gene *cbu_1651*, which is unique to *C. burnetii*, is of interest. Using *in silico* analyses, *cbu_1651* is predicted to have a transmembrane domain and is likely co-regulated with the T4BSS since it is located between the T4BSS genes *icmW* and *icmX* and suggests a possible important function for CBU_1651 during pathogenesis. The overall goal of this study was to characterize the localization of CBU_1651 during *in vivo* and *in vitro* growth. We have successfully developed polyclonal antisera against CBU_1651 and are using it for subsequent assays. We have found by western blot that CBU_1651 is secreted in a T4BSS dependent and lipid dependent manner into the growth medium. In tissue culture, indirect fluorescent antibody (IFA) assays indicated CBU_1651 to localize to the PV lumen. This data suggests that CBU_1651 is mediated by a mechanism that senses environmental stimuli.

5. **Characterization of the role of redox-active cysteines in the regulatory function of DksA in the Lyme disease spirochete *Borrelia burgdorferi.*** Sam Koshy (Undergraduate)*, William Boyle, and Travis J. Bourret, Creighton University School of Medicine, Omaha, NE

Borrelia burgdorferi is the causative agent of Lyme disease, and is estimated to infect as many as 300,000 individuals per year in the United States. *B. burgdorferi's* life cycle includes the white-footed mouse (*Peromyscus leucopus*), ticks of the genus *Ixodes*, and humans. As it is transmitted, it undergoes a variety of environmental changes that include shifts in pH, temperature, and nutrient availability. This project aims to explore the relevance of cysteines encoding the zinc finger domain in the transcription factor DksA (DnaK suppressor protein) that is known to play a key role in the stringent response. The zinc finger will be assessed by performing site-directed mutagenesis of cysteines within a *B. burgdorferi dksA* allele encoded on plasmids. The effects of these point mutations on DksA function will be determined by introducing the library of mutant plasmids into *dksA*-deficient *B. burgdorferi* strains. The ability of these strains to control the expression of stringent response genes, survive nutrient limitation and oxidative stress, and infect potential hosts will be conducted, highlighting the importance of the zinc finger motif within DksA in regulating the stringent response, and to gain insight into the how *B. burgdorferi* survives and proliferates in hostile environments.

6. **Identifying Intracellular Tick-borne Illnesses in Bison*, Bison bison,* via Blood Cell Staining and PCR.** Robertson, A. D. (Undergraduate)*, and A.R. Oller. Department of Biology and Agriculture, University of Central Missouri.

Previous tick vector studies have confirmed *Babesia, Anaplasma,* and *Ehrlichia* in Central Missouri ticks. Each microorganism is known to cause zoonotic cases of disease in mammalian hosts like ruminants via ticks. Although some tick studies utilized cattle, Bison studies are lacking. With bison, becoming an increasing fixture in pastures due to various dietary changes in meat consumption practices, the presence of tick-borne illnesses in captive bison herds is greatly needed. The veterinarian collected approximately 40 blood samples via tail vein venipuncture using EDTA as an anticoagulant, and was refrigerated. Sample numbers contained gender, age, and weight. This project looked at blood parasitemia (%) rates to confirm the presence of circulating blood parasites, as it is important to establish expected parasites and co-infections within bison herds in Missouri. Blood smears were made and stained with a filtered Wright-Geimsa stain that would also allow parasite visualization. Out of a total of 38 Bison, five have been thoroughly examined for pathogens. 4 thus far have suspected cases of *Anaplasma*, three *Ehrlichia*, and one *Babesia*. Some of the bison have coinfections; further examination of blood smears, along with PCR testing will yield more concise results. IACUC approval of UCM #928 was already obtained and granted for this pathogen survey.

7. **Generating Deletion and Point Mutations to Study a Novel Phytase-like Protein, CarP, That Protects *Pseudomonas aeruginosa* from Elevated Calcium.** Daniel McLeod **(**Undergraduate)*, Michelle King, Dr. Marianna Patrauchan. Oklahoma State University, Stillwater, OK.

