Meeting of the Missouri Valley Branch of the American Society of Microbiology

March 10-11, 2023

Kansas State University

Manhattan, KS

Welcome to Manhattan!

We are excited to welcome you to Manattan Kansas for the 2023 Meeting of the Missouri Valley Branch of the American Society of Microbiology. This meeting is an excellent platform for undergraduate and graduate students to present their research, network with other students and faculty, and interact with our ASM Distinguished Lecturer and Branch Speakers. This year our meeting is in person and we have more than 70 abstracts submitted by undergraduate and graduate students from Oklahoma, Kansas, and Nebraska. We hope you all enjoy this meeting and that it will lead to new friendships, collaborations, and new opportunities. Once again, welcome to Manhattan!

Erika Lutter
President ASM Missouri Valley Branch
Associate Professor
Department of Microbiology and Molecular Genetics
Oklahoma State University

PARKING GUIDE

The K-State Parking Garage and connecting skywalk, at the south entrance of the Union, enable us to become the “pathway to campus.”

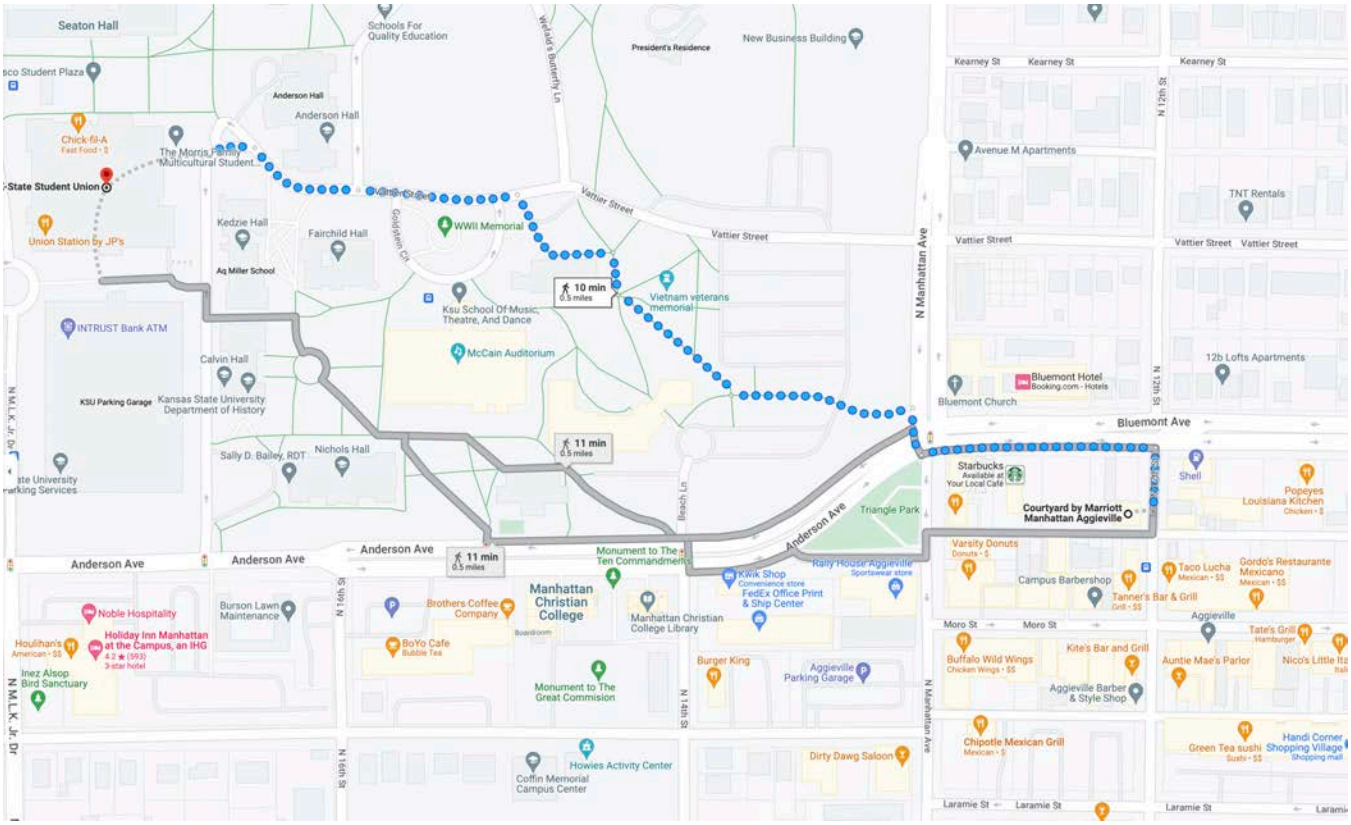
K-State parking garage is free on Saturday and Sunday.

The parking capacity for our Union visitors and convenience of the connecting skywalk allows easier, direct access to your Union’s great facilities, services and programs.

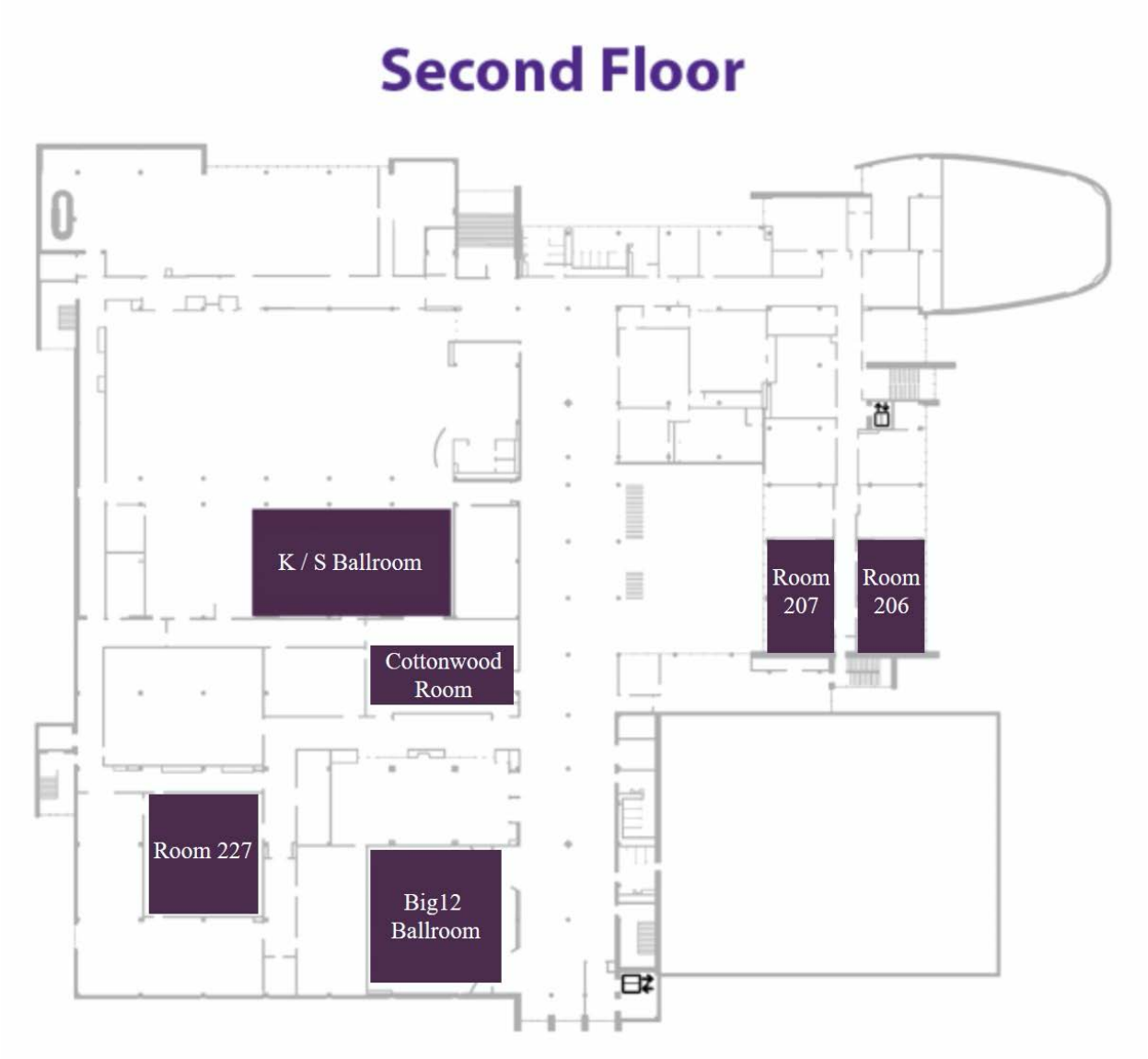
Visit the Parking Services website for current parking guidelines and information.

Address: 918 N. Martin Luther King Jr. Drive Manhattan, KS 66506

Directions from hotel to K-state Union



Map of K-State Union rooms on the second floor:



SCHEDULE OVERVIEW

Friday, March 10th

ADDRESS: 918 N. Martin Luther King Jr. Drive Manhattan, KS 66506

Note there is a **mixer** from 6-9PM (first drink ticket provided, additional drinks available at cash bar).

Friday, March 10, 2023	**At K-State Union**
6:00 - 9:00 PM	Mixer and snack buffet, K/S Ballroom
6:15 – 6:30 PM	Welcome and introductions
7:00 – 9:00	Poster session in K/S Ballroom

Saturday March 11th

ADDRESS: 918 N. Martin Luther King Jr. Drive Manhattan, KS 66506

Saturday, March 11, 2023	**At K-State Union**
8:00 - 8:30 AM, K/S Ballroom	Coffee and breakfast
8:30 – 10:45 AM	Student oral presentations *(see room assignments below)*
10:45 – 11:00 AM	Break
11:00 – 12:00 PM, Big12 Ballroom	Key Note: Dr. Miriam Braunstein *Title:* The Bacterial Protein Export Zoo
12:00-1:00 PM, K/S Ballroom	Lunch
1:00 -2:00 PM, K/S Ballroom	Poster session
2:00-2:15 PM	Break/ Business Meeting *Snacks in the K/S Ballroom*
2:15-2:45 PM, Big12 Ballroom	Dr. Jeonghoon Lee *Title:* Chlamydial Bactofilin Functions as a Cell Shape Determinant
2:45-3:15 PM, Big12 Ballroom	Dr. Carolyn Ibberson *Title:* Exploring microbe-microbe interactions in chronic infection sites with ‘omics approaches
3:15-3:45 PM, Big12 Ballroom	Sarah Nickel *Title:* Lessons Learned: My Path to Lab Director
3:45-4:00 PM	Break
4:00-4:15 PM, K/S Ballroom	Awards and closing remarks

ASM Distinguished Lecturer

Dr. Miriam Braunstein, PhD

Professor, Microbiology and Immunology
University of North Carolina School of Medicine

Biographical Sketch

Miriam Braunstein, Ph.D., a professor at the University of North Carolina School of Medicine, studies therapeutic development for *Mycobacterium tuberculosis*.

Personal statement: I am passionate about training the next generation of scientists and in helping trainees of all levels achieve their career goals. I am the Principal Investigator of the National Science Foundation-funded Summer Undergraduate Research Experience in Biological Mechanisms Program at the University of North Carolina (UNC). This program provides opportunities for students from groups underrepresented in the sciences or from schools with limited research capacity to work in UNC laboratories for the summer. I also founded the Southeastern Mycobacteria meeting, which provides students and postdoctoral trainees with opportunities to present their research and to network with other scientists. I am excited to be an ASM Distinguished Lecturer because it will allow me to contribute more broadly to the career development of future scientists through interactions at ASM Branch meetings. I am an ASM member since 1996. I served as the ASM Division U (Mycobacteriology) Chair, I am a Fellow of the American Academy of Microbiology, I am a member of the ASM *Journal of Bacteriology* editorial board, and I am one of the editors of the ASM Press *Gram Positive Pathogens* book published in 2019. As a PI who still does experiments, I welcome the opportunity to speak about my research, career path, and experiences with student and postdoctoral trainees.

Dr. Carolyn Ibberson, PhD

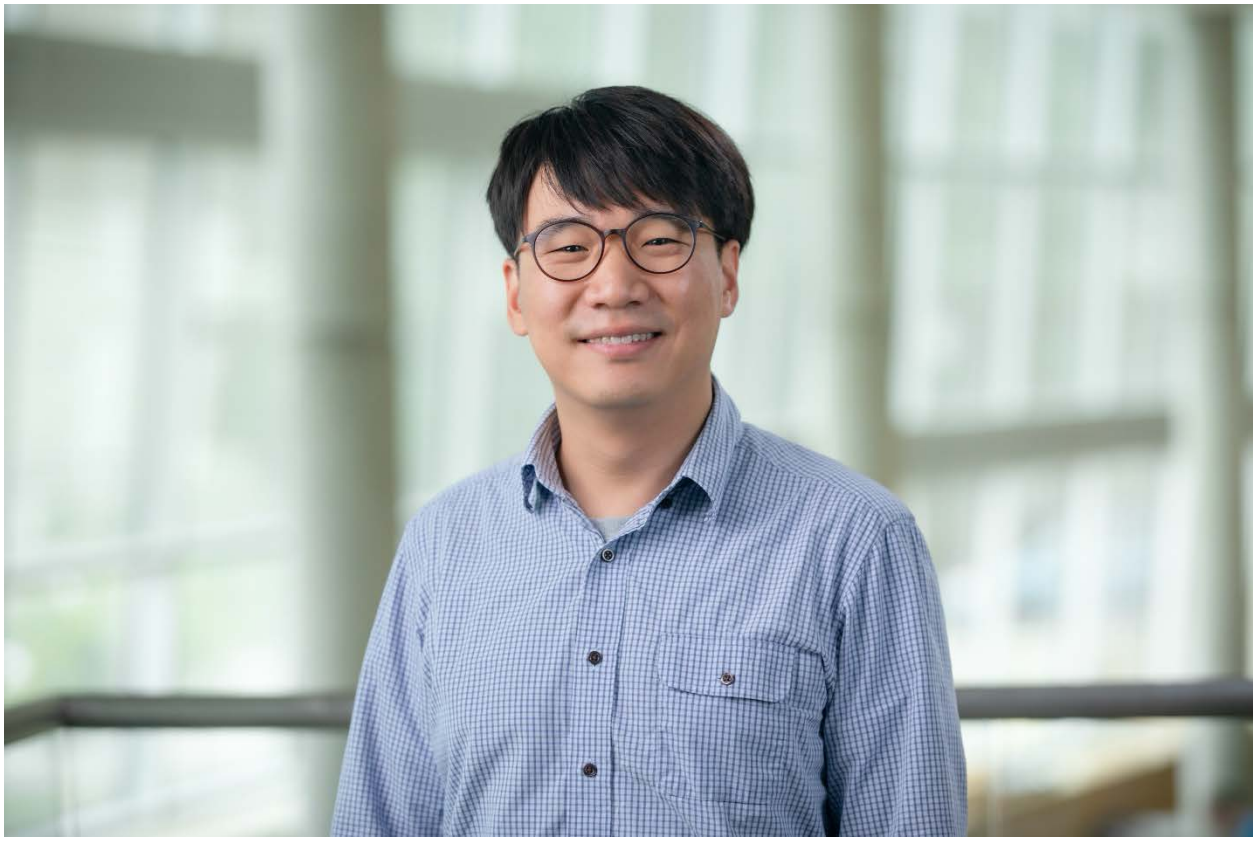
Assistant Professor
Department of Microbiology and Plant Biology
University of Oklahoma

Biographical information and research interests:

Polymicrobial communities are ubiquitous and the interactions between microbes are critical drivers of overall community function. Thus, a fundamental question in human health and disease is to understand how microbes interact and the spatial constraints to these interactions from the macro to micron to chemical scale. My lab uses a range of chronic infections, particularly cystic fibrosis and chronic wounds, to study microbe-microbe interactions across spatial scales. Our research centers around understanding bacterial physiology and behavior in situ in chronic human infection, with a focus on elucidating the mechanistic links between co-infecting microbes and disease severity. Our work is at the cutting edge of assessing bacterial physiology in human infection, and leverages classic microbiological techniques in combination with -omics approaches to ask foundational questions about how the prominent pathogen, *Staphylococcus aureus*, causes disease and persists in human infection sites.

Dr. Jeonghoon Lee, Ph.D.
Senior scientist
Department of Pathology and Microbiology

University of Nebraska Medical Center



Biographical information and research interests:

I am a Senior Scientist in the Department of Pathology and Microbiology at the University of Nebraska Medical Center. I got my Bachelor's degree in Life Science from Sogang University in Korea, and my Ph.D. in Biological Science from the Korean Advanced Institute of Science and Technology in Korea. Currently, I have been investigating how *Chlamydia trachomatis* divides and maintains its cell shape.

Sarah Nickel, MS, MLS(ASCP)^{CM}

Director, Molecular Diagnostics Laboratory
Assistant Professor, Medical Laboratory Sciences
Wichita State University

Biographical information and research interests:

Sarah Nickel is the director of the Wichita State Molecular Diagnostics Laboratory and an assistant professor in the Medical Laboratory Sciences at Wichita State University in Wichita Kansas. She has worked as a bench level clinical microbiologist for over 15 years and has taught in higher education for over 5 years. Sarah's teaching responsibilities have included Clinical Microbiology, Advanced Clinical Microbiology, Medical Immunology, and Molecular Diagnostic Techniques. She has been certified as a Medical Laboratory Scientist by the American Society for Clinical Pathology since 2006. Sarah completed her master's degree in Microbiology and Cell Science from the University of Florida in 2016. In 2020, Sarah co-founded the Wichita State Molecular Diagnostics Laboratory (MDL) to meet the community need for COVID-19 testing. MDL is a CLIA certified high complexity clinical laboratory that has performed over half a million PCR tests. Sarah is a passionate educator that loves applied research including new test development, laboratory process improvement, and digital transformation of the clinical laboratory.