Pseudomonas aeruginosa is an opportunistic pathogen that infects wounds and the lungs of cystic fibrosis patients. Previously, we showed that several virulence factors in *P. aeruginosa* are induced by elevated calcium (Ca^{2+}). We identified a putative phytase, CarP, and determined its role in protecting cells against Ca^{2+} and regulating Ca^{2+}-induced virulence factors. Therefore, we hypothesized that this protein binds Ca^{2+} and belongs to the Ca^{2+} regulatory network in *P. aeruginosa*. To study the role of *carP* in Ca^{2+} regulation, we used a transposon mutant and a complementation strain, where *carP* is cloned under an arabinose-inducible promoter. Here we aim to generate a clean *carP* deletion strain and a complemented strain where *carP* will be regulated by its native promoter. Sequence analyses showed no known Ca^{2+} binding motifs in CarP, and we aim to identify the responsible residues, which will likely present a novel Ca^{2+}- binding motif. We are using inverse PCR to replace E183 and E291 predicted to bind Ca^{2+} with a glutamine, which will prevent binding. We will then measure the proteins' ability to bind Ca^{2+} using isothermal chromatography. Obtaining these mutants will also enable future functional studies, characterizing the role of CarP in *P. aeruginosa* virulence.

8. **Soil Microbe Isolation Surrounding Native and Invasive Grasses to Test for Antimicrobial Properties against E.S.K.A.P.E. relatives.** Georgie O. Tauber (Undergraduate)*, Claudia M. Carvalho, and Mitchell J. Greer Department of Biological Sciences, Fort Hays State University, Hays, Kansas

As non-native species (such as *Bothriochloa ischaemum* and *B. blahdii*) continue to invade native lands, they alter the landscape. *Bothriochloa* spp. have been shown to have allelopathic effects. These allelochemicals need more investigation as to whether or not these biochemicals alter the microbes in the surrounding soil and/or have antimicrobial properties. If so, these antimicrobial properties could lead to new antibiotics. Antibiotic and antimicrobial resistance such as in the E.S.K.A.P.E pathogens, has begun to run rampant around the globe and has caused great dilemma to many physicians. The E.S.K.A.P.E. pathogens present a real threat to our society today. Samples were taken from soil surrounding *Bothriochloa ischaemum, B. blahdii*, and *Andropogon gerardii* - a native grass. After initial serial dilution and selection of colonies, the isolated colonies were tested against E.S.K.A.P.E. pathogen relatives for antimicrobial properties. There were zero zones of inhibition from samples taken near the invasive species, while two colonies from *Andropogon gerardii* samples showed zones of inhibition (both against *Staphylococcus epidermidis*). The bacteria were found to be a gram-postive. Further testing will be performed to determine the identity of the bacteria.

9. **Longevity Analysis of Germ-free *Drosophila melanogaster*.** Makayla Nemecek (Undergraduate)*, Rebecca Best, Shelby Peters, Carlie Prososki, Lesley Towery, Brad L. Ericson, Darby J. Carlson, & Kimberly. A. Carlson, Department of Biology, University of Nebraska at Kearney, Kearney, NE 68849

Gastrointestinal microbiota and viruses are a key component in characterizing gut health and longevity. Nora virus, a persistent virus that replicates in the gut of *Drosophila melanogaster,* shows no lethality to the organism. Viruses similar in nature to Nora virus interact with gut microbiota, and the health of the organism and longevity may be dependent on a persistent viral infection. Our hypothesis is that Nora virus may be needed within the gastrointestinal tract of *D. melanogaster* to maintain a favorable environment for gut microbiota and increase the lifespan. Germ free *D. melanogaster* were generated with the use of antibiotics and divided into four treatment groups: Nora virus positive/bacteria positive, Nora virus negative/bacteria positive, Nora virus negative/bacteria negative, and Nora virus negative/bacteria negative. The presence of Nora virus was detected with the use of RT-PCR and bacterial species was determined by plating homogenized flies on Luria Broth plates. A longevity study was conducted on each of the treatment groups and demonstrated that Nora virus does not enhance longevity, but microbiota is needed when virus is present to live. This data suggests that Nora virus infection is not beneficial to the microbial environment within the gastrointestinal tract, but further testing is needed.

10. **Silver Nanoparticles on Yeast Viability with Bioinformatics Analysis.** Cullen Horstmann (undergraduate)*, and Kyoungtae Kim. Missouri State University Department of Biology, Springfield, Missouri.

Nanoparticles have become common in many commercially used products such as zinc sunscreen and water resistant clothes. They may also be utilized in the targeted treatment of cancer, printable monitoring systems, and cost effective phones in the future. The effects nanoparticles have on biological organisms is crucial for the responsible use of these technologies. We investigated the effects of silver (Ag) nanoparticles on budding yeast (Saccharomyces cerevisiae) using growth assays, FUN-1 staining for metabolic activity, RNAseq, and RTPCR. Our growth assay showed that Ag has an inhibitory effect with its concentrations above 5μg/ml. Hundreds of genes in Ag treated cells were differentially expressed according to our transcriptome investigation. A large fraction of upregulated genes are identified to regulate ribosomal biogenesis and RNA processing, whereas downregulated genes are known to be responsible for mitochondrial functions based on our analysis of gene ontology terms. Furthermore, we validated the RNAseq results using an RTPCR assay. The resulting expression profile leads us to suspect that Ag nanoparticle exposure creates a stress environment in the cell.