Oral Presentation Schedule: Saturday March 11th

Category: I. General Microbiology (Session I)
Moderator:
Room: Big 12 Ballroom

Time	Student	Trainee	Title
8:30-8:45	Adrienne L. Jones	MS	Temporal progression of anaerobic fungal population in dairy calves from birth to maturity
8:45-9:00	Ashley Foltz	PhD	Microbiome perturbations moderately modulate *Caenorhabditis elegans* health and life history traits
9:15-9:30	Noopur Dasgupta	PhD	*Chlamydia trachomatis* recruits PKA and phosphorylated PKA substrates during infection
9:30-9:45	Abigail R. Swoboda	UG	The tail-specific protease, CT441, is essential for secondary differentiation in *Chlamydia trachomatis*
9:45-10:00	Ramee G. Aranda	PhD	Defining functional regions of atypical MAPK Erk2 iin *Dictostelium discoideum*
10:00-10:15	Rajendra K. Angara	Post-doc	*Coxiella burnetii* effector protein with FFAT motif (*Cb*EPF1) mediates membrane contact sites between host lipid droplets and endoplasmic reticulum
10:15-10:30	Rosalie L. Dohmen	PhD	Using photoactive yellow protein to develop novel FTIR spectroscopic methods for probing three protonation states in histidine side chains
10:30-10:45	Nate Korth	PhD	Using the Human Gut Microbiome as a Phenotype of Sorghum in a Genetic Mapping Study

Category: I. General Microbiology (Session II)
Moderator:
Room: K/S Ballroom

Time	Student	Trainee	Title
8:30-8:45	Sara M. Hopkins	PhD	Identification and Characterization of *C. elegans* genes that *Stenotrophomonas maltophilia* targets to evade host insulin-like DAF-2/16 pathway defenses
8:45-9:00	Vandana Singh	Post-doc	Altering the redox status of *Chlamydia trachomatis* impacts its developmental cycle progression
9:15-9:30	Nathan D. Hatch	PhD	Defining the regulons of two minor sigma factors in *Chlamydia trachomatis*
9:30-9:45	Matthew D. Romero	PhD	TmeA-mediated signaling operates during later stages of *Chlamydia trachomatis* invasion and is necessary for efficient dynamin-dependent closure of *Chlamydia*-containing vacuoles
9:45-10:00	Ryan M. Singh	PhD	seNOS-mediated regulation of Hmp toxicity enhances fitness of *Staphylococcus epidermidis*
10:00-10:15	Sasmita Panda	Post-doc	An essential role of alanine racemase in overcoming organic anion intoxication in *Staphylococcus aureus*
10:15-10:30	Nicholas A. Wood	PhD	Substrate profile of *Chlamydia trachomatis* ClpXP protease provides insight into its function during secondary differentiation

Category: II. Environmental Microbiology
Moderator:
Room: Room 206

Time	Student	Trainee	Title
8:30-8:45	Brianne D. Edwards	MS	Role of soil pH in driving selection rhizobial endophytes within soybean root nodules
8:45-9:00	Carrie J. Pratt	PhD	Tortoises as novel hosts for deep-branching anaerobic gut fungi: exploring phylogenetic diversity, community structure, isolation, and evolutionary history
9:15-9:30	Damilare Ajagbe	PhD	Growth and tolerance of *Modicisalibacter* sp. at the intersection of high salinity, heavy metals and hydrocarbons: a case study for bioremediation of produced water
9:30-9:45	Samuel Miller	PhD	Novel taxa with bio-industrial potential isolated from alpaca fecal material
9:45-10:00	Samiskshya Giri	PhD	Microbial isoprene reduction tied to greenhouse gas removal in deep-sea carbonates and Eucalyptus-leave sediments
10:00-10:15	Nicole A. Fiore	PhD	A chemolithotrophic, methanogenic, and moderately alkaline enrichment community maintained with calcium carbonate as a sole source of inorganic carbon

Category: III. Medical Microbiology (Session I)
Moderator:
Room: Cottonwood Room

Time	Student	Trainee	Title
8:30-8:45	Joshua A. Mettlach	PhD	*Salmonella* regulates lipopolysaccharide biosynthesis by controlling LapB interactions with LpxC
8:45-9:00	Noah Shackelford	UG	The prevalence of tick-borne disease-causing pathogens in South Central Nebraska
9:15-9:30	Aaron A. Jensen	PhD	Examining the function of the ClpC AAA+ ATPase in the biology of *Chlamydia*
9:30-9:45	Megolhubino Terhuja	PhD	Combining virus-like particles and live-attentuated virus to induce broad antiviral immunity against Respiratory Syncytial Virus
9:45-10:00	Rayssa Duraes Lima	PhD	*Cutibacterium acnes* produces molecules with antivirulence activity agains *Staphylococcus* spp.
10:00-10:15	Inyeong Lee	MS	CUL3 negatively regulates NLRP12-mediated inhibition of the NF-kB signaling pathway
10:15-10:30	Priscilla Chatman	UG	Antifungal activity of novel compound EIPE-1 against the fungal pathogen *Cryptococcus neoformans*

Category: III. Medical Microbiology (Session II)
Moderator:
Room: Room 227

Time	Student	Trainee	Title
8:30-8:45	Robin Cesur	MSc	Distribution of PERV (Porcine Endogenous Retrovirus)-C among feral pigs in Eastern Kansas
8:45-9:00	Armando I. Lerma	PhD	Microbial and host factors that modulate differences in host's clinical outcome to *Clostridioides difficile* infection
9:15-9:30	Francis Fontanilla	PhD	*Chlamydia trachomatis* modulates expression of JAK-STAT signaling components to attenuate the type II interferon response in epithelial cells
9:30-9:45	Morgan Cade	PhD	Investigating the effects of the infant probiotic *B. infantis* and human milk oligosaccharides on the severity of anaphylaxis in a mouse model of peanut allergy
9:45-10:00	Kyle Dittmer	UG	Determining antimicrobial activity of metal-*N*-heterocyclic carbene complexes against *Staphylococcus aureus*
10:00-10:15	Kari Heck	PhD	Assessment of commensal *E. coli* outer membrane vesicles for application in a bacterial-derived oral gene delivery system
10:15-10:30	Anthony F. Juritsch	PhD	Dietary fiber from sorghum flour protects mice harboring human gut microbiotas against chemically-induced colitis
10:30-10:45	Sam M. Koshy	PhD	Fast axonal transport of infectious prion protein

Faculty and Category: V. Flash Talks
Moderator:
Room: Room 207

Time	Student	Trainee	Title
8:45-9:05	Amanda Brinkworth	faculty	Novel adaptation of *in vitro* cultured organotypic skin for tick feeding and pathogen transmission
9:10-9:30	Ralph S. Tanner	faculty	False positives in a commercial fecal indicator bacteria assay
9:45-9:55	Sukaina Al-Hamedi	UG	Optimization of two peroxidaase activity assays to determine pro-inflammatory activities of innate immune cell populations
9:55-10:05	Kenan Brodd	UG	*Staphylococcus aureus* persister cells exhibit higher tolerance to innate immune components
10:05-10:15	Shengfeng Ruan	PhD	Exploring arabinose metabolism impairment in cells overexpression ParE toxins
10:15-10:25	Xu Shi	PhD	The Gut Microbiota Modulates the Severity of Experimental Autoimmune Myocarditis

Poster Presentation Schedule: Friday Night March 10th

Number	Name	Trainee	Title
1	Ray E. Widner	PhD	Inc Proteins Facilitate VAMP3 Recruitment to the *Chlamydia trachomatis* inclusion membrane
2	Baishakhi Biswas	PhD	Molecular Mechanisms of Mucosal Colonization by *C. difficile*
3	Yafan Yu	PhD	The structural bais for DNA-uptake by *Acinetobacter*
4	Tanisha Goyal	MSc	*Chlamydia trachomatis* inclusion membrane protein CT226 interaction with host proteins TMOD3 and FL2
5	Mason S. Mandolfo	UG	*Bacteroides rodentium* limits tumor progression in mouse model of melanoma
6	Imam Taskin Alam	PhD	Characterization of halophilic microbial methanotropic community in the Zodletone Spring, OK
7	Chinemerem Onah	MSc	The CGRP-Ramp1 signaling pathway enhances anti-*Aspergillus fumigatus* immune response
8	Adreana Aquino	UG	SSA_0809 is a homotrimeric, reactive intermediate deaminase A (RidA) from an opportunistic pathogen, *Streptococcus sanguinis*
9	Elisa M. Rouse	UG	Characterizing the oral mycobiome of domestic dogs
10	Abigail G. Hall	PhD	The Spx redox swithc is not essential for the thiol homeostasis in *Staphylococcus aureus*
11	Taylor Rosso	PhD	Nitrate stimulated iron reduction in unsaturated soil
12	Bodhi Jelinek	PhD	A new DNA extraction method for tick nymphs
13	Carmen E. Perez-Donado	PhD	Cadmium has differential effects on microbial short chain fatty acid production depending on fecal donor
14	McKenna Cruikshank	UG	The role of *slsA* in *Staphylococcus lugdunensis* biofilm formation
15	Elizabeth Pascual	Med Stu	*Engyodontium album* empyema in an immunocompromised patient

16	Brooke D. Espquivel	unspecified	Characterization of the unique biology and drug resistance mechanisms of *Candida auris*
17	Katya A. Faber-Quimby	UG	Surveillance for *Candida auris* on commercially available fruit
18	Felipe Aveline Ferreira	UG	Mapping interaction sites of ParE toxin with ParD antitoxin and with DNA gyrase
19	Mariam Garcia	UG	Characterizing high persister phenotypes in *Staphylococcus epidermidis* clinical isolates
20	David Gomez Quintero	PhD	Combining *Lactobacillus taiwanensis* and *Gordonibacter urolithinfaciens* decreases body weight gain and increases lean mass in a mouse model of diet-induced obesity

Poster Presentation Schedule: Saturday March 11th

Number		Name	Trainee	Title
21		Paiton Hancock	UG	Correlation of *Veillonella* bacteria with oral health
22		Hugh C. McCullough	PhD	Defined gastrointestinal community responses to vancomycin perturbation in a bioreactor system
23		Chih-Han Tu	PhD	TA system ParE-mediated gyrase inhibition invokes toxicity and increases mutagenic frequency without impacting antibacterial susceptibility
24		Marshall "Julius" Koons	UG	Purification and preliminary crystallization of SSA_0908, a substrate-binding protein from *Streptococcus sanguinis*
26		McKenzie J. Bietz	UG	Flint Hills phages: analysis and annotation of novel lysogenic bacteriophage
27		Eliana Pendergrass	UG	Replication and genome characterization of v_Bsu_Adastra, a lytic bacteriophage of *Bacillus subtilis*
28		William Durstock	MSc	A microbial source tracking study to identify fecal contamination in a Karst water system
29		Yingxin Zhang	PhD	Manipulation of PI3K/Akt pathway and downstream host targest by *Pseudomonas aeruginosa* to promote invasion

List of Abstracts (*alphabetical by presenter's last name*)**:**

Growth and Tolerance of *Modicisalibacter* sp. at the Intersection of High Salinity, Heavy Metals and Hydrocarbons: A Case Study for the Bioremediation of Produced Water

Damilare Ajagbe[1] (Doctoral)*, Marian Nimeh[1], Ashton Davis[1], Mark krzmarzick[2], Babu Fathepure[1]
[1] Dept. of Microbiology and Molecular Genetics, Oklahoma State University, Stillwater, Oklahoma
[2] Dept. of Civil and Environmental Engineering, Oklahoma State University, Stillwater, Oklahoma

Oil and gas extraction operations generate large quantities of polluted wastewater known as produced water (PW), with approximately 21 billion barrels generated annually in the United States alone. In addition to the disposal challenge, there is an increasing need to treat PW for beneficial re-use due to population growth, rising energy demand, and water scarcity episodes. Due to its high salinity and toxic constituents (hydrocarbons, heavy metals, radionuclides), bioremediation is considered a potentially viable alternative compared to existing PW treatment technologies. For this purpose, we have isolated hydrocarbon-degrading halophiles including *Modicisalibacter* sp. strain Wilcox and *Arhodomonas recens*. Using a variety of culture-based techniques and molecular methods, we investigated their metabolic capabilities to degrade hydrocarbon in the presence of high salt and heavy metals. Results showed that both bacteria can degrade different aliphatic and aromatic compounds at salinities as high as 4.5 M NaCl. Strain Wilcox can also metabolize several aromatic compounds in the presence of high levels of heavy metals and can remove 17-100% of added metals through bioaccumulation and biosorption mechanisms. Transcriptomic analyses suggest that Strain Wilcox can respond to metal stress via a combination of specific and non-specific mechanisms. These findings highlight strain Wilcox's potential for PW cleanup.

Category: II. Environmental Microbiology Oral Presentation (9:15 AM, Room 206)

Optimization of Two Peroxidase Activity Assays to Determine Pro-inflammatory Activities of Innate Immune Cell Populations.

Sukaina Al-Hamedi (undergraduate)*[1], Anthony Juritsch[1], Dulcie Archuleta[2], and Amanda Ramer-Tait[1].
[1]University of Nebraska-Lincoln, Lincoln, Nebraska; [2]Nebraska Wesleyan University, Lincoln, Nebraska.

Excessive infiltration of pro-inflammatory neutrophils into the intestinal mucosa is a hallmark of inflammatory bowel disease. We recently observed that germ-free mice colonized with distinct human microbiomes presented with variable populations of infiltrating neutrophils and eosinophils during colitis. We therefore wanted to assess the functional significance of these observations by measuring the myeloperoxidase (MPO) activity of the neutrophils infiltrating the inflamed colon. However, existing MPO activity assay protocols also non-specifically measure the activities of other relevant peroxidases, such as eosinophil peroxidase (EPX). Thus, this study aimed to optimize two peroxidase activity assays that specifically measure MPO and EPX. Assay reaction time and sensitivity were optimized prior to demonstrating assay specificity. To demonstrate the MPO and EPX assay specificity, we used recombinant mouse MPO and bone marrow-derived eosinophils, respectively. Our assays successfully measured MPO and EPX activity from low numbers of immune cells. Furthermore, reaction product absorbance when using MPO and EPX standards was significantly higher when tested under their respective assay conditions. Altogether, these results demonstrate that our assays are sensitive and specific enough to distinguish between MPO and EPX activity. Future studies will use these assays to quantify microbiome-dependent differences in innate immune cell infiltration in mice with colitis.

Category: V. Flash Talk (9:45 AM, Room 207)

Characterization of Halophilic Microbial Methanotrophic Community in the Zodletone spring, OK

Imam Taskin Alam (Doctoral)*, Babu Fathepure
Oklahoma State University, Stillwater, Oklahoma

Abstract

Methane is a greenhouse gas projected to be 28-fold more potent than CO_2. This study focuses on abandoned gas and oil wells (AOG) which are often high in salinity. In the US, estimated three million AOGs emitted about 0.28 million metric tons of methane in 2018. This study aims characterize microbes that can oxidize methane under hypersaline condition. Sediment samples were collected from Zodletone spring, a sulfur-rich spring in Oklahoma. Microcosms containing sediment sample in mineral salt medium were set up to enrich methane oxidizing community by spiking the headspace with 1% (v/v) of methane. Methane was supplied periodically in headspace. The enrichment degrades methane at NaCl concentration ranging from 0 M to 2.5 M with highest rate at 1 M. Amplicon sequencing of 16s rRNA performed from extracted DNA of both sediment and enrichment revealed the phylum *Balneolota* (64%) and *Proteobacteria* (28%) to be the most abundant in the enrichment, while in the original sediment their abundance was relatively low (18% and 13% respectively). The most abundant known methane oxidizer was the genus *Methylohalobrius*. In conclusion, the enrichment process resulted in the enrichment of microbial community involved in aerobic oxidation of methane which are also common in halophilic environment.

Category: IV. Poster Presentation (#6, presenting Friday evening)

***Coxiella burnetii* Effector Protein with FFAT Motif (*Cb*EPF1) Mediates Membrane Contact Sites between Host Lipid Droplets and Endoplasmic Reticulum.**

Rajendra K. Angara (Postdoc)* and Stacey D. Gilk.
University of Nebraska Medical Center, Omaha, Nebraska.

Membrane contact sites (MCS) are regions of close proximity between two membranes that facilitate exchange of small metabolites, ions, and lipids. FFAT motif-containing proteins bind the VAP proteins of the endoplasmic reticulum (ER) and mediate MCS between the ER and various cell organelles. Several bacterial pathogens, including *Coxiella burnetii*, hijack VAP to establish MCS between the host ER and the bacteria-containing vacuole. *Coxiella* causes Q fever endocarditis, which is hard to treat and often fatal. *Coxiella* is found in lipid droplet (LD)-rich macrophages in the cardiac valves of Q fever endocarditis patients, and *Coxiella*-infected macrophages accumulate LDs *in vitro*. We recently discovered that *Coxiella* Type 4B Secretion System (T4BSS) effector proteins modulate LD metabolism in macrophages; however, the specific T4BSS effector proteins and their role in modulating host LD homeostasis is unknown. Here, we show that *Coxiella* secretes a T4BSS effector protein with FFAT motif (*Cb*EPF1) that localizes to the host ER at LD biogenesis sites and translocates to the mature LD surface. Further, *Cb*EPF1 interacts with VAP proteins in the host ER and establishes MCS between the host ER and LDs. *Cb*EPF1-mediated membrane contact sites could be a novel strategy to regulate host LD metabolism to support *Coxiella* infection.