11. **Site Directed Mutagenesis of Known Vps1 Ubiquitination Sites.** Ryan Windish (Undergraduate)*, Kyoungtae Kim. Missouri State University, Springfield, Missouri.

Ubiquitination is a cellular process that is important for protein degradation. It occurs through a series of enzymatic reactions in which ubiquitin, a key factor ubiquitously expressed in eukaryotic organisms, tags the substrate proteins to be degraded via proteasomes. Vacuolar Protein Sorting 1 (Vps1), which is yeast's homologue to mammalian dynamin, has five known ubiquitination sites. This protein is crucial to the retrieval of both Vps10 and Snc1, which play significant roles for intracellular vesicular trafficking pathways. We have performed experiments by mutating the known ubiquitination sites of Vps1 through site directed mutagenesis. A mutant strain harboring Vps1 K561N mutation displayed severe defects in the trafficking of Snc1 and Vps10, suggesting that the Vps1 ubiquitination sites are pivotal for their trafficking toward the Golgi and that proper turnover of Vps1 regulates these cargo trafficking processes.

12. **Initial Characterization of the Two ClpP Isoforms of *Chlamydia trachomatis* Suggests Independent Functionality for Each.** Nicholas A. Wood (Undergraduate)*[1,2], Krystal Chung[3], Nathalia Rodrigues de Almeida[1], Martin Conda-Sheridan[1], Derek J. Fisher[3], Scot P. Ouellette[1]
[1]University of Nebraska Medical Center, Omaha, Nebraska.
[2]University of South Dakota, Vermillion, South Dakota.
[3]Southern Illinois University, Carbondale, Illinois.

Chlamydia trachomatis is an obligate intracellular bacterium that differentiates between two distinct forms during its developmental cycle: elementary bodies (EBs) and reticulate bodies (RBs). The EB is the electron dense, infectious form. Within the cell, the EB differentiates into the RB, which replicates and develops within a host membrane derived vesicle, termed an inclusion. RBs replicate within this inclusion and eventually differentiate back into EBs before release from the host cell. Given the unique functional and morphological forms of *Chlamydia*, we are interested in proteomic regulation and turnover through protein degradation. We hypothesize that the Clp protease system plays an integral role in proteomic turnover by degrading specific proteins from one developmental form or the other. *Chlamydia* encodes five *clp* genes: *clpX*, *clpC*, two *clpP* paralogs, and *clpB*. ClpC and ClpX are chaperone proteins that unfold and feed proteins into the ClpP protease to be degraded, and ClpB is a deaggregase. Our initial characterization focuses on the two ClpP paralogs. We demonstrate their developmental expression, potential for oligomerization, importance during infection by antibiotics targeting ClpPs, and the effect of overexpression of inactive ClpP mutant proteins. Together, these data indicate an important role for ClpP proteins in chlamydial growth and pathogenesis.

13. **Using interpretable neural network models of *Pseudomonas aeruginosa* gene expression to reveal potential targets of an unstudied transcription factor implicated in high resistance to a novel antimicrobial peptide.** Christopher Johnson (Undergraduate)* and Dr. Donald Rowen. University of Nebraska at Omaha, Omaha, Nebraska

Antimicrobial peptides (AMPs) are an intriguing alternative to currently available antibiotic therapies. DASamP2, an AMP developed by the Wang lab at the University of Nebraska Medical Center, is effective against *Pseudomonas aeruginosa* and *Staphylococcus aureus,* but the mechanism of action is not currently understood. To characterize the genetic factors affecting susceptibility to this AMP, our lab has previously used transposon mutagenesis to construct numerous mutant strains of *P. aeruginosa* demonstrating increased resistance to DASamP2. Analysis of one of our most resistant mutants revealed that upregulation of the transcription factor PA5189 may be responsible for producing a resistant phenotype capable of surviving over 10x MIC. Though this gene is unstudied, we can extract biologically meaningful insights from publicly available gene expression datasets by using denoising autoencoders or disentangled deep variational autoencoders to produce a compressed representation of all gene expression datasets. Analyzing a trained neural network allows us to connect raw expression data to higher-level biological features. Early results suggest PA5189 may be involved in the regulation of numerous efflux pumps, porins, and other genes – most of which are also unstudied, potentially indicating that we are looking at a new facet of how *P. aeruginosa* adapts to antimicrobial assault.

Poster Session 2 (Graduate Students)

1. **Sodium Pyruvate Alters the Immune Response to Influenza A Virus Infection in Macrophages** Hazar Abu Salamah (Graduate)*. Dr. Christopher Lupfer. Department of Biology, Missouri State University, Springfield, MO 65897

Pyruvate is the end product of glycolysis. It can either be transported into the mitochondria for use in the TCA cycle or be used to regenerate NAD^+ during aerobic glycolysis. We recently discovered that addition of sodium pyruvate to the culture medium during infection of macrophages with influenza A virus affects the production of cytokines involved in immune signaling. The purpose of the present study was to determine whether sodium pyruvate's role in energy production in the macrophages may alter the immune response to the infection. While infection of macrophages with influenza A virus resulted in high levels of cytokines (IL-6, IL-1β, and TNF-α) in the absence of sodium pyruvate, the addition of sodium pyruvate significantly impaired cytokine production. Furthermore, sodium pyruvate did not affect virus growth, suggesting the effect of sodium pyruvate is on the immune response produced by the macrophages and not the viability of the virus.

2. **Characterization of the Role of PA5189 of *Pseudomonas aeruginosa* in Resistance to an Antimicrobial Peptide.** Zachary Scott (Masters)*, Christopher Johnson, and Donald Rowen Department of Biology, University of Nebraska at Omaha, Omaha, Nebraska

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.

3. **CHARACTERIZATION OF TWO NOVEL ANTIMICROBIALS AGAINST *Acinetobacter baumannii*.** Infencia Xavier Raj (Masters)*[1], Anuradha Roy[2], and Indranil Biswas[1].
   [1] Department of Microbiology, Molecular Genetics and Immunology, University of Kansas Medical Center, Kansas City, KS 66160. [2] High Throughput Screening Lab, University of Kansas, Lawrence, KS 66045

Acinetobacter baumannii belongs to a group of ESKAPE pathogens; which is an opportunistic pathogen prevalent in nosocomial infections. This pathogen can cause a wide range of infections from minor skin and soft-tissue infections to more severe invasive diseases such as bacteremia, meningitis and ventilator-associated pneumonia. *A. baumannii* has the ability to acquire antibiotic resistance cassettes from the environment and this trait has allowed the organism to persist in health care settings and also facilitated global emergence of multidrug resistance (MDR). The organism is becoming resistance to most of the common antibiotics including aminoglycosides, β–lactams and quinolones. To fight against the increasing infections caused by ESKAPE pathogens, there has been an increased effort in the last five years to identify novel small anti-infective molecules or to modify existing compounds to increase their potency. Using a high throughput screening assay, we recently examined several small-molecule libraries at the IDAD Core Facility at KU, Lawrence. We identified several potential candidates among ~20000 compounds that either specifically inhibited *A. baumannii* strains or displayed broad-spectrum inhibitory activity against *A. buamannii* and other gram-negative ESKAPE pathogens. The main goal of this study is to characterize two such anti-infective molecules to determine their inhibitory spectra, mode of action, and synergistic activities with other antibiotics.

4. **The Host Protein Kinase C is manipulated by *Chlamydia trachomatis* During Infection**
   Prakash Sah[1] (Doctoral)*, Ted Hackstadt [2], Erika Lutter[1]
   [1]*Department of Microbiology and Molecular Genetics, Oklahoma State University, Stillwater, OK, USA;* [2]*Laboratory of Intracellular Parasites, NIAID, NIH Rocky Mountain Laboratories, Hamilton, USA*

Chlamydia trachomatis is responsible for causing a range of diseases such as blinding trachoma and urogenital infections leading to serious complications. Inside a host cell, *C. trachomatis* lives in a parasitophorous vacuole called an inclusion from where it is able to secrete various effectors to manipulate host-cellular functions to its benefit. Currently, not much is known about Chlamydial manipulation of host kinases such as Protein Kinase C (PKC). PKCs are members of AGC family of kinases and involved in regulating various cellular functions such as, growth and proliferation, migration, survival and apoptosis. We hypothesize that *C. trachomatis* manipulates PKC pathways to regulate intracellular development inside the host, as PKCs are important in regulating various cellular functions. Indirect immunofluorescence of infected cells verified recruitment of multiple PKC isoenzymes to microdomains (Src-family kinases rich regions) on the inclusion of *C. trachomatis* L2/434. Recruitment of PKC substrates, including Marcks, was also confirmed. Recruitment of PKCs and PKC substrates was found to be species specific. Inhibition of PKC activity with Staurosporine and Go6983 resulted in decreased recoverable infectious progeny. Western blot analysis revealed differential activation of PKC at different time points during infection. These results confirm PKCs are important for intracellular growth and development of *C. trachomatis*.