Category: I. General Microbiology Oral Presentation (10 AM, Big 12 Ballroom)

Defining Functional Regions of Atypical MAPK Erk2 in *Dictyostelium discoideum.*

Ramee G. Aranda (Doctoral)*, Jeffrey Hadwiger. Oklahoma State University Main Campus, Stillwater, Oklahoma.

In eukaryotic cell signaling, mitogen-activated protein kinases (MAPKs) mediate cellular processes like cell growth, differentiation, and movement. In *Dictyostelium discoideum*, a social amoeba, there are only two of these MAPKs, Erk1 and Erk2. These MAPKs are involved in a developmental lifecycle where starved cells aggregate to form multicellular structures including fruiting bodies. Erk1 is a typical MAPK responsible for developmental kinetics and aggregate size while Erk2 is an atypical MAPK required for chemotaxis and multicellular development and the translocation of a transcription factor GtaC. Little is known about atypical MAPKs like Erk2 however, it's known that Erk2 is activated within 30 seconds after stimulation by a chemoattractant and that its function is required for a burst of Erk1 activation in a secondary response to chemoattractants. Atypical MAPKs have a conserved C-terminal motif (CTM) not found in other MAPKs. To test the specificity and function of this motif and other regions of MAPKs, a series of MAPK chimeras have been created and expressed in strains lacking one or the other MAPK. Testing the function and regulation of these chimeras will help define regions of typical and atypical MAPK specificity. Recent results suggest the CTM motif is necessary for most Erk2 functions.

Category: I. General Microbiology Oral Presentation (9:45 AM, Big 12 Ballroom)

SSA_0809 is a Homotrimeric, Reactive Intermediate Deaminase A (RidA) from an Opportunistic Pathogen, *Streptococcus sanguinis*.

Adreana Aquino (Undergraduate)*[,1], Alexa Benedict[1], Brandi Buckner[2], Leonard Thomas[3], Rakhi Rajan[3], Diana Downs[2] and Vijayakumar Somalinga[1]

[1]Department of Biological & Biomedical Sciences, Southwestern Oklahoma State University, Weatherford, Oklahoma. [2]Department of Microbiology, University of Georgia, Athens, Georgia. [3]Department of Chemistry and Biochemistry, University of Oklahoma, Norman, Oklahoma.

Reactive intermediate deaminase A (RidA) is a low-molecular weight protein in YjgF/YER057c/UK114 superfamily. The archetypal RidA subfamily is involved in amino acid metabolism and shown to catalyze the neutralization of toxic 2-amino acrylate (2AA) intermediates produced during amino acid catabolism. In *Salmonella enterica*, mutants lacking *ridA* exhibit physiological defects from the antagonistic interaction of 2AA with pyridoxal phosphate (PLP)-dependent enzymes. The importance of RidA and the incomplete understanding of metabolic networks affected by RidA led us to investigate its role in *Streptococcus sanguinis*, an opportunistic pathogen and the leading cause of subacute infective endocarditis in humans. BLAST analysis of *S. sanguinis* genome revealed a protein SSA_0809 with 50% identity to RidA from *S. enterica*. Biochemical studies on *S. sanguinis* SSA_0809, henceforth SsRidA, revealed its capacity of accelerating 2AA neutralization to pyruvate. To better understand SsRidA activity, the first crystal structure in a holoenzyme confirmation was solved at 1.97Å. The overall structure of SsRidA revealed a homotrimeric arrangement with active sites formed at the monomer interfaces, typical for this family. Active site electron density revealed the presence of ligand in only one active site leaving two active sites unoccupied. This incomplete ligand occupancy in SsRidA is still under investigation.

Category: IV. Poster Presentation (#8, presenting Friday evening)

Flint Hills Phages: Analysis and Annotation of Novel Lysogenic Bacteriophage

McKenzie J. Bietz*(Undergraduate), Bre L. Elliot, Kylie C. Howell, Brendan C. Newcomer, Craig J. Ollendick, Grace M. Schieferecke*(Undergraduate), Sidney A. Wilson, Martha Smith Caldas, Chris Herren
Division of Biology, Kansas State University, Manhattan, KS

Millions of microorganisms occupy the soil beneath us, and one of those is *Gordonia terrae* CAG4, an actinobacteria that can opportunistically infect immunocompromised hosts. Cohabitating with those bacteria are millions of bacteriophages, icosahedral viruses that can lyse bacteria. One such phage, Crater, was isolated, purified, and amplified from a residential area near the campus of Kansas State University. Crater's genome was sequenced and compared with other bacteriophage genomes on Phamerator, placing it in the DN3 subcluster of temperate phages. Its genome is made up of 52,539 base pairs and contains 94 genes. Using DNA Master, GeneBank, Starterator, Glimmer, PhagesDB, NCBI Blast, HHPred, and Phamerator, the genome was annotated, identifying a function for 43 genes. The genes necessary for lysogeny were annotated in the genome, proving Crater has the ability to integrate within its host. Crater's genome was compared to that of other annotated DN phages: Apricot, Kuwabara, and Leroy, and insertions, deletions, inversions, and frameshift were identified. By employing these strategies to annotate Crater's genome, more can be understood about it and other temperate phages' mechanisms of infection and lysogeny, as well as its potential to be used in phage therapy.

Category: IV. Poster Presentation (#26, presenting Saturday)

***Staphylococcus aureus* Persister Cells Exhibit Higher Tolerance to Innate Immune Components**

Kenan Brodd (Undergraduate)*, Emma Weis, Alexis Hobbs, Kim Carlson, and Austin Nuxoll
University of Nebraska at Kearney, Kearney, NE

Staphylococcus aureus is a pathogenic bacterium capable of causing serious infection in humans, like skin lesions, endocarditis, and sepsis. Difficulty treating infections may be due to the presence of persister cells. Persister cells are defined by surviving antibiotic treatment, however, it is unclear whether they have a fitness advantage to other stressors. Specifically, survival to innate immunity remains largely unexplored. Previous experiments show that a *fumC* (fumarase C, a tricarboxylic acid cycle gene) knockout exhibits increased survival to antimicrobial peptides. These experiments prompted further investigation of persister survival to other components of innate immunity such as reactive oxygen (ROS) and nitrogen (RNS) species. *S. aureus* strains of wild type HG003 and *fumC::N* were grown to mid-exponential phase, challenged with paraquat (induces ROS) and $NaNO_2$ (induces RNS), and survival was measured over 72 hours. Based on the finding that the *fumC::N* strain had increased survival in the presence of ROS and RNS, survival within a macrophage was examined. RAW264.7 macrophages were infected with HG003 and *fumC::N* (multiplicity of infection of 10) and bacterial survival was measured over 48 hours. The *fumC::N* strain exhibited increased survival suggesting persisters may provide a survival advantage to components of innate immunity in addition to antibiotics.

Category: V. Flash Talk (9:55 AM, Room 207)

Investigating the Effects of the Infant Probiotic *B. infantis* and Human Milk Oligosaccharides on the Severity of Anaphylaxis in a Mouse Model of Peanut Allergy.

Morgan Cade[1]*(Doctoral), Tasneem Ali[1], Emily Plotnik[1], Anthony Juritsch[1], Kristin Beede[1], Robert Schmaltz[1], Jeffrey Price[1], Bethany M. Henrick[2], Amanda Ramer-Tait[1]
[1]University of Nebraska–Lincoln, Lincoln, Nebraska, [2]Evolve Biosystems, Davis, California

Interactions between gut microbes and early-life immune programming may influence food allergy development, thus potentially contributing to rapidly increasing incidences of pediatric peanut allergy. *Bifidobacterium* induce oral tolerance in conventional mouse models of food allergy; however, they have not been tested in mice harboring an early-life human microbiome. We hypothesized that *Bifidobacterium infantis* EVC001 plus human milk oligosaccharides (HMO) would limit anaphylaxis severity in an infant microbiome-associated mouse model of peanut allergy. After receiving an infant microbiome lacking *Bifidobacterium,* germ-free mice were orally sensitized to peanut. Mice were gavaged daily with *B. infantis* or PBS and given drinking water with or without 5% HMO throughout the study. *B. infantis* + HMO treatment significantly decreased fecal pH and cecal acetic acid levels, increased abundances of *B. infantis,* and lowered anaphylactic scores after challenge compared to *B. infantis* alone. No differences were observed in plasma MCP-1 and IgE or in splenic Treg numbers. These results suggest that HMO enhances *B. infantis'* ability to limit innate immune responses related to anaphylaxis but does not alter peripheral Treg responses to peanut. Moreover, our infant microbiome-associated mouse model of peanut allergy provides a novel framework for testing the efficacy of probiotics/prebiotics in limiting allergic responses.

Category: III. Medical Microbiology/Immunology Oral Presentation (9:30 AM, Room 227)

Distribution of PERV (Porcine Endogenous Retrovirus)-C among Feral Pigs in Eastern Kansas

Robin Cesur (Masters)* and Eric Gillock. Fort Hays State University, Hays, KS

Allotransplantation can sometimes be tough in the United States due to a lack of donors. Xenotransplantation can be used to help alleviate these shortages. Pigs are an animal that may be used for this but are problematic when it comes to Porcine Endogenous Retrovirus (PERV). This is a retrovirus that can become introduced into the germ line of a pig. There are three subtypes based on cell tropism, sequence variation, and receptor interference. A and B are present in all pigs and infect pigs and humans. C is only present in some pigs and only infects pigs. A and C can recombine in two known varieties, long and short. Since A can infect humans, these hybridizations can also infect humans. By looking at recombinant envelope regions in PERV, it can be hypothesized how prevalent this hybridization is in feral and domestic samples. This study is using PCR to detect the presence of PERV-C as well as the two known varieties of hybridizations in a sample size of 53 in Eastern Kansas. It was found that 44/53 (83%) were positive for PERV-C, 13/31 (42%) were positive for PERV A/C hybrid long. The short variety is still being examined.

Category: III. Medical Microbiology/Immunology Oral Presentation (8:30 AM, Room 227)

Antifungal Activity of Novel Compound EIPE-1 against the Fungal Pathogen *Cryptococcus neoformans*

Priscilla Chatman (undergraduate)*, Brittney Conn, Emma Maritz, Toby L. Nelson, Karen L. Wozniak. Oklahoma State University, Stillwater, Oklahoma.

Cryptococcus neoformans is an opportunistic fungal pathogen that affects immunocompromised individuals. Antifungal drugs have been used to treat fungal infections for many decades. However, due to similarities between fungal and mammalian cells, these drugs are often toxic. In these last few decades, the fungi have also become resistant to the antifungal drugs. EIPE-1 was synthesized from vanillin, and was shown to have activity against methicillin resistant *S. aureus* (MRSA) and other gram-positive bacterial pathogens. We hypothesized that EIPE-1 could be used to kill fungal pathogens. For this study, we tested EIPE-1 against *C. neoformans* using a minimum inhibitory concentration (MIC) assay and an in vitro model of intracellular fungal growth using RAW macrophages. EIPE-1 has antifungal activity against *C. neoformans* in our MIC assay, with an MIC value of 1.749 µg/ml. In addition, following phagocytosis of *C. neoformans* by RAW macrophages, treatment with EIPE-1 had significant antifungal effects on *C. neoformans* compared to *C. neoformans* alone and *C. neoformans* with RAW macrophages (without treatment). We have also shown that EIPE-1 has antifungal activity against additional cryptococcal strains. Future studies will examine the mechanism behind antifungal activity of EIPE-1 following phagocytosis by macrophages.

Category: III. Medical Microbiology/Immunology Oral Presentation (10:15 AM, Cottonwood Room)

Novel adaptation of *in vitro* cultured organotypic skin for tick feeding and pathogen transmission.

Carley Conover; Matthew White; Amanda Brinkworth*. Department of Pathology & Microbiology, University of Nebraska Medical Center, Omaha, NE.

Serious illnesses such as Lyme Disease, Rocky Mountain Spotted Fever and Granulocytic Anaplasmosis are caused by bacteria transmitted via tick-bite. To date no effective therapies have been identified to prevent pathogen dissemination immediately following an arthropod bite on human skin. A major hurdle in identifying therapeutic targets to prevent tick-borne diseases is the lack of a source material for studying human skin infection following a tick-bite. Here we use an *in vitro* cultured organ`otypic skin model composed of epidermal (stratified keratinocytes) and dermal layers (fibroblasts embedded in collagen) for a completely novel application of studying transmission of the Lyme Disease agent *Borrelia burgdorferi* by *Ixodes scapularis* ticks. We have demonstrated that *Ixodes* nymphs attach (41.7%) to our skin rafts when human blood supplemented with ATP is present below the skin raft, and nymphs partially become engorged (23.2%) under conditions of high humidity. We introduce *B. burgdorferi* into nymphs by the immersion method, feed them on skin rafts, and detect pathogen transmission by PCR. This is the first demonstration of arthropod feeding on *in vitro* generated organotypic human skin, and its development will enable new avenues of research not only for the Lyme Disease field, but also for other tick-borne diseases.

Category: III. Medical Microbiology/Immunology Oral Presentation (8:45 AM, Room 207**)**

The Role of *slsA* in *Staphylococcus lugdunensis* Biofilm Formation.

McKenna Cruikshank (Undergraduate)* Justine M. Pitzer, and Austin S. Nuxoll
Department of Biology, University of Nebraska at Kearney, Kearney, NE

S. aureus has been the primary focus of the medical community, regarding staphylococcal related infections. However, there have been new concerns that a related bacterium, *S. lugdunensis*, is responsible for biofilm-induced infections, similar to those caused by *S. aureus* and *S. epidermidis*. Contributing to the pathogenic nature of this organism is its ability to form a biofilm, the culprit behind cases of endocarditis and severe prosthetic joint infections. To identify important factors involved in biofilm formation, *S. lugdunensis* was treated with ethyl methanesulfonate (EMS) and individual mutagenized cells were isolated through cell sorting. Cells exhibiting both decreased and increased biofilm formation were sequenced and a mutation in the gene coding for a surface protein, *slsA* was identified in a low biofilm former. A knockout of the *slsA* gene is currently being conducted to confirm these screening results. Additionally, to confirm that *S. lugdunensis* forms a protein-mediated biofilm, it was treated with proteinase K. Proteinase K treatment resulted in dispersal, further suggesting *S. lugdunensis* forms protein-mediated biofilms and that the *slsA* gene likely plays a major role in this process. This study helps us to understand the factors involved in biofilm formation in *S. lugdunensis.*

Category: IV. Poster Presentation (#14, presenting Friday evening)

***Chlamydia trachomatis* recruits PKA and Phosphorylated PKA Substrates during Infection.**

Noopur Dasgupta (Doctoral)*, Prakash Sah and Erika Lutter**.** Oklahoma State University, Stillwater, OK, USA.

Chlamydia trachomatis is an obligate intracellular pathogen and the most common leading cause of bacterial sexually transmitted disease in the United States which also results in various long-term complications. Given its intracellular nature it is known to depend on the host for its replication and survival; however, the extent of host cell signaling modulation is not known. The goal of this project is to determine the extent of Protein Kinase A (PKA) modulation during *C. trachomatis* infection. PKA is an important host kinase regulating transcription, protein expression and cell survival. Here we show that PKA subunits are recruited to the vicinity of the inclusion during *C. trachomatis* infection and that PKA substrate phosphorylation is increased during *C. trachomatis* infection. Using increasing concentrations of known PKA pharmacological inhibitors, while assessing viability of the host cells, we demonstrate that PKA is important for *C. trachomatis* replication and contributes to extrusion production. Overall, PKA modulation may be important during *C. trachomatis* infection and further studies will elucidate the host factors and mechanisms targeted through PKA activation.

Category: I. General Microbiology Oral Presentation (9:15 AM, Big12 Ballroom)

Determining Antimicrobial Activity of Metal-*N*-Heterocyclic Carbene Complexes against *Staphylococcus aureus.*

Kyle Dittmer (Undergraduate)*, Justine Pitzer, Hector Palencia, and Austin Nuxoll.
University of Nebraska at Kearney, Kearney, NE

Antibiotic resistance is becoming a major concern with an estimated 1.27 million deaths yearly and a contributing factor in nearly 5 million deaths, which is expected to rise to 10 million by 2050. However, recent research found molecules containing metal-*N*-heterocyclic carbene complexes (i.e. silver) inhibited biofilm formation in multiple pathogenic bacteria. The objective of this study was to screen compounds containing silver complexes for increased effectiveness against *Staphylococcus aureus* in hard-to-treat environments such as biofilms and persister cells. To assess antimicrobial activity of compounds against *S. aureus*, minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) were determined for 13 unique compounds. Time-dependent kill assays were performed over 72 hours with C1, C3, C8, C10, C11, and C12 compounds eradicating *S. aureus* within 24 hours. Compounds were also tested against biofilms which are notoriously difficult to eradicate. C1, C3, C8, and C10 reduced bacterial burden by 1-log while all other compounds, including vancomycin, were unable to reduce bacterial burden. Further characterization is needed to assess whether these compounds are suitable antibiotics, but preliminary results are encouraging.

Category: III. Medical Microbiology/Immunology Oral Presentation (9:45 AM, Room 227)

Using Photoactive Yellow Protein to Develop Novel FTIR Spectroscopic Methods for Probing Three Protonation States of Histidine Side Chains

Rosalie L. Dohmen (Doctoral)[1]*, Sarah Teeman[1], Elizabeth Schneider[1], Riddhi Patel[1], Jake Mulready[2], Emily Hurst[2], Salma Sultana Priya[2], Wouter D. Hoff[1], and Aihua Xie[2]

[1]Department of Microbiology and Molecular Genetics, Oklahoma State University, Stillwater, OK, United States, [2]Department of Physics, Oklahoma State University, Stillwater, OK, United States.

Proton transfer is a widely occurring process central to a range of protein functions. We study protein structure-function relationships involving functionally important proton transfer. However, few current experimental tools are sensitive to changes in the proton locations during protein function. Histidine is a common active site residue that often functions by changing its protonation state. Our specific aim is to develop generally applicable strategies for using Fourier transform infrared (FTIR) spectroscopy to unambiguously determine histidine side chain protonation state. We use photoactive yellow protein (PYP), a bacterial photoreceptor that has emerged as a powerful model system for this study. The PYP from *Halorhodospira halophila* contains two histidine residues: one is solvent exposed (His3), and the other is buried in a hydrophobic pocket (His108). Building on our previous computational studies, we performed measurements to identify vibrational modes of His3 and His108 in PYP. We achieved specific assignments of infrared modes using point mutants and side chain-specific isotope editing of histidine. The interpretation of static FTIR measurements on these samples at several pH values provides both assignment of the vibrational modes and protonation state of histidine's. This work offers a generally applicable approach for such studies on histidine side chains.

Category: I. General Microbiology Oral Presentation (10:15 AM, Big12 Ballroom)

A Microbial Source Tracking Study to Identify Fecal Contamination in a Karst Water System
William Durstock (Masters)*,: Missouri State University; Saki Urushidani: Environmental Services Department, City of Springfield, MO, Babur S Mirza: Missouri State University

Waterborne pathogens originating from human fecal material of infected individuals are one of the major areas of health concern in karst environments where water can easily flow from old leaky septic tanks and broken sanitary sewer lines into rivers and streams. The current study was focused on temporal monitoring of fecal indicator bacteria (FIB) in Sequiota Spring. Based on an initial Microbial Source Tracking (MST) study, we observed a high abundance of human fecal indicator bacteria (HFIB) (up to 110,000 cells/L water) in July 2020. The City of Springfield initiated a detailed assessment and repair plan for the upstream sanitary sewer lines as a remediation solution. Through this remediation effort, the HFIB significantly decreased (55 times reduction) in June 2022. We also assessed the waterfowl fecal indicator bacteria which were low ~300 cells/L and remained unchanged from the year 2020 to 2022. This suggests that the sewer repairs completed in the recharge area of Sequiota Spring were a primary cause of the reduction in HFIB. This study demonstrated a successful remediation effort in reducing human fecal contamination to reduce potential health risks at this site.
Category: IV. Poster Presentation (#28, presenting Saturday)

Role of Soil pH in Driving Selection Rhizobial Endophytes within Soybean Root Nodules.
Brianne D. Edwards (Masters)* and Babur S. Mirza. Missouri State University, Springfield, Missouri.

Soybean plants fulfill most of their nitrogen requirement by developing symbiotic associations with four different rhizobial genera, including *Bradyrhizobium*, *Sinorhizobium*, *Mesorhizobium*, and genus *Rhizobium*. In general, members of *Bradyrhizobium* and *Sinorhizobium* have been reported as root nodule endophytes under acidic soils and alkaline soil conditions, respectively. So far, it is unknown whether the selection of rhizobial endophytes is regulated by their ability to survive under different soil pH or primarily driven by host plants regardless of their relative abundance in soil. This study was focused on the assessment of the potential role of soil pH in selecting rhizobial endophytes and determining whether the selection of rhizobial endophytes is controlled by their relative abundance in rhizosphere soil. In a greenhouse study, we inoculated soybean plants with different cell densities of *Bradyrhizobium japonicum* and *Sinorhizobium fredii* cultures. Plants were grown under three soil pH conditions. We assessed the distribution of rhizobial endophytes within root nodules and rhizosphere using high-throughput DNA sequencing of 16S rRNA gene amplicons. We observed significant differences in plant growth and selection of root nodules endophytes across different soil pH conditions. These results will be helpful in identifying better rhizobial bioinoculants under various soil pH conditions.
Category: III. Environmental Microbiology Oral presentation (8:30 AM, Room 206)

Characterization of the Unique Biology and Drug Resistance Mechanisms of *Candida auris*

Brooke D. Esquivel* and Theodore C. White.
Division of Biology and Biomedical Systems, School of Science and Engineering, University of Missouri Kansas City, Kansas City, Missouri

We analyzed one hundred *Candida auris* isolates with a variety of drug resistance profiles and representing each clade to gain a greater understanding of *C. auris* biology, antifungal resistance, and adaptations to stressful conditions.

We measured intracellular fluconazole accumulation, revealing a strong correlation between fluconazole resistance and drug uptake. Fluconazole-resistant isolates had reduced levels of intracellular fluconazole accumulation compared to fluconazole-susceptible isolates.

We found that changing the environmental pH can alter the uptake of azole drugs and other compounds in *C. auris* cells. Most commonly there is increased compound uptake and hypersusceptibility when the pH is increased. We are investigating whether this response to pH includes modification of the cell wall and plasma membrane, or the effect of transporter activity.

We measured transporter activity related to MFS and ABC transporters in the *C. auris* isolates using alanine β-naphthylamide accumulation and Rhodamine 6G efflux assays. We found a diversity of efflux capabilities between the isolates.

Finally, we are working on gene expression analyses to identify candidate genes correlated to our phenotypic characterizations of these isolates.

Further characterization of, and associations between, intracellular drug accumulation, membrane composition, efflux transporters and gene expression, could help define *C. auris*-distinctive mechanism of drug resistance.

Category: IV Poster Presentation (#16, presenting Friday evening)

Mapping interaction sites of ParE toxin with ParD antitoxin and with DNA gyrase

Felipe Avelino Ferreira (Undergraduate)*, Kevin Snead, Christina Bourne.
University of Oklahoma Department of Chemistry and Biochemistry, Norman, Oklahoma.

Toxin-Antitoxin (TA) systems are widespread in prokaryotes. In the Type II TA systems, the toxin is a protein that reduces the host metabolism, and the antitoxin is a protein that directly binds the toxin to counteract its activity. ParE toxins comprise a subfamily that has been demonstrated to inhibit gyrase-mediated supercoiling, resulting in the accumulation of DNA breaks, promoting lethality in plasmid segregation killing (PSK) models, and thus invoking the SOS response *in vivo*. Our long-term goal is to disrupt the TA interaction to provide insights for an anti-gyrase method of controlling bacterial cell growth.

We are interested in understanding which regions are critical for the interaction of ParE with its cognate antitoxin ParD, and how this interaction blocks the ability of ParE to inhibit DNA gyrase. This will also help us understand mechanistic details for ParE inhibition of gyrase, which remains largely known. We have constructed N-terminally truncated ParD antitoxins and measured their affinity to ParE, which revealed a minimal unit of antitoxin that maintains affinity for the ParE toxin. My project carries on by assaying if these truncated ParD antitoxins still neutralize the action of ParE with respect to gyrase inhibition. We are currently building suitable clones to introduce the ParE toxin complexed with minimal ParD peptides into an *in vitro* gyrase activity assay. The results will give us insights into which portions of the ParE toxin must be exposed to interact with, and thus inhibit, DNA gyrase.

Category: IV. Poster Presentation (#18, presenting Friday evening)

A Chemolithotrophic, Methanogenic, and Moderately Alkaline Enrichment Community Maintained with Calcium Carbonate as a Sole Source of Inorganic Carbon.

Nicole A. Fiore (Doctoral)[1], Rebecca A. Daly[2], Kelly C. Wrighton[2], Sanjay Antony-Babu[1], Daniel N. Miller[3], Rebecca V. Kiat[1], Donald Pan[1], Caitlin Lahey[1], Nicole R. Buan[4], and Karrie A. Weber[1, 5].*
[1]School of Biological Sciences, University of Nebraska-Lincoln, Lincoln, NE; [2]Department of Crop and Soil Sciences, Colorado State University, Fort Collins, CO; [3]Agroecosystem Management Research Unit, United States Department of Agriculture, Lincoln, NE; [4]Department of Biochemistry, University of Nebraska-Lincoln, Lincoln, NE; [5]Department of Earth and Atmospheric Sciences, University of Nebraska-Lincoln, Lincoln, NE.

Most of the carbon on Earth is sequestered as sedimentary carbonates, which are considered a metastable carbon reservoir under alkaline conditions. Here, we demonstrate the use of calcium carbonate as a sole inorganic carbon source for a methanogenic microbial consortium at moderately alkaline pH. Saline wetland soil was inoculated in minimal medium (100% argon, pH 8.3) yielding an enrichment continuously maintained on hydrogen gas and calcium carbonate. The culture was geochemically characterized under five alkaline pH conditions (from 7.6 to 8.5) in sealed serum bottles with parallel heat-killed or uninoculated reactors as negative controls. In live cultures, methane increased with a concurrent decrease in headspace hydrogen. Methane concentration was inversely proportional to pH, but could be standardized against cell density. Dissolved calcium increased significantly in live cultures with no change in pH, demonstrating microbially mediated mineral dissolution. Shotgun metagenomic sequencing identified a methanogen (*Methanobacterium* sp.) and five bacterial community members. Two methanogenic strains, ACI-5 and ACI-7, were isolated from the enrichment for long-read sequencing and physiological characterization. Overall, these results indicate that carbonate minerals may be subject to microbial dissolution yielding methane, even under alkaline conditions, and demonstrate the potential for carbonate sustained lithoautotrophic communities.

Category: II. Environmental Microbiology Oral Presentation (10 AM, Room 206)

Microbiome Perturbations Moderately Modulate *Caenorhabditis elegans* Health and Life History Traits.

Ashley Foltz (Doctoral)* and Michael Herman
School of Biological Sciences, University of Nebraska – Lincoln, Lincoln, NE

Bacteria are the sole food source of *C. elegans* and many must be advantageous to their health, but some are detrimental. *Stenotrophomonas* bacteria are abundant members of the natural *C. elegans* microbiome described by Zhang *et al*. (2017). Surprisingly, we and others have shown that many *Stenotrophomonas* isolates are detrimental to *C. elegans* health (Samuel *et al*. 2016, White *et al*., 2016, Radeke and Herman, 2020). This has led us to ask several questions about the relevant interactions that individual microbiome members have. We are characterizing the role of *Stenotrophomonas* in the microbial community to better understand these interactions. Our approach is to use an experimental microbiome (CeMbio) consisting of representative bacteria (Dirksen *et al*., 2020) to determine the effects of perturbing microbiome composition. We found that *C. elegans* survivorship is not significantly affected by most microbiome perturbations, although communities with more pathogenic *Stenotrophomonas* strains resulted in reduced survivorship. We also found that development time and fecundity, used as measures of host fitness, are mostly unaffected as compared to the intact community. This suggests that the CeMbio community may function to buffer the effects of detrimental members on host health and fitness.

Category: I. General Microbiology Oral Presentation (8:45 AM, Big12 Ballroom)

***Chlamydia trachomatis* Modulates the Expression of JAK-STAT Signaling Components to Attenuate the Type II Interferon Response in Epithelial Cells.**

Francis Fontanilla (Doctoral)*, Rey Carabeo, and Amanda Brinkworth.
University of Nebraska Medical Center, Omaha, Nebraska

Chlamydia trachomatis, an obligate intracellular pathogen, subverts host signaling processes to ensure its successful intracellular development. Following infection, lymphocyte-derived interferon- (IFN) induce epithelial cells to produce indoleamine-2,3-dioxygenase (IDO1) that starve *Chlamydia* of tryptophan. However, relative to mock-infected cells, IFN -induced expression of IDO1 is down-modulated and is concomitant with lower nuclear localization of its transcription factor Signal Transducer and Activator of Transcription 1 (STAT1) in infected cells, suggesting an altered interferon response. Thus, we hypothesize that *Chlamydia* targets the components of the IFN -JAK/STAT pathway to attenuate signaling. To test this, we infected HEp-2 cells with *C. trachomatis* serovar L2 for 24 hours before exposing to IFN . We observed a reduced phospho-activation of both STAT1 and its kinase Janus Kinase 2 (JAK2) in infected cells. This lower activation correlated with lower expression of both total STAT1 and JAK2 during infection. This decrease was rescued when we inhibit *de novo* chlamydial protein synthesis, restoring IFN -induced activation and expression of STAT1 target genes. Taken together, our findings suggest a mechanism for *Chlamydia* to dampen the interferon response by modulating its host cell to respond poorly to the cytokine. These findings provide insight into how *Chlamydia* can circumvent host immune responses by attenuating cytokine signaling.

Category: III. Medical Microbiology/Immunology Oral Presentation (9:15 AM, Room 227)

Characterizing High Persister Phenotypes in *Staphylococcus epidermidis* Clinical Isolates.

Mariam Garcia (Undergraduate)*, Kaitlyn Pineda, and Austin Nuxoll
University of Nebraska at Kearney, Kearney, Nebraska.

Staphylococcus epidermidis is an opportunistic pathogen that typically resides within our normal skin flora and is primarily associated with causing disease in immunocompromised individuals. Often these infections are biofilm-mediated and associated with indwelling medical devices. Antibiotic treatment of these infections is often unsuccessful, leading to poor patient prognosis. One possible explanation for these observations is the presence of persister cells (a subpopulation of dormant cells). High persister isolates have been observed in other microbial pathogens such as *Pseudomonas aeruginosa* and *Candida albicans*. Recent work in the related pathogen, *S. aureus*, demonstrates that persister formation is dependent on energy depletion through the tricarboxylic acid (TCA) cycle. We hypothesized that high persister isolates occur in *S. epidermidis* clinical isolates through an energy-dependent mechanism. To observe the possibility of a correlation between high persister formation and a dysfunctional TCA cycle, extracellular acetate (as an indicator of TCA cycle activity) was measured in high and low persister isolates. Of the 17 isolates screened, seven correlated with high extracellular acetate concentrations and exhibited high antibiotic tolerance, and four of them exhibited low antibiotic tolerance. Preliminary data has demonstrated the correlational relationship between a dysfunctional TCA cycle and increased persister formation.

Category: IV. Poster Presentation (#19, presenting Friday evening)

Microbial isoprene reduction tied to greenhouse gas removal in deep-sea carbonates and Eucalyptus-leave sediments

Samikshya Giri[a](Doctoral)*, Nancy G. Prouty[b], Sabrina Beckmann[a]
a: Oklahoma State University, Stillwater, Oklahoma.
b: USGS Pacific and Coastal Marine Science Centre

Isoprene is the most abundantly produced biogenic volatile organic compound and a critical climate-active atmospheric gas of similar abundance to the potent greenhouse gas methane. Isoprene is also the forgotten piece of the puzzle to tackling climate change. Every form of life produces isoprene but up-to-date, data rarely exist about its global biogeochemical cycle. It is highly reactive in the atmosphere affecting methane concentrations and increasing detrimental negative implications on climate, air quality, and health. On the contrary, we know that methane is abundant in the marine and terrestrial subsurface environment. Deep sea carbonates as well as river sediments are hot spots of microorganisms that carry out anaerobic oxidation of methane which plays an important role in the long-term storage and removal of methane from these habitats. Nothing is known about the fate of isoprene and about potential microbial communities with the ability to metabolize isoprene in deep-sea carbonates and eucalyptus-leave sediments potentially affecting methane metabolism. We are seeking to unravel the anaerobic pathways of microbial isoprene degradation and the effect of isoprene abundance and degradation on microbial methane production and oxidation. Here we present the initial results of the reductive metabolism of isoprene in an anaerobic marine and terrestrial environment. Our data show that isoprene is reduced in a methane-oxidizing environment in deep-sea carbonate enrichment cultures and eucalyptus leave-rich sediments. In methanogenic environments, isoprene even in small concentration, is found to inhibit methanogenesis. We are currently characterizing the microorganism's metabolizing methane and isoprene in the enrichment cultures.
Category: II. Environmental Microbiology Oral Presentation (9:45 AM, Room 206)

***Chlamydia trachomatis* Inclusion Membrane Protein CT226 Interaction with Host Proteins TMOD3 and FL2.**

Tanisha Goyal (Masters)*[1], Christina Bourne[2], Erika Lutter[1]
[1]Oklahoma State University, Stillwater, OK, USA
[2]Oklahoma University, Norman, OK, USA

Chlamydia trachomatis is an obligate intracellular pathogen that is transmitted sexually in humans. It is estimated that it results in more than 3 million STI infections in USA including pelvic inflammatory disease, ectopic pregnancy, and reduced fertility. Bacteria has a distinctive biphasic life cycle, its reproductive stage creates a parasitophorous vacuole, also known as an inclusion. *Chlamydia* produces inclusion membrane proteins which decorate the inclusion membrane and mediate host-pathogen interactions. Previous studies have identified that the Inc CT226 interacts with the host actin remodeling protein TMOD3 and the host inflammasome associated protein FL2. We hypothesize that Inc CT226 is interacting with host proteins FL2 and TMOD3 at the leucine rich regions. The aim is to study is to verify the regions of Inc CT226, FL2 and TMOD3 that interact using the bacterial two hybrid system and pulldowns. Purified proteins of predicted interacting regions of CT226, FL2 and TMOD3 will be purified for structural analysis, binding assays, and crystallography.
Category: IV. Poster Presentation. (#4, presenting Friday evening)

False Positives in a Commercial Fecal Indicator Bacteria Assay

Grant M. Graves[1], Jason R. Vogel[1], Kara B. De Leon[2], Alex W. Walls[2] and Ralph S. Tanner[2,3*]
[1]Civil Engineering and Environmental Science, University of Oklahoma, Norman, OK
[2]Microbiology and Plant Biology, University of Oklahoma, Norman, OK
[3]Presenter, Professor of Microbiology

Enterolert is an assay for the enumeration of enterococci in environmental samples based on an esculin positive phenotype. Identification of species recovered from individual windows of Enterolert panels showed the presence of non-FIB (fecal indicator bacteria), primarily esculin positive *Paenibacillus* spp. A number of other spp., including some Gram negative bacteria, were also responsible for false positive results. While overall false positive results were only 19% of the total spp. identified and the false positive rate for aqueous samples was only 11%, false positive results could be 100% for enumerations of some environmental samples. Stream sediment sampled over a 10 week period showed essentially random fluctuations in the % false positives windows during this time period. Study of the population composition of a stream sediment in a microcosm study also showed that populations could shift from >90% FIB to >90% false positives, while the apparent enterococci concentration remained stable over a 31 day period. Work is needed to improve the enumeration of enterococci in environments of interest, since these results have real world impacts, such as using this method for assessing waterbodies for recreational water quality criteria.