5. **A Prokaryotic dCTP Deaminase in the Eukaryote *Dictyostelium discoideum.*** Heng Liang (Doctoral)*, PI: Catherine P. Chia, School of Biological Sciences, University of Nebraska-Lincoln, Lincoln, NE, USA.

DNA synthesis is crucial in all living organisms. Deoxycytidine triphosphate (dCTP) deaminase (EC 3.5.4.13) is an enzyme in the pathway which converts dCTP into deoxythymidine triphosphate (dTTP), one of the building blocks of DNA. Although typically found only in gram-negative prokaryotic cells, two genes encoding predicted dCTP deaminases (*dcd1*:DDB_G0293580 and *dcd2*:DDB_G0268194) are identified in the annotated genome of *Dictyostelium discoideum,* a eukaryotic soil amoeba. *D. discoideum* also has genes for dCMP deaminase and thymidylate synthase, enzymes for pyrimidine biosynthesis in eukaryotes. We are investigating whether *dcd1* and *dcd2* are expressed, and whether the activities and location of their respective proteins are regulated either by the cell cycle or during development. Phylogenetic analyses of the predicted protein sequences with prokaryotic dCTP deaminases indicate that *dcd1* and *dcd2* have bacterial ancestors that likely entered the *D. discoideum* genome through two independent gene transfer events. Consistent with its suggested bacterial origin, the *D. discoideum dcd1* rescued the slow growth phenotype of an *E. coli* dCTP deaminase knockout, indicating that *dcd1* encodes an enzyme capable of functioning in the prokaryotic pyrimidine biosynthesis pathway. Construction of a *dcd1* knockout in *D. discoideum* is in progress to examine the specific roles of dCTP deaminase in *D. discoideum*.

6. **Characterization of the IS3 Family of Insertion Sequences in the Genome of *Halanaerobium hydrogeniformans*.** Ronald L. Frank, Kody A. Bassett (Masters)*, Melanie R. Mormile. Missouri University of Science and Technology, Rolla, Missouri.

Halanaerobium hydrogeniformans is an anaerobic, gram-negative, rod-shaped, haloalkaliphilic bacterium isolated from Soap Lake in Washington State. The genome sequence was determined in 2011 as part of an effort to reveal some of the adaptations that enable this bacterium to grow in a high salt, high alkaline environment. One prong of that effort is a genome-wide survey of an unusually abundant number of transposable elements. The results reported here are limited to one of nine families of bacterial insertion sequences found in this 2.6 Mb genome. The *H. hydrogeniformans* genome contains 29 loci that harbor either functional, defective, or fossil remnants of the IS3 family of bacterial insertion sequences. The elements have been divided into five groups (ISHahy2, 3, 4, 5, and a solo partial element). Each of the five groups appears to have originated from an independent invasion of the genome by a unique IS3-related element. The transposase of typical IS3 elements is encoded by two overlapping out-of-frame orfs A and B by a programmed translational frameshift at a slippery site upstream of the stop codon in orfA. We describe here the characteristics of the five groups of IS3-related elements in *Halanaerobium hydrogeniformans* as they relate to that model.

7. **The Fatty Acid Kinase FakA Shapes the Metabolome of *Staphylococcus aureus*.** Zachary DeMars (Doctoral)* and Jeffrey L. Bose. University of Kansas Medical Center, Kansas City, Kansas.

Staphylococcus aureus is capable of phosphorylating exogenous fatty acids which are then able to be incorporated into the bacteria's membrane via the fatty acid kinase FakA. Additionally, FakA plays a significant role in virulence factor regulation and skin disease. We previously showed that a *fakA* mutant displays altered growth kinetics *in vitro,* observed during late-exponential phase of growth. Here, we show that this is both glucose and aeration-dependent, indicating an altered acetate switch. Consistent with this, acetate production was altered and the growth benefit was eliminated with inactivation of the acetate-generating enzyme AckA. Using a mass spectrometry-based approach, we identified altered concentrations of TCA cycle intermediates and both intracellular and extracellular amino acids. Together, these data demonstrate a redirection of carbohydrate carbon flow and altered amino acid metabolism in the *fakA* mutant. While ATP levels were similar, inactivation of *fakA* increases the NAD/NADH ratio, indicating a more oxidized cellular environment. Finally, we suggest that the global metabolic regulatory proteins CcpA and CodY as being important regulatory mechanism for the altered growth in a *fakA* mutant. Together, this data identifies a previously unidentified role for *fakA* in the global physiology of *S. aureus* that links the fatty acid and central metabolism.