Category: II. Environmental Microbiology Oral Presentation (9:10 AM, Room 207)

The Spx Redox Switch is Not Essential for Thiol Homeostasis in *Staphylococcus aureus*.

Abigail G. Hall (Doctoral)*[1], Abdulelah A. Alqarzaee[1], Sujata S. Chaudhari[1], Dorte Frees[2] and Vinai C. Thomas[1]
[1]Department of Pathology and Microbiology, University of Nebraska Medical Center, Omaha, Nebraska, USA
[2]Department of Veterinary and Animal Sciences, University of Copenhagen, Denmark

Spx is a redox-sensitive transcriptional regulator that responds to disulfide stress and maintains thiol homeostasis in low GC gram-positive bacteria. The formation of an oxidized disulfide in the Spx redox switch enables it to bind the alpha subunit of the RNA polymerase to facilitate gene regulation. Here, we investigate the physiological role of the Spx redox switch in the gram-positive pathogen *Staphylococcus aureus*. Although *spx* is essential for the growth of *S. aureus*, we successfully engineered a *spx*^{C10A} mutation that encoded an oxidation-insensitive Spx^{C10A} variant. The turnover rate of Spx^{C10A} was identical to the native variant and was mediated by the intracellular ClpP protease. The *spx*^{C10A} mutant was more sensitive to disulfide stress than the wildtype strain. However, a kinetic analysis of intracellular redox status using a redox-sensitive GFP reporter indicated that thiol homeostasis was unaffected in the *spx*^{C10A} mutant, and it recovered from disulfide stress at a similar rate to the WT strain. Transcriptomic analysis revealed multiple metabolic pathways to be impacted in the *spx*^{C10A} mutant, suggesting the Spx redox switch's function extended beyond thiol homeostasis. These findings indicate that the Spx redox switch's role in disulfide stress response may involve regulating cellular functions distinct from thiol homeostasis.

Category: IV. Poster Presentation (#10, presenting Friday evening)

Correlation of *Veillonella* Bacteria with Oral Health.

Paiton Hancock (Undergraduate)* and Dawn Simon. University of Nebraska at Kearney, Kearney, Nebraska.

Veillonella is a common bacteria found within the oral microbiome, particularly in association with dental caries in children. Thus, the presence of *Veillonella* may be indicative of an individuals' overall oral health. In this study, we aim to understand the prevalence of *Veillonella* in healthy college-aged individuals and determine if there is a correlation between prevalence and self-reported oral health. Previous studies have been conducted in a different demographic, primarily younger children in other countries. We are using one-step PCR to identify species of *Veillonella* within the oral microbiome from tongue biofilm samples. Thus far we have examined 15 participants. Preliminary results suggest the presence of multiple *Veillonella* species. Based on previously published results, we hypothesize that there will be an increased likelihood of *V. parvula, V.denticariosi*, *and V.tobetsuensis* bacteria in individuals with lower oral health compared to those with good oral health. If a correlation does exist, it may suggest a biological indicator of oral health. The oral microbiome composition, including Veillonella can change in the presence of other diseases, such as GERD. Thus, better understanding *Veillonella's* prevalence in the oral microbiome may have consequences beyond oral health.

Category: IV. Poster Presentation (#21, presenting Friday evening)

Defining the Regulons of the Two Minor Sigma Factors in *Chlamydia trachomatis.*

Nathan D. Hatch (Doctoral)*, Scot P. Ouellette.
University of Nebraska Medical Center, Omaha, Nebraska.

Chlamydia trachomatis encodes three sigma factors: σ66, σ54, and σ28. σ66 is considered the major sigma factor and responsible for most transcription initiation during early and mid-cycle development. The roles of the minor sigma factors, σ54 and σ28, have not been well characterized to date – however, there are data to suggest each plays a role in late-stage development and secondary differentiation. As the process of secondary differentiation itself is poorly characterized, clarifying the roles of these sigma factors and subsequently identifying the genes regulated by them will further our understanding of chlamydial differentiation. We hypothesize that σ54 and σ28 have non-redundant and essential functions for initiating late gene transcription and mediating secondary differentiation in *Chlamydia*. Here, we demonstrate the necessity of each minor sigma factor in successfully completing the developmental cycle. We have implemented and validated multiplexed CRISPRi techniques novel to the chlamydial field to examine effects of knocking down both alternative sigma factors simultaneously. Knocking down or overexpressing transcript levels for either or both alternative sigma factors resulted in a severe defect in EB production as compared to controls. Furthermore, RNA sequencing revealed significant changes in many late genes hypothesized to be essential for differentiation.

Category: I. General Microbiology Oral Presentation (9:15 AM, K/S Ballroom)

Assessment of Commensal *E. coli* Outer Membrane Vesicles for Application in a Bacterial-Derived Oral Gene Delivery System.

Kari Heck (Doctoral)*, Amanda E. Ramer-Tait, Angela K. Pannier
University of Nebraska-Lincoln, Lincoln, Nebraska.

Gene delivery via the oral route is desirable due to the high rate of patient compliance and ease of administration. To overcome challenges associated with oral gene delivery, we are developing a novel delivery system by loading outer membrane vesicles (OMVs) with plasmid DNA to create DNA-loaded OMV nanocarriers (DNA-OMV NCs). Commensal bacteria residing in the human GI tract, including *E. coli*, produce OMVs, which act similarly to exosomes by protecting and trafficking DNA, RNA, protein, and other small molecule cargo. Additionally, OMVs can survive gastric transit, cross the mucus barrier, and be internalized by intestinal epithelial cells, thus making them an ideal biomaterial for oral delivery. To identify OMV sources to be utilized as DNA-OMV NCs, we have screened OMVs isolated from an existing collection of 30 human commensal *E. coli* strains for cytokine production and internalization into Caco2 and J774 cells. Notable differences were observed among the various OMVs in terms of their internalization and cytokine production from cells. The data collected in this screen demonstrate that OMV properties such as internalization and cytokine production can differ among strains of bacteria and could allow for customization of the DNA-OMV NC platform for a variety of oral delivery applications.
Category: III. Medical Microbiology/Immunology Oral Presentation (10 AM, Room 227)

Identification and Characterization of *C. elegans* Genes That *Stenotrophomonas maltophilia* Targets to Evade Host Insulin-Like DAF-2/16 Pathway Defenses.

Sara M. Hopkins (Doctoral)*, Leah J. Radeke, and Michael A. Herman. School of Biological Sciences, University of Nebraska-Lincoln, Lincoln, Nebraska.

The bacterivorous nematode *Caenorhabditis elegans* has been developed as a model to study host immune responses to bacterial pathogens, including the emerging human nosocomial pathogen *Stenotrophomonas maltophilia*. Upon exposure to pathogenic bacteria, the nematode utilizes several highly conserved innate immunity pathways, such as the insulin-like DAF-2/16 pathway that is protective against most bacterial pathogens. Interestingly, normally long-lived *daf-2* mutant animals are susceptible to pathogenic strains of *S. maltophilia*. To understand how *S. maltophilia* is able to evade the DAF-2/16 pathway, we used transcriptional profiling in *wild-type* and *daf-2* mutants to identify candidate *C. elegans* genes that may be targeted by *S. maltophilia* to defeat host defenses. We hypothesize that *S. maltophilia* may interfere with the functions of these target genes to shorten the lifespan of normally long-lived *daf-2* mutants, and thus expect that candidate target genes should be required for *daf-2*-mediated lifespan extension in the absence of the pathogen. To test this hypothesis, we are using RNA-mediated interference to knock-down candidate target genes in *wild-type* and *daf-2* backgrounds and evaluating the effect on lifespan. Future characterization of these candidate target genes may illuminate the underlying mechanisms that enable pathogenic *S. maltophilia* to bypass the nematode's innate immune responses.
Category: I. General Microbiology Oral Presentation (8:30 AM, K/S Ballroom)

A NEW DNA EXTRACTION METHOD FOR TICK NYMPHS

Bodhi Jelinek (Doctoral)*, Keith Geluso[1], Julie Shafer[1]
Department of Biology, University of Nebraska at Kearney, Kearney, NE

The collection of tick nymph DNA to use for polymerase chain reaction (PCR) does not currently have a standardized method that will provide enough DNA from a single nymph. However, through the use of the Extracta DNA Prep for PCR-Tissue kit a new technique has been established. Rodents were trapped using live traps, and tick nymphs removed from the rodents with tweezers. The nymphs were placed in 70% ethanol and taken back to the laboratory where DNA was extracted using the Extracta DNA Prep for PCR-tissue DNA extraction kit. After extraction they underwent DNA quantification, and it was found that 117 out of 120 samples had ample DNA for PCR. These samples were subjected to multiplex and singleplex PCR looking for pathogenic bacteria. Those that were found to be positive for particular bacteria were purified using a GeneJET PCR Purification kit and were sent to the University of Nebraska Medical Center for sequencing. Once we obtain the sequences, NCBI BLAST will be used to confirm the identification. This technique will allow us to use individual nymphs for analysis rather than pooling samples and will increase our understanding of tick nymphs as disease vectors.

Category: IV. Poster Presentation. (#12, presenting Friday evening)

Examining the function of the ClpC AAA+ ATPase in the Biology of *Chlamydia*

Aaron A. Jensen (Doctoral)*, Scot P. Ouellette
University of Nebraska Medical Center, Omaha, Nebraska

Bacterial AAA+ unfoldases are crucial for bacterial physiology by recognizing specific substrates and, typically, unfolding them for degradation by a proteolytic component. The caseinolytic protease (Clp) system is one example where a hexameric unfoldase (e.g. ClpC) complexes with a tetradecameric protease (e.g. ClpP). Unfoldases can have both ClpP-dependent and ClpP-independent roles in bacterial biology. Since ClpC is typically found in Gram-positive bacteria, its presence within the obligate intracellular Gram-negative pathogen *Chlamydia*, an organism with a highly reduced genome, is interesting. Previously, we showed that chlamydial ClpC functions as a bona fide AAA+ unfoldase. Furthermore, *in vivo* dysregulation of chlamydial ClpC revealed a significant reduction in growth, highlighting its importance in chlamydial development. Interestingly, our data show overexpression of ClpC leads to an increase in glycogen accumulation significantly earlier in development than normally detected. This effect was not observed when overexpressing a mutant isoform of ClpC, suggesting a ClpC substrate may impact this pathway. We have taken a direct approach to analyze the function of ClpC within the glycogen pathway using gene knockouts and transcript analyses. Overall, these findings will provide us with a better understanding of the regulation of glycogen metabolism and insights into the cellular function of chlamydial ClpC.

Category: III. Medical Microbiology/Immunology Oral presentation (9:15 AM, Cottonwood Room)

Temporal progression of anaerobic fungal population in dairy calves from birth to maturity

Adrienne L. Jones[1*] (Masters), Jordan Clayborn[1], Elizabeth Pribil[1], Andrew Foote[1], Dagan Montgomery, Noha H. Youssef[l], and Mostafa S. Elshahed*[1]
[1]Oklahoma State University, Stillwater, Oklahoma

Anaerobic gut fungi (AGF) inhabit the alimentary tract of Bovidae, but little is known regarding the progression of AGF in cattle from birth till maturity. We followed the AGF community in six dairy cattle, where fecal samples were collected pre-weaning (day1-48), during the weaning phase (day 49-60), and post-weaning (3-12 months). We aimed to document changes in AGF diversity and community structure, and to correlate the community dynamics to salient events in alimentary tract anatomical development and feeding regiments. Surprisingly, the microbial communities in newly birthed calves were distinct from their mothers, and more diverse, with a community dominated by genera mostly associated with hindgut fermenters (e.g. *Khoyollomyces, AL3*). A drastic change in community structure was observed post-weaning, with a significant decrease in alpha diversity, and a shift towards typical cattle AGF communities (e.g. *Orpinomyces, Pecoramyces*). Community structure shift coincided with age-related anatomical development (e.g. the development of the rumen occurring 2-3 months post birth), and nutritional transitions to plant substrates associated with weaning.

Category: I. General Microbiology Oral Presentation (8:30 AM, Big12 Ballroom)

Dietary Fiber from Sorghum Flour Protects Mice Harboring Human Gut Microbiotas Against Chemically-Induced Colitis.

Anthony F. Juritsch (Doctoral)*[1], Kristin Beede[1], Morgan Cade[1], Sukaina al-Hamedi[1], Dulcie Achuleta[2], Qinnan Yang[1], Robert Schmaltz[1], Jeff Price[1], Devin Rose[1], Stephen Kachman[1], Scott Sattler[1,3], Andrew Benson[1], Amanda Ramer-Tait[1]
Affiliations: [1]University of Nebraska-Lincoln, Lincoln, Nebraska; [2]Nebraska Wesleyan University, Lincoln, Nebraska; [3]United States Department of Agriculture – Agriculture Research Service (USDA-ARS), Lincoln, Nebraska

Although gut microbiota composition can influence the efficacy of clinical dietary fiber interventions, few preclinical studies of inflammatory bowel disease have assessed the effects of baseline microbial community variation on disease outcomes during dietary interventions. Therefore, this study sought to validate a model of dextran sulfate sodium (DSS) induced colitis in human-microbiome-associated (HMA) mice to evaluate microbiome-mediated effects of dietary interventions. Germ-free C57BL/6 mice were colonized with one of three healthy human fecal microbiotas to generate three cohorts of HMA mice. Mice were then fed either a fiber-free diet or a diet containing 30% sorghum flour (w/w). After two weeks, mice were provided DSS (1.0 to 2.5%, w/v) or control water for seven days. We observed that 1.25% DSS was sufficient to induce a robust, microbiome-dependent colitis injury in HMA mice characterized by body weight loss, anorexia, colon shortening, increased microscopic disease, elevated *ex vivo* cytokine secretion, and accumulation of lipocalin-2 in cecal contents. Notably, these colitis parameters were significantly decreased in mice consuming the 30% sorghum flour diet. In conclusion, our results suggest that this preclinical HMA mouse model is sufficient to further investigate the effects of microbiome-targeted dietary therapies on intestinal inflammation.

Category: III. Medical Microbiology/Immunology Oral Presentation (10:15 AM, Room 227)

Purification and Preliminary Crystallization of SSA_0908, a Substrate-Binding Protein from *Streptococcus sanguinis*.

Marshall "Julius" Koons (Undergraduate)*[,1], Camille Goerend[1], Leonard Thomas[2], Rakhi Rajan[2] and Vijayakumar Somalinga[1]

[1]Department of Biological & Biomedical Sciences, Southwestern Oklahoma State University, Weatherford, Oklahoma. [2]Department of Chemistry and Biochemistry, University of Oklahoma, Norman, Oklahoma.

Streptococcus sanguinis is a pathobiont that is the leading cause of subacute infective endocarditis (SIE) in humans. Blood transit and attachment to cardiac vegetation is a prerequisite for SIE pathogenesis. While numerous studies have identified cell-surface adhesins in *S. sanguinis*, many suggested to be involved in SIE remain uncharacterized. One being SSA_0908, a putative ABC-transporter substrate binding proteins (SBP) with homology to CD0837, a SBP from *Clostridiodes difficle* implicated in host colonization and aromatic amino acid transport. Sequence analysis showed that residues involved in aromatic amino acid ligand binding is highly conserved in SSA_0908. Homology modeling of SSA_0908 revealed a type 1 periplasmic SBP fold with two a-b-a sandwich domains connected via a hinge-loop. The ligand binding pocket at the interface of the sandwich domains shows active site architecture similar to other aromatic amino acid SBP's. Sequence and structural homology of SSA_0908 to other characterized aromatic amino acid transporters indicated that this protein may be involved in similar function in *S. sanguinis*. In order to further characterize SSA_0908, we have successfully over-expressed and purified this protein using affinity chromatography. Preliminary crystallization trials resulted in microcrystals in several conditions. We are currently optimizing crystallization conditions to grow diffraction quality crystals.

Category: IV. Poster Presentation (#24, presenting Saturday)

Using the Human Gut Microbiome as a Phenotype of Sorghum in a Genetic Mapping Study.

Nate Korth (Doctoral)*, Qinnan Yang, Mallory Van Haute, Michael Tross, Ravi Mural, James C. Schnable, and Andrew Benson. Nebraska Food for Health Center; University of Nebraska-Lincoln; Lincoln, NE

The composition of the human gut microbiome is associated with many aspects of human health and disease and is shaped by diet. Staple grains, which comprise a significant percentage of calories consumed by humans, consist of few elite hybrid lines that have been artificially selected for crop yield and resilience traits. We developed an automated *in vitro* microbiome screening platform as a high-throughput method for phenotyping the effects of grains on gut microbes to employ a quantitative genetics approach to identify novel plant traits that impact human nutrition mediated by the gut microbiome in a diverse population of sorghum. In a genome-wide analysis, we identified genetic loci in sorghum associated with gut microbes from two human subjects. Genome-wide mapping of various sorghum biochemical and agronomic traits revealed traits likely causal for microbiome associations. An identified locus on chromosome four encompasses the *tan1* gene known to regulate condensed tannin accumulation in sorghum, which has been shown impact gut microorganisms tremendously. This work demonstrates that genetic factors affecting sorghum can drive significant effects on gut microbes, particularly on those considered to be beneficial. Understanding these relationships will enable targeted breeding strategies that can improve human health through modulation of the gut microbiome.

Category: I. General Microbiology Oral Presentation (10:30 AM, Big12 Ballroom)

Fast Axonal Transport of Infectious Prion Protein

Sam M. Koshy (Doctoral)*, Ronald A. Shikiya, Anthony E. Kincaid, Jason C. Bartz. Creighton University School of Medicine, Omaha, Nebraska.

Peripherally acquired disease-causing prions (PrP^{Sc}) invade the brain via defined neuroanatomical pathways. PrP^{Sc} transport has been approximated as slow axonal transport, but the methods utilized lack sensitivity and measure both inoculum PrP^{Sc} and newly replicated PrP^{Sc}, confounding observed rates. To increase accuracy, we utilized highly sensitive protein misfolding cyclic amplification (PMCA) to measure hamster PrP^{Sc} transport in the mouse sciatic nerve (ScN), a hamster prion replication deficient system. We also purified and fluorescently labeled PrP^{Sc} to directly measure transport in mouse ScN explants using two photon confocal microscopy. After PMCA, we failed to detect PrP^{Sc} in the uninoculated ScN 24 hours p.i. (post infection) but PrP^{Sc} was detected in the inoculated ScN and lumbar spinal cord (SC) 24 hours p.i. Based on the distance from the inoculation point to the lumbar SC, PrP^{Sc} transport rate was calculated as at least 25 mm/day, well above established slow transport rates (0.3-8 mm/day). Fluorophore conjugated PrP^{Sc} was successfully imaged in ScN explants, and multiple timeseries were acquired. Analysis of PrP^{Sc} transport velocities using the ImageJ plugin Trackmate were consistent with fast axonal transport. These data suggest that PrP^{Sc} can use fast axonal transport, having implications for prion disease pathogenesis and treatment.

Category: III. Medical Microbiology/Immunology Oral presentation (10:30 AM, Room 227)

CUL3 Negatively Regulates NLRP12-Mediated Inhibition of the NF-kB Signaling Pathway.

Inyeong Lee (Masters)*, Cathy Rippe, Abbi Brown, and Christopher Lupfer.
Missouri State University Biology, Springfield, Missouri.

Nod-like receptor family pyrin domain-containing protein 12 (NLRP12) is mainly known for its inhibitory function on NF-kB signaling in innate immune cells, and more recently, for its ability to regulate chemokine signaling and ubiquitination of the immune receptor RIG-I. Through a yeast-2 hybrid screen, the Lupfer lab discovered that NLRP12 interacts with other ubiquitin-associated proteins including CUL3. We report here the further characterization and functional significance of these interactions in human cells. Throughout the study, we observed that NLRP12 interacted with CUL3, and expression of CUL3 resulted in ubiquitination of NLRP12. This was associated with increased NF-kB activation, and increased IL-8 production. These data suggest that CUL3 negatively regulates NLRP12, preventing it from inhibiting NF-kB signaling.

Category: III. Medical Microbiology/Immunology Oral presentation (10 AM, Cottonwood Room)

Chlamydial Bactofilin Functions as a Cell Shape Determinant.

Jeonghoon Lee*[1], John V. Cox[2], and Scot P. Ouellette[1]
[1] University of Nebraska Medical Center, Omaha, NE
[2] University of Tennessee Health Science Center, Memphis, TN

Bacterial cell morphology is usually determined by peptidoglycan (PG) synthesis. However, *Chlamydia trachomatis* has no PG sacculus. Therefore, how cell size and morphology are determined in *Chlamydia* is unknown. We recently identified and characterized a gene encoding a chlamydial bactofilin homolog, *bacA*. Using a CRISPRi conditional-knockdown system, we decreased endogenous *bacA* transcripts and observed that the cell morphology of *bacA*-knockdown bacteria was abnormal, displaying a larger size with larger PG rings at the septum and unevenly distributed major outer membrane protein. These phenotypes were complemented by ectopic expression of BacA_6xHis, suggesting that BacA is crucial for maintaining optimal cell shape. Interestingly, BacA has an extended N-terminus showing high disorder probability. The region immediately after this is equivalent to the membrane binding domain of other bactofilin homologs. We hypothesize the BacA N-terminus is crucial for membrane localization to regulate the function of BacA. We observed that ΔN81_BacA lost membrane localization and filamentation, whereas the ΔN50_BacA appeared more stable. Furthermore, the region 51-81aa, which is equivalent to the membrane binding domain of other bactofilin homologs, directs GFP to the membrane. Based on these data, we suggest the extended N-terminus is critical for membrane localization, filamentation, and the dynamic properties of BacA.

Peggy Cotter Branch Travel Award winner (2:15 PM, Big12 Ballroom)

Microbial and Host Factors that Modulate Differences in Host's Clinical Outcome to *Clostridioides difficile* Infection.

Armando I. Lerma (Doctoral)*, Thomas Auchtung, and Jennifer Auchtung.
University of Nebraska-Lincoln, Lincoln, Nebraska

Clostridioides difficile is one of the most important pathogens in hospital and community healthcare settings. The clinical outcome of infection of toxigenic *C. difficile* infection (CDI) can fall within a wide range of disease severity from asymptomatic colonization to fulminant pseudomembranous colitis and death. In recent studies, it has been suggested that a high proportion of nosocomial CDI cases are transmitted from asymptomatic carriers which might be acting as infection reservoirs. Investigating what causes the different responses to infection could lead to the development of novel prevention and treatment strategies. Although several explanations have been proposed to explain variations in susceptibility, understanding of the exact mechanisms that underlie the spectrum of variation in CDI disease severity remains limited and further research is needed to determine what factors are responsible for these variations. In this work, we establish different human microbiota-associated (HMA) mouse models. By analyzing innate immune responses to CDI, we demonstrate that these models reproduce differences in disease severity during infection that were largely based on mouse strain and independent from *C. difficile* burden or toxin activity. Altogether, our ^{HMA}mouse models demonstrated the potential to study interactions between microbiome, pathogen and host inflammatory responses in the context of CDI.

Category: III. Medical Microbiology/Immunology Oral Presentation (8:45 AM, Room 227)

***Cutibacterium acnes* produces molecules with antivirulence activity against *Staphylococcus* spp.**
Rayssa Durães Lima[1] (Doctoral)*, Gabrielle Antunes dos Reis[2], Juliana da Silva Reviello[2], Thaís Glatthardt[2], Larissa da Silva Coimbra[2], Carla Ormundo Gonçalves Ximenes Lima[2], Marcos Filipe Muniz Faria[2], Luis Caetano Martha Antunes[1], Rosana Barreto Rocha Ferreira[1]. [1]University of Kansas, Lawrence, Kansas, [2]Federal University of Rio de Janeiro, Rio de Janeiro, Brazil

Staphylococcus species are often isolated from human and animal infections. The rapid spread of antimicrobial resistance among *Staphylococcus* indicates the importance of discovering new alternatives for the treatment of such infections. The human microbiome has been studied as a source of new compounds with activity against pathogens. The aim of this study was to test the activity of molecules secreted by *Cutibacterium acnes* against *Staphylococcus*. Analysis of growth curves, biofilm formation assays, microscopic observations, preliminary characterization of bioactive molecules, characterization of colony morphology, and biofilm matrix composition were performed. Among the staphylococci species tested, *S. lugdunensis* and *S. hominis* displayed significant reduction in biofilm formation by *C. acnes* compounds and, in the case of *S. lugdunensis*, this effect was lost after treatment with sodium periodate, indicating a polysaccharidic nature of the active molecule. *C. acnes* supernatant also dispersed mature biofilms of *S. aureus*, *S. epidermidis*, and *S. pseudintermedius*. Furthermore, *C. acnes*-produced molecules changed the morphology of colonies recovered from *S. lugdunensis* biofilms. Understanding the interactions between different microorganisms could shed light on new compounds with potential applications to treat bacterial infections.

Category: III. Medical Microbiology/Immunology Oral presentation (9:45 AM, Cottonwood Room)

***Bacteroides rodentium* Limits Tumor Progression in a Mouse Model of Melanoma.**
Mason S. Mandolfo (Undergraduate)*[1], Kristin A. Beede[1], Robert Schmaltz[1], Jeff Price[1], Ze'ev Ronai[2], and Amanda Ramer-Tait[1]. [1]University of Nebraska-Lincoln, Lincoln, Nebraska; [2]Sanford Burnham Presby, San Diego, California.

Growing evidence points to an important role for the gut microbiome in anti-tumor immunity for multiple types of cancers, including melanoma. Our team previously identified twelve gut bacterial strains, including species from *Bacteroides*, *Parabacteroides*, and *Alistipes*, that elicited anti-tumor immune responses and limited melanoma tumor growth when given to mice. During those studies, *Bacteroides rodentium* was found to be the most abundant strain, thus prompting us to ask whether *B. rodentium* alone was sufficient to limit melanoma tumor progression and whether it changed the abundance of other members of the gut microbiota. To test this, we colonized germ-free C57BL/6 mice with an eight-member, defined microbial community (Altered Schaedler Flora, ASF) alone or in combination with *B. rodentium*. After two weeks, mice were injected subcutaneously with YUMM1.5 melanoma cells, and tumor growth was monitored until necropsy on day 22. Mice colonized with *B. rodentium* had significantly smaller tumors compared to control mice. Experiments are ongoing to quantify the abundance of *B. rodentium* and each ASF member throughout the study using species-specific primers and qPCR. Our results show an important role for *B. rodentium* in limiting melanoma progression. Further studies are needed to identify the specific mechanisms underlying *B. rodentium*-mediated anti-tumor immunity.

Category: IV. Poster Presentation (#5, presenting Friday evening)

Defined Gastrointestinal Community Responses to Vancomycin Perturbation in a Bioreactor System

Hugh C. McCullough (Doctoral)*[1,2], Hyun-Seob Song[1,2,3], and Jennifer M. Auchtung[1,2]
[1]Nebraska Food for Health Center, University of Nebraska-Lincoln, Lincoln, NE;
[2]Department of Food Science and Technology, University of Nebraska-Lincoln, Lincoln, NE;
[3]Department of Biological Systems Engineering, University of Nebraska-Lincoln, Lincoln, NE

The gastrointestinal tract is home to many communities of microbes, which can be associated with food in the lumen or the host mucosal layer. As microbial function can be dependent on the context of the microbe, we and others have hypothesized that the mucus layer and the microbiota which colonize it may play a role in microbiome stability. To improve insights into the resistance and resilience of mucin-associated communities, we modified a bioreactor system to hold sampleable mucin-coated coverslips. Culturing a defined consortium of fecal bacteria, we tracked taxon abundances of communities associated with the coverslips and in the planktonic phase over time. To better understand their interactions, we fit the data to generalized Lotka-Volterra models, inferring the ecological interactions driving the population dynamics of each taxon within each community. Using these interaction networks, we will narrow down what interactions may influence the persistence of different taxa facing antibiotic treatment in the planktonic and sessile communities of the cultured metacommunities.
Category: IV. Poster Presentation (#22, presenting Saturday)

Salmonella **Regulates Lipopolysaccharide Biosynthesis by Controlling LapB Interactions with LpxC.** Joshua A. Mettlach (Doctoral)*, Melina B. Cian, and Zachary D. Dalebroux. University of Oklahoma Health Sciences Center, Oklahoma City, Oklahoma.

S*almonella enterica* serovar **T**yphi**m**urium (*S*Tm) is a prevalent human pathogen that regulates the abundance and structure of outer membrane **l**ipo**p**oly**s**accharide (LPS) to survive in hazardous host environments. A critical inner membrane regulatory network composed of PbgA, LapB, and FtsH functions to control LpxC proteolysis, the rate-limiting enzyme of LPS biosynthesis; however, the molecular mechanisms between these proteins are unresolved. We proposed that LapB functions as an adaptor protein directly interacting with PbgA and LpxC to facilitate LpxC proteolysis by FtsH and in turn control LPS biosynthesis. Deletion of *lapB* (Δ*lapB*) attenuates growth, stabilizes LpxC levels, and results in hyperproduction of short LPS molecules. The Δ*lapB* phenotype is consistent with dysregulated LPS biosynthesis and rescued by expression of LapB *in trans.* LapB devoid of its transmembrane segment ($LapB^{cyto}$) binds to LpxC *in vitro* but does not rescue Δ*lapB* defects *in vivo*, establishing that LapB functions as an inner membrane-bound adaptor protein. Furthermore, specific LapB amino terminal tetratricopeptide repeats are critical for LpxC interaction with current work focused on defining the essential interaction residues. These data support the hypothesis that LapB functionally controls LpxC levels through a direct protein-protein interaction which is critical for *S*Tm growth and LPS biosynthesis regulation.
Category: III. Medical Microbiology/Immunology Oral presentation (8:30 AM, Cottonwood Room)

Novel Taxa with Bio-industrial Potential Isolated from Alpaca Fecal Material.

Samuel Miller (Doctoral)* [1,2], Meredith Hendry [1], Jacobey King [1], Krithivasan Sankaranarayanan PhD [1,2], and Paul A. Lawson PhD [1]

[1] Department of Microbiology & Plant Biology, University of Oklahoma, Norman.

[2] Laboratories of Molecular Anthropology & Microbiome Research, University of Oklahoma, Norman.

The characterization of mammalian microbiomes has important implications for the health and disease of the host organism and agriculture/industry. Alpaca (*Vicugna pacos*) are pseudoruminants that rely on their gut microbiome (a reservoir for fermentative lignocellulosic bacteria) to access energy and nutrients from the plant material they consume. In this study, two novel strains designated "*Clostridium tannerae"* A1-XYC3^T sp. nov. and "*Bacteroides pacosi"* A2-P53^T sp. nov. were isolated from the fecal material of two alpaca from a ranch in Newcastle, Oklahoma. Whole-genome and 16S rRNA gene-based analyses determined that the *Clostridium* isolate was phylogenetically related to well-characterized acetogens *Clostridium magum, Clostridium carboxidivorans*, and *Clostridium aciditolerans*, and the *Bacteroides* isolate was related to *Bacteroides* koreensis, *Bacteroides* kribbi, and *Bacteroides ovatus.* Genomic data demonstrated that the *Clostridium* isolate possesses enzymes in the Wood–Ljungdahl pathway, associated with producing acetate and ethanol, which is important in industrial processes. Similarly, the *Bacteroides* isolate contains numerous well-defined xylanases, enzymes extensively employed in the paper and pulp industries. Identifying novel microbial isolates capable of producing industrially valuable end products and enzymes highlights the need to further explore the uncharacterized diversity of mammalian microbiomes.

Category: II. Environmental Microbiology Oral Presentation (9:30 AM, Room 206)

Surveillance for *Candida auris* on Commercially Available Fruit.

FNU Monika, Creighton University, Omaha, NE; Katya A. Faber-Quimby (Undergraduate)*, University of Nebraska, Lincoln, NE; David Quimby, Creighton University, Omaha, NE

Candida auris has emerged as a global pathogen of significant medical concern. Due to large increases in the amounts of azole fungicides used in agriculture and medical environments, there may be selective pressure leading to the increased prevalence of azole-resistant *Candida* spp, including *C.auris*. Previous studies have investigated – and revealed the presence of – pathogenic yeast on the surface of commercially available produce. As many related studies have been performed in southeast Asia, the goal of this study was to investigate the possible presence of pathogenic yeast on produce in the United States. Peaches were collected from local supermarkets, underwent surface culturing, and had colonies of yeast species identified. Although all the yeast species that could be identified are known to cause human disease, only one isolate (*Candida parapsilosis*) is among the most common species involved with human infections. Inability to isolate *C.auris* from our samples could be due to the less hospitable temperate climate in peach cultivation, lower human worker colonization with *C.auris* in the United States to colonize the fruit after harvest, or testing constraints.

Category: IV. Poster Presentation (#17, presenting Friday evening)

The CGRP-Ramp1 Signaling Pathway Enhances Anti-*Aspergillus fumigatus* Immune Response.
Chinemerem Onah (Masters)*, Michael Bartkoski, and Pankaj Baral. Kansas State University Division of Biology, Manhattan, Kansas.