8. **Characterization of the Nitrogen Assimilation Regulator (glnG) Role in *Escherichia coli* Colonization of the Mammalian Intestines**. Justin Bowen (Master's)*, Tyrell Conway, Jerreme Jackson. Oklahoma State University Microbiology and Molecular Genetics, Stillwater, OK.

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 protein, NR1, which activates the genes responsible for survival during ammonia limitation. Under nitrogen-limited growth conditions NR1 activates transcription of glutamine synthetase which, along with additional proteins, facilitates transport and degradation of nitrogen-containing compounds. 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 will use 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 will be prepared and sequenced by differential RNA-seq analysis, which will allow mapping of all *gln*G-dependent promoters. We expect the *glnG* mutant *E. coli* to fail to colonize the intestines if the *glnG* gene is vital to colonization of the mammalian intestine.

9. **GENERATION OF YEAST 2-HYBRID CLONES TO EXAMINE THE ROLE OF NUCLEOTIDE OLIGOMERIZATION AND BINDING DOMAIN (NOD)-LIKE RECEPTORS.** Abbigale Mabary (Masters)*, Faculty Advisor: Dr. Christopher Lupfer. Missouri State University. Springfield, Missouri.

NOD-like receptors (NLRs) are a class of cytoplasmic proteins essential for the initiation and regulation of immune responses to infectious disease, metabolic and cellular damage and cancer. The human genome encodes for 22 NLR proteins. However, only about half of the 22 NLRs have known functions, and the mechanisms by which they function are even more ambiguous. Previous research indicates that some NLRs activate inflammation, while others, like NLRP12, functions as regulators of inflammation, thus serving as a negative feedback mechanism. NLRP12 suppresses inflammation by inhibiting the transcription factor NF-κB, which activates transcription for cytokines that activate the immune response cascade. Inhibition of NF-κB by NLRP12 is important in the prevention of a hyper-inflammation, which is involved in severe infections as well as cancer development. Although the general function of NLRP12 is known, how it is activated is not known. We are, therefore, embarking on a journey to find novel proteins that interact with NLRP12 to decipher the mechanisms by which they function. We are generating a yeast 2-hybrid system to examine the interaction of NLRP12 with a human cDNA library. Novel interactions discovered through this 2-hybrid screen should provide insight into the function of this NLR protein and help us understand the immune response to infectious and non-infectious disease.

10. **Ecology and prevalence of ticks and tick-borne bacterial pathogens in a peri-urban landscape of the Midwestern U.S.** Abrar Alzahrani (Masters)*[1], Nicholas A. Burnett[1], Ram Raghavan[2], and Anuradha Ghosh[1] 1. Dept. of Biology, Pittsburg State University (Pittsburg, KS), 2. Dept. of Diagnostic Medicine/Pathobiology, Kansas State University (Manhattan, KS)

Ticks transmit a wide variety of pathogens including viruses, bacteria, protozoa, and helminthes to vertebrates. Their life cycle depends on blood meals from various hosts as well as on environmental conditions such as the temperature and habitat type. The present study proposed to assess the prevalence of various tick species and infection prevalence of bacterial pathogens causing various diseases within the tick community of southeast Kansas and adjacent area. Over 2000 ticks were collected during warmer months of 2016 and 2017 (May-August) from three types of tick habitats (woodland, open grassland and woodland/grassland ecotones) using the flag-drag method. All the ticks (adults and nymphs) were sexed and identified in the laboratory. Majority of these were identified as *Amblyomma* spp. followed by *Dermacentor* spp. and rarely *Ixodes* spp. The ticks were surface-sterilized and total genomic DNA is currently being extracted from the adult ticks; and will be subjected to PCR amplification using bacterial species-specific primers. Microclimate data as well as landscape fragmentation pattern will be analyzed using GIS-based monitoring method. It is comprehensible that a better understanding of the variations in tick-pathogen prevalence is crucial for implementing sound surveillance and management programs and to understand risk for human/animal diseases.