Aspergillus fumigatus opportunistic infections are currently on the rise due to increasing proportion of immunocompromised and chronic lung disease patients. These individuals develop Invasive Pulmonary Aspergillosis (IPA) and lethal pneumonia caused by *A. fumigatus* leading to dysregulated inflammation and altered immune responses. The lungs are heavily innervated by nociceptive neurons which help maintain homeostasis and immune cell functions. Although *A. fumigatus*-specific therapeutics exist, they fail to provide absolute protection against IPA and come with huge clinical limitations. Therefore, this study aims to understand the crosstalk between lung-innervating neurons and the immune system to identify new anti-fungal therapeutic targets through the signaling of these two systems. To understand the role of Calcitonin Gene Related Peptide (CGRP – released by nociceptive sensory neurons) in neutrophil, bone marrow-derived macrophages (BMDMs) and monocyte anti-fungal response, we employed immunoassay, intracellular killing and flow cytometry analyses and found that CGRP increases the level of phagocytosis and intracellular killing of *A. fumigatus* conidia by these cells. These effects were eliminated in immune cells that lack the Ramp1 receptor (for CGRP). Altogether, our data support the notion that nociceptive neurons and their secreted signaling molecule (CGRP) act through the Ramp1 receptor to increase anti-fungal immunity during *A. fumigatus* infection.
Category: IV. Poster presentation (#7, presenting Friday evening)

An Essential Role for Alanine Racemase in Overcoming Organic Anion Intoxication in *Staphylococcus aureus*.
Sasmita Panda[1] (Postdoc)*, Yahani P. Jayasinghe[2], Dhananjay D. Shinde[1], Emilio Bueno[3], Amanda Stastny[1], Blake P. Bertrand[1], Sujata S. Chaudhari[1], Tammy Kielian[1], Felipe Cava[3], Donald R. Ronning[2] and Vinai C. Thomas[1].
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Organic weak acids excreted as byproducts of host and pathogen metabolism are significant determinants of infection outcome. Due to their lipid permeable nature, weak acids can accumulate in the cytoplasm as anions to alter cellular homeostasis and inhibit growth. Intriguingly, the gram-positive pathogen *Staphylococcus aureus* can produce, tolerate, and grow in millimolar amounts of the weak acid, acetate. Here we explore the basis of staphylococcal tolerance to acetate intoxication. We demonstrate that the acetate anion inhibits D-alanyl-D-alanine ligase (Ddl) activity and perturbs intracellular D-ala-D-ala pools to compromise peptidoglycan crosslinking. However, the acetate-mediated inhibition of Ddl negatively impacted staphylococcal growth only when alanine racemase (*alr*) was inactivated, and the intracellular D-ala pools were substantially lowered. In the absence of *alr*, the native levels of D-alanine amino transferase (Dat) activity could not sufficiently replete intracellular D-ala pools to restore growth. Furthermore, the *alr* mutant was sensitive to different biologically relevant weak acids indicating a broad conservation of inhibitory mechanism. Our findings suggest that Alr activity may have evolved as a mechanism to maintain high intracellular D-ala concentrations and counter weak acid anion intoxication.
Category: I. General Microbiology Oral Presentation (10 AM, K/S Ballroom)

Replication and Genome Characteristics of v_Bsu_Adastra, a Lytic Bacteriophage of *Bacillus subtilis*.

Eliana Pendergrass (Undergraduate)* and Andrew F. Herbig
Department of Biology, Washburn University, Topeka, KS

Bacillus subtilis is a Gram positive, endospore forming bacterium that is commonly found in soil and the gastrointestinal tract of humans. Bacteriophages infecting *B. subtilis* can influence the population dynamics of this bacterium and, by extension, its ecological niche. The bacteriophage v_Bsu_Adastra (hereafter Adastra) is a lytic phage that was isolated from soil and is host restricted to *B. subtilis*. Adastra is a tailed phage with morphology similar to myophages in the SPO1 family. We characterized infection and replication characteristics of Adastra by determining its total adsorption constant, latent period, and burst size. After forty minutes Adastra replicates within and lyses its bacterial host producing 93 new phages. Sequencing of the phage genome revealed a 136,306 bp chromosome coding for 198 proteins and three tRNAs. Adastra is 98% identical to bacillus phage SP8 at the nucleotide level and clusters phylogenetically with other members of the SPO1 family of phages. We identified a putative +1 programmed ribosomal frameshift within a tail chaperone gene and an intron splitting a DNA polymerase gene. A host cell lysis cassette with an endolysin gene flanked by two holin genes was also identified in the genome.

Category: IV. Poster Presentation (#27, presenting Saturday)

Cadmium has differential effects on microbial short chain fatty acid production depending on fecal donor

Carmen E. Perez-Donado (Doctoral)*. Jennifer Auchtung. Devin Rose. University of Nebraska Lincoln, Lincoln, Nebraska.

Cadmium (Cd) is a toxic heavy metal widely distributed in the environment and the food chain. When involuntarily consumed through food, Cd displays low bioavailability in the small intestine, and can interact with the gut microbiota. We hypothesized that Cd would inhibit growth of some members of the gut microbiota which would lead to altered production of short chain fatty acids (SCFA) depending on microbiota composition. The aim of this study was to identify the effects of Cd exposure on production of SCFA by the gut microbiota obtained from twenty-one fecal donors. Across all microbiomes, only butyrate production was significantly affected by Cd ($p=0.002$), which cut butyrate production by more than half (15.1±1.38 mM versus 7.23±0.80 mM). When analyzing butyrate production by microbiome, 19 out of 21 (90%) showed decreases in butyrate production ranging from 11-95% reduction. Two microbiomes had increased butyrate production of 15% and 32% in the presence of Cd. These findings demonstrate that Cd has detrimental effects on butyrate production by the microbiome, but the impact of Cd varies among gut microbial communities. Future work should determine unique microbial features that enable Cd-tolerance of a microbiota.

Category: IV. Poster presentation (#13, presenting Friday evening)

Tortoises as Novel Hosts for Deep-Branching Anaerobic Gut Fungi: Exploring Phylogenetic Diversity, Community Structure, Isolation, and Evolutionary History

Carrie J. Pratt (Doctoral)*[1], Yan Wang[2], Jason E. Stajich[3], Noha H. Youssef[1], Mostafa S. Elshahed[1]; [1]Oklahoma State Univ., Stillwater, OK, [2]Univ. of Toronto, Toronto, ON, Canada, [3]Univ. of California, Riverside, Riverside, CA

Although anaerobic gut fungi (AGF, phylum Neocallimastigomycota) have been well-documented in mammalian hosts, their presence in non-mammalian hosts is still poorly understood. Here, we investigated the occurrence of AGF in tortoises (family Testudinidae) and examined their phylogenetic diversity, community structure, isolation, and evolutionary history. By using culture-independent analysis, we identified three novel deep-branching AGF genera that accounted for over 90% of sequences in 14 out of 16 fecal samples from nine tortoise species. We isolated two of these dominant genera across various temperatures and confirmed their deep-branching position through phylogenomic analysis, with the basal genus *Khoyollomyces* as their closest relatives. Molecular clock timing of the two genera suggests that the evolution of Neocallimastigomycota occurred in the early Cretaceous period (105 and 112 Mya) rather than the late Cretaceous period (67 Mya). These early branching AGF genera associated with tortoises may possess distinctive patterns of horizontal gene transfer and lignocellulolytic genes, which have been linked to the establishment of AGF in the mammalian gut. Our findings provide evidence of AGF occurrence in a non-mammalian host, expanding our understanding of the phylogenetic diversity, ecological distribution, and evolutionary history of the phylum Neocallimastigomycota.

Category: II. Environmental Microbiology Oral Presentation (8:45 AM, Room 206)

Combining *Lactobacillus taiwanensis* and *Gordonibacter urolithinfaciens* Decreases Body Weight Gain and Increases Lean Mass in a Mouse Model of Diet-induced Obesity.

David Gomez Quintero (Doctoral)*, Ashley M. Toney, Kristin Beede, Jeff Price, Robert Schmaltz, Amanda E. Ramer-Tait. University of Nebraska-Lincoln, Lincoln, Nebraska.

In the United States, one in three adults have obesity, and $147 billion is spent annually on obesity-related health care. New dietary strategies are therefore being evaluated to treat obesity, including supplementation with gut bacteria with putative health benefits (probiotics). Previous studies in our lab have shown that providing *Gordonibacter urolithinfaciens* to mice limited body weight gain, decreased white adipose tissue, and lowered insulin levels. These beneficial effects were strongly and negatively correlated with an increased abundance of *Lactobacillus*, including *Lactobacillus taiwanensis*. Here, we isolated *L. taiwanensis* from mice and tested whether the combination of *L. taiwanensis* and *G. urolithinfaciens* was required to provide health benefits. Germ-free mice were colonized with a *Lactobacillus*-deficient microbiome and provided a high-fat diet with 20% fructose drinking water. Mice received either *L. taiwanensis* only, *G. urolithinfaciens* only, both bacteria, or no treatment. Mice treated with both *L. taiwanensis* and *G. urolithinfaciens* gained significantly less body weight and had more lean mass compared to control mice or mice receiving only *G. urolithinfaciens*. These results demonstrate that *G. urolithinfaciens* provides health benefits when *L. taiwanensis* is present. Such a finding provides useful insight into how personalized probiotic strategies can serve as treatments for obesity.

Category: IV. Poster Presentation (#20, presenting Friday evening)

TmeA-mediated Signaling Operates During Later Stages of *Chlamydia trachomatis* Invasion and is Necessary for Efficient Dynamin-dependent Closure of *Chlamydia*-containing Vacuoles.

Matthew D. Romero (Doctoral)*, Rey A. Carabeo. University of Nebraska Medical Center, Omaha, Nebraska

During invasion, *Chlamydia trachomatis* deploys the secreted effectors, TarP and TmeA, which collaboratively modulate actin kinetics and facilitate pathogen uptake. Previously we demonstrated that TmeA deletion (ΔTmeA) reduced uptake efficiency and specifically altered actin turnover, suggesting that TmeA signaling operates during later stages of pathogen engulfment, such as closure and scission of *Chlamydia*-containing vacuoles. The host GTPase Dynamin mediates scission of endocytosed vesicles and contributes to *Chlamydia* development via nutrient acquisition. However, it remains unclear whether dynamin participates in *Chlamydia* invasion, or whether TmeA signaling is involved. We found that invading wild-type EBs were fully encapsulated by actin, whereas actin was restricted to the base of ΔTmeA EBs, leaving bacteria partially exposed. Inhibition of dynamin prior to invasion caused defective encapsulation of wild-type EBs, but had no effect on ΔTmeA EBs. Likewise, dynamin inhibition restricted entry of wild-type *Chlamydia*, but not ΔTmeA EBs. Finally, dynamin inhibition altered actin kinetics during wild-type entry, resulting in prolonged actin turnover and retention. Altogether, we conclude that TmeA signaling is spatiotemporally resolved, driving later stages of actin recruitment necessary for full encapsulation of EBs. Additionally, we report that host dynamin activity is important for invasion and operates in a manner sensitive to TmeA signaling.

Category: I. General Microbiology Oral Presentation. (9:30 AM, K/S Ballroom)

Nitrate Stimulated Iron Reduction in Unsaturated Soil.

Taylor Rosso[1] (Doctoral)*, Dan Miller[3], Karrie A. Weber.[1,2,4] School of Biological Sciences, University of Nebraska, Lincoln, NE[1]; Department of Earth and Atmospheric Sciences, University of Nebraska, Lincoln, NE[2]; Agroecosystem Management Research Unit, Agricultural Research Service, United States Department of Agriculture, Lincoln, NE[3]; Daugherty Water for Food Institute, University of Nebraska, Lincoln, NE[4].

Coupled iron, nitrogen, and carbon redox cycling has been primarily studied in saturated systems. The potential significance of these coupled cycles also exists in unsaturated soils following precipitation or irrigation. Following irrigation, soil was collected and homogenized. MPN enumeration of heterotrophic iron reducing bacteria revealed 3.3 x 10^4 cells/g. A series of soil slurries were initiated in bicarbonate-buffered medium under anoxic conditions to follow Fe(III) reduction in the presence of nitrate. Following pre-incubation, nitrate was added to soil slurries to a final concentration of 0.3 mM (low) or 50 mM (high). Control treatments remained unamended. Fe(III) reduction was observed in all treatments after 24 hours. Interestingly, the high nitrate amendment treatment resulted in the most Fe(III) reduced, 6.4 mmol•L^{-1}, relative to the low and unamended treatments, 1.2 mmol•L^{-1}, and 1.73 mmol•L^{-1}, respectively. Nitrate reduction was observed concomitant with Fe(III) reduction in the amended treatments. In the no nitrate and low amendment treatments, minimal, transient accumulation of N_2O was observed, compared to high amendment, which reached 47 μmol/L by the end of the incubation. These results show that iron reduction is stimulated by influxes of nitrate, highlighting the potential significance of iron redox cycling in unsaturated soils.

Category: IV. Poster Presentation (#11, presented Friday night)

Characterizing the Oral Mycobiome of Domestic Dogs.

Elisa M. Rouse (Undergraduate)*, Brooke D. Esquivel, Brandon Holder, Butch KuKanich, Kate KuKanich, Allison M. Schweiker, Dariyan Springfield, Theodore C. White.
Division of Biology and Biomedical Systems, School of Science and Engineering, University of Missouri-Kansas City, Kansas City, Missouri.
College of Veterinary Medicine, Kansas State University, Manhattan, Kansas.

Understanding the oral and gut mycobiome is a relatively new field of study in humans and animals. The purpose of this study is to characterize the oral mycobiome of domestic dogs to identify commensal and potentially pathogenic fungi present. 253 buccal swabs were obtained in collaboration with Kansas State University's College of Veterinary Medicine and a local animal shelter, and were struck onto a chromogenic fungal growth medium that distinguishes between fungal species based on colony color and morphology. After isolating and harvesting colonies, DNA was extracted from each species. PCR was used to amplify a fungus-specific variable region of the genome (ITS-1), which was then sequenced. NCBI BLAST database was used to identify each species present. After the isolates were speciated, we began antifungal drug susceptibility testing against common drugs such as fluconazole, ketoconazole, and terbinafine.
Exploring the oral mycobiome of dogs as well as the corresponding drug susceptibility of isolates will allow researchers to assess the appropriateness of antifungal use as it relates to the development of drug resistant species. These findings will improve our understanding of the microorganisms within our pets, and thus the organisms that humans are commonly exposed to through our canine companions. **Category: IV. Poster Presentation** (#9, presented Friday night)

Engyodontium album **Empyema in an Immunocompetent Patient**

Rubenstein, Jane D.O.[1], Harris, Kelley D.O.[1], Pascual, Elizabeth M.S. (Medical Student)[2]*
Department of Internal Medicine, Oklahoma State University, Tulsa, OK[1]
Oklahoma State University College of Osteopathic Medicine, Tulsa, OK[2]

The majority of organisms isolated from pleural fluid infections include viridans streptococci in community-acquired infections and methicillin-resistant *Staphylococcus aureus* in hospital-acquired infections. Gram negative bacilli such as *Pseudomonas, Klebsiella,* and *Enterobacter* have also been identified. Fungal etiologies are rare and typically present in an immunocompromised host. In these cases, *Candida albicans* is often isolated. In this report, we discuss a case of an immunocompetent patient presenting with *Engyodontium album* empyema.
Category: IV. Poster Presentation (#15, presented Friday night)

Exploring Arabinose metabolism Impairment in Cells Overexpressing ParE Toxins.
Shengfeng Ruan (Doctoral)*, Christina Bourne, University of Oklahoma, Norman, Oklahoma.

The type-II ParDE toxin-antitoxin (TA) system is a small bicistronic genetic element found in many prokaryotes, encoding a pair of non-secreted proteins. The toxin, ParE, inhibits DNA gyrase and can lead to cell death in the absence of the protein-protein interactions with the cognate antitoxin, ParD. In previous studies, at least 0.1% of cells survived despite plasmid-based overexpression of the *Mycobacteria tuberculosis* ParE1 toxin under control of an arabinose-inducible promoter. Upon reinoculation, they were found to exhibit resistance to a secondary induction of arabinose, leading to the hypothesis that arabinose metabolism may be impaired. To test this hypothesis, the cells were "cured" of the ParE-containing plasmid by sequential non-selective passages and a plasmid containing the same-promoter-controllable fluorescent reporter protein (mCherry) was used to assess arabinose uptake in the "resistant" cells. A lack of signal confirmed that arabinose was likely the cause for the resistance phenotype. The arabinose transporter AraE was constitutively expressed in the cells with the reporter, which only restored arabinose uptake in some cells as noted by fluorescence microscopy, and is likely due to heterogeneous expression of AraE,. Future experiments will include bacterial whole genome sequencing to investigate possible genotypic changes underlying the observed resistance. Based on these studies, it seems likely that bacterial cells will take the "path of least resistance" to escape the potent toxicity of TA system proteins.
Category: V. Flash talk (10:05 AM, Room 207)

The Prevalence of Tick-Borne Disease-Causing Pathogens in South Central Nebraska.
Noah Shackelford (Undergraduate)*, Darby Carlson, and Julie Shaffer. University of Nebraska at Kearney Department of Biology, Kearney, Nebraska.

Dermacentor variabilis, commonly known as the American dog tick, is the foremost native tick species in Nebraska and is a known vector of several pathogenic bacteria. This study sought to determine the pathogen prevalence of *D. variabilis* ticks collected during the 2022 tick season in areas of south central Nebraska both along and isolated from the Platte River. DNA was extracted from 272 male and 260 female *D. variabilis* ticks for endpoint PCR testing; the presumptive positives from which were further tested through amplicon testing to verify pathogen identity. Sequencing results indicated that 13% (34) of males and 17% (43) of females tested were positive for one or more pathogenic bacteria, for a combined 15% (77) of ticks infected. This percentage is much higher than the previously identified 4% rate of infection for *D. variabilis* ticks collected in Nebraska. Increases in bacterial prevalence and thus the environmental risks of exposure are likely due to changing tick distributions, including spillover from *Amblyomma americanum*, Lone star ticks. As the incidence of tick-borne disease has been increasing across the country, Nebraska is just another example of an area that needs further tick surveillance to identify and stop this dangerous trend.
Category: III. Medical Microbiology Oral Presentation (8:45 AM, Cottonwood Room)

Molecular Mechanisms of Mucosal Colonization by *C. difficile.*

Ben Sidner, Baishakhi Biswas (Doctoral)*, Armando Lerma, Leslie A. Ronish, Hugh McCullogh, Jennifer M. Auchtung, Kurt H. Piepenbrink. University of Nebraska-Lincoln, Lincoln Nebraska.

Clostridioides difficile is a Gram-positive, spore-forming anaerobe and is the most common cause of antibiotic-associated diarrhea, representing a major public health threat. Although intestinal dysbiosis and immune deficiency are known to favor *Clostridioides difficile* infection, the underlying host-pathogen interactions that promote colonization and persistence are unknown. Recent studies provided compelling evidence that *C. difficile* associates with the colonic mucus layer during infection and could be a putative site of colonization. Using an improved quantitative model of *in vitro* adherence to mucus, we explored the molecular mechanisms underlying mucus association. Specifically, we tested whether two extracellular appendages, Type IV pili (T4P) and flagella, mediate mucosal adherence. We found flagella were important for adhesion while T4P were not necessary. We are currently testing specificity of *C. difficile* flagella to mucin through competitive exclusion of microbe-mucus interactions by protein blocking with isolated and purified flagellar extract. We also observed that O-linked glycans facilitate adhesion through chemical modification of an *in vitro* mucus layer. Finally, by comparing multiple strains of *C. difficile* and mucins derived from human and animal origin, we observed the ability of mucin structural and chemical variation to impact *C. difficile* attachment with possible insights into host tropism.

Category: IV. Poster Presentation (#2, presented Friday night)

seNOS-mediated regulation of Hmp toxicity enhances fitness of *Staphylococcus epidermidis*.

Ryan M. Singh (Doctoral)*, Sujata S. Chaudhari, Elizabeth H. Hutfless, Cortney E. Heim, Dhananjay Shinde, Abdulelah A. Alqarzaee, Sasmita Panda, Margaret Sladek, Vineet Kumar, Matthew C. Zimmerman, Paul D. Fey, Tammy Kielian, and Vinai C. Thomas. University of Nebraska Medical Center, Omaha, Nebraska.

Staphylococcus epidermidis is an opportunistic human pathogen that cannot maintain redox homeostasis in the presence of host-derived nitric oxide (NO). Despite this, *S. epidermidis* possesses a genetically encoded nitric oxide synthase (seNOS) that produces endogenous NO. In this study, we investigated the role of seNOS in *S. epidermidis* and found that it is necessary for countering the toxicity of the flavohemoglobin Hmp. Hmp is required for detoxifying NO and is essential for the pathogenesis of *S. epidermidis* in vivo. However, the heme prosthetic group in Hmp generates superoxide ($O_2^{\bullet-}$) that inhibits *S. epidermidis* growth if *hmp* expression is dysregulated. We found that nitrite derived from seNOS activity triggers the CymR-CysK mediated regulation of *hmp* transcription to limit Hmp toxicity. Our findings reveal a fundamental mechanism by which the NOS-Hmp axis enhances staphylococcal fitness and contributes to the physiology of this opportunistic pathogen.

Category: I. General Microbiology Oral Presentation (9:45 AM, K/S Ballroom)

Altering the Redox Status of *Chlamydia trachomatis* Impacts Its Developmental Cycle Progression

Vandana Singh* and Scot P. Ouellette
Department of Pathology and Microbiology, College of Medicine, University of Nebraska Medical Center, Omaha, NE

Chlamydia trachomatis is an obligate intracellular pathogen. Its unique developmental cycle differentiates between two distinct forms: elementary body (EB) and reticulate body (RB). EBs and RBs differ in size, infectivity, transcriptome, and proteome. Intriguingly, they also differ in redox status: EBs are oxidized and RBs are reduced. We hypothesize that alterations in redox can trigger secondary differentiation. Like other obligate pathogens, *C. trachomatis* encounters reactive oxygen species (ROS). However, what mechanism(s) *Chlamydia* uses to maintain its redox homeostasis is unknown. In this study, we examined the function of alkyl hydroperoxide reductase subunit C (AhpC) in chlamydial biology. AhpC, a well-known member of the peroxiredoxins family, is involved in antioxidant defense. According to our hypothesis, ablation of *ahpC* modulates bacterial redox status and will trigger early secondary differentiation. To test our hypothesis, we generated and confirmed using RT-qPCR a CRISPRi *ahpC* knockdown strain of *C. trachomatis*. Under conditions in which *ahpC* transcripts are reduced, we measured higher levels of EB associated genes at an earlier time post-infection (14 hpi) compared to the control. IFA and IFU assays indicated enhanced sensitivity of *ahpC* knockdown against oxidizing agents. These data suggest *ahpC* knockdown creates an oxidizing environment and may reposition developmental cycle progression.

Category: I. General Microbiology Oral Presentation (8:45 AM, K/S Ballroom)

The Tail-Specific Protease, Ct441, Is Essential for Secondary Differentiation in *Chlamydia trachomatis*.

Abigail R. Swoboda (Undergraduate)[1*], Nicholas A. Wood[1], Elizabeth Saery[2], Derek J. Fisher[2], and Scot P. Ouellette[1].
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[2]Southern Illinois University Carbondale, Carbondale, Illinois.

The obligate intracellular pathogen, *Chlamydia trachomatis* (Ctr), undergoes a complex developmental cycle where the bacterium differentiates between two functionally and morphologically distinct forms: the elementary body (EB) and the reticulate body (RB). The EB is the smaller, infectious, and non-dividing form that initiates infection of a host cell whereas the RB is the larger and non-infectious form that replicates within a membrane-bound vesicle called an inclusion. The mechanism(s) driving differentiation between these forms is poorly understood. Bulk protein turnover is likely required for differentiation given the differences in the protein repertoires and functions of the EB and RB. We hypothesize that periplasmic protein turnover is critical for the reorganization of an RB to an EB during secondary differentiation. Ct441 is a periplasmic protease ortholog of tail-specific proteases (i.e. Tsp, Prc) and is expressed during secondary differentiation. We investigated the effect of altering Tsp expression on Ctr development. Through the assessment of bacterial morphology and infectious progeny production, we found that overexpression and CRISPRi-mediated knockdown of Tsp negatively impacted chlamydial development. Electron microscopic assessments during knockdown experiments revealed a defect in EB morphology, directly linking Tsp function to secondary differentiation. These data implicate Ct441/Tsp as a critical factor in secondary differentiation.

Category: I. General Microbiology Oral Presentation (9:30 AM, Big12 Ballroom)

Combining virus-like particles and live-attenuated virus to induce broad antiviral immunity against Respiratory Syncytial Virus

Megolhubino Terhüja (Doctoral)* and Antonius G.P. Oomens. Department of Veterinary Pathobiology, College of Veterinary Medicine, Oklahoma State University, Stillwater, OK

Respiratory Syncytial Virus (RSV) is a major cause of acute lower respiratory disease worldwide and a vaccine is not available. We previously developed a unique live single-cycle mucosal RSV vaccine, termed RSV-Mnull, aimed to protect the pediatric population. To focus the immune response induced by RSV-Mnull on the most relevant vaccine targets of major antigens F and G, the prefusion form of F (preF) and the central conserved domain of G (GCR), we designed a novel RSV-based virus-like particle (VLP) displaying only the preF and GCR antigens (termed VLP-preF/GCR). In a novel combined vaccine approach, we tested whether a booster with VLP-preF/GCR could enhance and focus the anti-G and -F immune response of RSV-Mnull primed mice. BALB/c mice were vaccinated intranasally with 0.6 million PFU of RSV-Mnull, and boosted with VLP-preF/GCR. Serum antibodies were examined by ELISA. The results show that intranasal boosting with VLP-preGCR strongly enhanced anti-G and F antibody levels. Moreover, relative to a double RSV-Mnull vaccination, boosting with VLP-preF/GCT should focus the response on preF and GCR, which we are currently testing. These findings suggest that VLPs can be used to enhance live-attenuated vaccines and have potential to induce a local mucosal response without use of adjuvants.

Category: III. Medical Microbiology/ Immunology Oral presentation (9:30 AM, Cottonwood Room)

TA System ParE-mediated Gyrase Inhibition Invokes Toxicity and Increases Mutagenic Frequency Without Impacting Antibacterial Susceptibility

Chih-Han Tu*(Doctoral); Shengfeng Ruan and Christina Bourne
Department of Chemistry and Biochemistry, University of Oklahoma, Norman, OK

DNA gyrase, an essential enzyme in bacterial cells, regulates DNA topology by temporarily rendering double-strand breaks during replication or transcription. Compounds capable of stabilizing gyrase-mediated double-strand breaks are valuable antibacterial therapeutics; however, this breaks can trigger an "SOS" response, which can induce DNA mutations. ParE toxin proteins, which belong to a family of non-secreted type II Toxin-Antitoxin (TA) systems, inhibit DNA gyrase and promote persistence of double-strand DNA breaks.

The goal for this study is to evaluate the extent of toxicity of selected chromosomal ParE toxins in their native bacterial cells, the frequency of mutations after inducing ParE expression, and the impact this has on susceptibility to clinically useful antibiotics. ParE toxins from *Mycobacterium tuberculosis* (Mt) and *Vibrio cholerae* (Vc), significantly increase the mutation frequency and VcParE toxins did not significantly impact the antibiotic susceptibility to clinically useful antibiotics except for the gyrase inhibitor, levofloxacin. Interestingly, only one of the ParE toxins in *Pseudomonas aeruginosa* (Pa) affects cell growth; additionally, both PaParE toxins have essentially no impact on mutation frequency but do alter susceptibility to the antibiotic, meropenem. These results demonstrate that ParE toxins are unlikely to drive resistance to clinically useful antibiotics despite a mechanism of toxicity that is correlated with increased mutation frequencies.

Category: IV. Poster Presentation (#23, presented Saturday)

The Gut Microbiota Modulates the Severity of Experimental Autoimmune Myocarditis.

Xu Shi (Doctoral)*, Paul Velander, Robert Schmaltz, Jeff Price, Amanda Ramer-Tait.
University of Nebraska-Lincoln, Lincoln, Nebraska.

Myocarditis is an inflammatory disease of the heart muscle caused by infectious agents or triggers that induce autoimmune responses toward heart-specific antigens. Although the gut microbiota has been linked to cardiovascular diseases, its role in the pathogenesis of inflammatory myocarditis has not yet been determined. We therefore investigated whether the microbiota modulates disease severity in a mouse model of Experimental Autoimmune Myocarditis (EAM). Germ-free male C3H/HeN mice were conventionalized with one of three distinct mouse microbiomes (M31B, W116 and MC608) prior to the induction of EAM via injection of adjuvanted myosin. After 21 days, heart tissues were evaluated for microscopic inflammatory lesions and cardiomyopathy, and autoimmune responses were assessed by qualifying myosin-specific IFN- and serum IgG levels. Compared to mice carrying the M31B microbiome, mice harboring the MC608 microbiomes exhibited significantly higher inflammatory heart scores characterized by moderate to marked chronic diffuse myocarditis. The presence of the MC608 microbiome also induced a stronger serum anti-myosin autoantibody response and greater production of myosin-specific IFN- from splenocytes compared to the M31B microbiome. Together, these results demonstrate that the gut microbiota influences EAM severity and autoreactive immune responses. The mechanisms underlying this microbiota-based regulation are currently being investigated using 16S rRNA gene sequencing.

Category: V. Flash Talk (10:15 AM, Room 207)

Inc Proteins Facilitate VAMP3 Recruitment to the *Chlamydia trachomatis* Inclusion Membrane.

Ray E. Widner (Doctoral)*, Lindsey A. Knight, and Elizabeth A. Rucks. University of Nebraska Medical Center, Omaha, NE.

Chlamydia trachomatis is a Gram-negative obligate intracellular pathogen that resides within a vacuole called the inclusion. *C. trachomatis* recruits SNARE proteins, which are a class of eukaryotic proteins that mediate membrane fusion events within intracellular trafficking pathways. However, mechanisms of SNARE recruitment to the inclusion membrane are unclear. One SNARE, VAMP3, interacts with five different Incs temporally during chlamydial development. Live cell imaging demonstrates that a subpopulation of VAMP3 stably associates with the inclusion membrane while another subpopulation dynamically traffics on and off. We hypothesize that specific VAMP3-Inc interactions favor dynamic or stable VAMP3 interactions at the inclusion as a mechanism to avoid triggering host cell defense or stress responses. We developed chlamydial strains to inducibly knockdown IncF and IncG, the first Incs with which VAMP3 interacts. Knockdown of *incF* and *incG* does not affect inclusion size and modestly decreases progeny production. Current experiments will determine whether knockdown affects VAMP3 localization at various timepoints post-infection to characterize if VAMP3 localization is dependent upon the initial interaction with these Incs. These studies will elucidate mechanisms of eukaryotic protein recruitment to the inclusion membrane, which is important towards understanding mechanisms of chlamydial pathogenesis.

Category: IV. Poster Presentation (#1, presented Friday night)

Substrate Profile of the *Chlamydia trachomatis* ClpXP Protease Provides Insight into Its Function during Secondary Differentiation

Nicholas A. Wood (Doctoral)*[1], Shiomi Kuwabara[2], Abigail R. Swoboda[1], Amanda M. Blocker[2], Derek J. Fisher[2], and Scot P. Ouellette[1]
[1]University of Nebraska Medical Center, Omaha, Nebraska.
[2]Southern Illinois University Carbondale, Carbondale, Illinois.

The ClpXP protease is a self-compartmentalized, proteolytic system for targeted degradation of substrates through both tag-dependent and tag-independent recognition mechanisms. Our lab previously characterized the role of this system during secondary differentiation, whereby attenuation of tagged substrate degradation prevents initiation of secondary differentiation through transcription-independent mechanisms. Accordingly, we hypothesize that accumulation of untagged ClpXP substrates drive the transition from RB to EB. We identified substrates of the ClpXP complex through affinity purification using catalytically inactive ClpX isoforms that were either sufficient ($ClpX_{E187A}$) or deficient ($ClpX_{R230A/E187A}$) in tag recognition. We leveraged DSSO, a mass spectrometry-labile crosslinker, to maintain complex stability during the initial purification. We then performed SDS-PAGE to enrich targets directly bound to ClpX, extracted protein bands, and utilized mass spectrometry for identification of peptides. We applied stringent cut-offs, filtered by SAINT analysis, to narrow down our list of high-probability targets to ~130 proteins. Ontology analysis of these proteins suggests that ClpX targets a broad array of biological processes, underscoring the central role of ClpXP in chlamydial development and secondary differentiation. We conclude that selective proteolysis of untagged substrates licenses the ClpXP system to play a crucial role during the chlamydial developmental progression.

Category: I. General Microbiology Oral presentation (10:15 AM, K/S Ballroom)

The structural basis for DNA-uptake by *Acinetobacter.*

Yafan Yu (Doctoral)*, and Kurt Piepenbrink.
University of Nebraska-Lincoln, Lincoln, Nebraska.

Natural transformation is one mechanism of horizontal gene transfer (HGT) in bacteria; it occurs through uptake of extracellular DNA (eDNA) by bacterial cells which can then incorporate this novel genetic material into their genomes. Bacteria from the genus *Acinetobacter*, are naturally-competent, which is thought to contribute to the spread of multidrug-resistant (MDR) *Acinetobacter* infections. Natural competence in *Acinetobacter* is dependent upon DNA-uptake mediated by type IV pili (T4P). T4P are extracellular helical polymers composed of thousands of protein subunits called pilins. The mechanism by which *Acinetobacter* T4P binds DNA, including which subunits serve as eDNA-receptors, is unknown. To identify the eDNA-receptors in *Acinetobacter*, we have recombinantly expressed *Acinetobacter* T4P subunits and directly measured their affinity for double-stranded DNA using EMSA (electrophoretic mobility shift assays) as well as measuring T4P function in *Acinetobacter baumannii* transposon mutants of T4P subunits. We found that *fimT* is not required for pilus assembly but promotes bacterial biofilm formation. A DNA-binding homologue of FimT has recently been identified in *Legionella pneumonia*. In addition to experimental testing of DNA binding by Ab FimT, we are currently using computational approaches, including structure prediction (AlphaFold) and docking (HADDOCK), to predict the interface of DNA recognition in *Acinetobacter* T4P.

Category: IV. Poster Presentation (#3, presented Friday night)

Manipulation of PI3K/Akt pathway and downstream host targets by *Pseudomonas aeruginosa* to promote invasion.

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Pseudomonas aeruginosa is an opportunistic pathogen causing chronic lung infections in cystic fibrosis (CF) patients, who have mutations in CF transmembrane conductance regulator (CFTR) gene. *P. aeruginosa* can invade and survive within airway epithelial cells. The activation of PI3K pathway to generate PIP3 and the recruitment of Akt are crucial for *P. aeruginosa* invasion. To determine how *P. aeruginosa* exploits this pathway and what downstream targets are involved, we infected CF bronchial epithelial cell line CuFi-5 and alveolar basal epithelial cell line A549 with mid-log grown PAO1 for 30 mins and 90 mins. Immunoblotting analysis and coomassie blue staining were performed to compare the signaling events in different cell lines. We observed the activation of Akt in CuFi-5 cells but not in A549 cells, as seen via the phosphorylation of Akt at Thr308 and Ser473 which increased in a time-dependent manner upon invasion and intracellular adaptation. We also observed increased phosphorylation of Akt substrates in CuFi-5 cells. Our next goal is to use mass-spectrometry to identify those Akt substrates, with validation by RNAi and kinase inhibitors. This study will help better understand the invasion mechanisms of *P. aeruginosa* and the role of CFTR in *P. aeruginosa* invasion.

Category: IV. Poster Presentation (#29, presented Saturday)