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

March 17-18, 2017

Missouri State University, Springfield, MO





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On behalf of Missouri State University, we would like to welcome you to Springfield, MO for the 2017 Joint Meeting of the Missouri and Missouri Valley Branches of the American Society for Microbiology. We have tried to capture the diverse interests of the members of branches as we created the program. Thank you all for your participation. This is a young scientist-focused meeting and we encourage the students not only to participate in presentations, but also to ask questions. We hope that this conference will serve as a platform to gather like-minded scientists from broad backgrounds with the goal of creating new connections and exchanging ideas.

Paul Schweiger and Chris Lupfer

**Missouri Branch Officers:**

John Steiert, President

Lea Daniel, President-Elect

Gretchen Thornsberry, Secretary-Treasurer

Anna Oller, Councilor

Phillip Lucido, Alternate Councilor

**Missouri Valley Branch Officers:**

President (Open)

Secretary-Treasurer (Open)

Councilor (Open)

Alternate Councilor (Open)

**ASM Branch Meeting – Missouri State University, Plaster Student Union, March 17-18, 2017**  
**Friday, March 17, 2017**

<b>5:00 – 5:15P</b>	Welcome by Drs. Schweiger and Lupfer	PSU Ballroom East
<b>5:15-5:45P</b>	<b>The Human Virome.</b> Kristie Wylie	PSU Ballroom East
<b>5:45-6:15P</b>	<b>Delignification of Plant Biomass by Bacteria for Bioethanol Production.</b> Babu Fathepure	PSU Ballroom East
<b>6:30-7:30P</b>	Dinner (Chicken Marsala and Veg Lasagna)	PSU Ballroom East
<b>7:45-8:45P</b>	<b>Keynote: Vibrio Ecology and Disease Etiology.</b> James Grimes	PSU Ballroom East
<b>9:00P</b>	Adjourn for evening	PSU Ballroom East

**Saturday, March 18, 2017**

<b>8:00-9:45A</b>	Coffee & Muffins/Bagels Poster Session 1 (Undergraduate posters)	PSU Ballroom West
<b>9:45-10:45A</b>	<b>Oral Session 1</b> I/II. General/Environ Microbiology Session 1 III. Medical Micro/Immun Session 1 IV. Undergraduate Session 1	Convener: Paul Schweiger Convener: Chris Lupfer Convener: Frank Champlin
		PSU 308 B/C PSU 315 A/B PSU 317
<b>10:45-11:00A</b>	<b>Break</b>	
<b>11:00A-12:00P</b>	<b>Oral Session 2</b> I/II. General/Environ Microbiology Session 2 III. Medical Micro/Immun Session 2 IV. Undergraduate Session 2	Convener: Paul Schweiger Convener: Chris Lupfer Convener: Frank Champlin
		PSU 308 B/C PSU 315 A/B PSU 317
<b>12:00-2:00P</b>	Lunch (Assorted sandwiches on brioche) Poster Session 2 (Graduate Students and Post-Graduates) Branch Business Meetings	PSU Ballroom West
<b>2:00-3:00P</b>	<b>Keynote: <i>Salmonella</i> in Food Production and Molecular Approaches for Understanding Its Ecology.</b> Steven Ricke	Parliamentary Rm (313)
<b>3:00-3:15</b>	<b>Break</b>	
<b>3:15-3:45P</b>	<b>Role of Thioredoxin in Sulfate and Uranium Reduction in Sulfate-Reducing Bacteria.</b> Erica Majumder	Parliamentary Rm (313)
<b>3:45-4:15P</b>	<b>Pairing multimedia with primary literature for active learning.</b> Dave Westenberg	Parliamentary Rm (313)
<b>4:15-4:45P</b>	Awards/Closing remarks	Parliamentary Rm (313)
<b>5:00P</b>	Adjourn	Parliamentary Rm (313)

## ASM Distinguished Lecturers

James Grimes, PhD



### **Vibrio Ecology and Disease Etiology**

The University of Southern Mississippi, Gulf Coast Research Laboratory

Bacteria belonging to the genus *Vibrio* and related genera are major carbon cycle drivers in marine and estuarine environments. As is the case for most carbon cycle participants, they metabolize easily degradable compounds such as carbohydrates and proteins; few degrade recalcitrant compounds such as hydrocarbons and lignins. Even fewer are pathogenic for animals, including humans. This presentation will cover *Vibrio* ecology and it will address the few *Vibrios* that are pathogenic for humans.

**Biographical Sketch:** D. Jay Grimes is Professor of Coastal Sciences at The University of Southern Mississippi (USM). From 2002 to 2007 he served as Provost and Vice President for Academic Affairs at USM and from 1997 to 2007 he was Director of the USM Gulf Coast Research Laboratory. Previously, Grimes served on the faculties of the University of Wisconsin-La Crosse (1971 to 1980), University of Maryland (1980 to 1987), and University of New Hampshire (1987 to 1990); he was also director of the New Hampshire Sea Grant College Program. In 1990, Grimes was selected for federal service as a microbiologist and program manager at the U.S. Department of Energy. Grimes is a fellow in the American Academy of Microbiology and in the American Association for the Advancement of Science. He chaired the American Society for Microbiology's Communications Committee for nine years and he chaired ASM's Environmental Microbiology Committee from 2012 to 2015. He is past-president of the U.S. Federation of Culture Collections, served as vice chair of the Consortium for Oceanographic Research and Education, was chair of the NASULGC Board on Oceans and Atmosphere, and served on the Science Advisory Panel to the U.S. Commission on Ocean Policy. Much of his research has focused on the ecology of waterborne human diseases, especially the *Vibrios*. Recently, Grimes investigated the applicability of satellite remotely sensed data to predict human health risks from waterborne pathogens, especially *Vibrio parahaemolyticus*. He is also examining antibiotic resistance in bacteria isolated from water, sediment, fish and bottlenose dolphins in Mississippi Sound. Grimes received his B.A. and M.A. in Biology from Drake University (1966 and 1968) and his Ph.D. in Microbiology from Colorado State University (1971).



## ***Salmonella* in Food Production and Molecular Approaches for Understanding Its Ecology**

Dept. of Food Science and Center for Food Safety, University of Arkansas

Salmonellosis is one of the most common foodborne diseases and is one of the more costly foodborne diseases in the United States. Given that foodborne *Salmonella* spp. can originate from a wide variety of food production environments, reduction of this organism at all stages of food production is critical. This talk explores the prevalence of *Salmonella* species in food production systems and how they survive the various environmental pressures it encounters. In particular, specific gene expression responses of *Salmonella* spp. under typical food production and processing conditions may be critical. This requires a variety of molecular approaches to identify specific genes and in some cases functions as well. Finally, more integrated approaches for controlling *Salmonella* spp. given the foodborne pathogen's ability to persist in food production environments may need to be developed.

**Biographical Sketch:** Dr. Steven Ricke's expertise is primarily focused on pathogenic characteristics of foodborne *Salmonella*. He received his B.S. and M.S. from the University of Illinois and his Ph.D. from the University of Wisconsin, and was a USDA-ARS postdoctoral fellow in Microbiology at North Carolina State University. He was a professor at Texas A&M University until 2005, when he became the first holder of the new Donald "Buddy" Wray Endowed Chair in Food Safety at the University of Arkansas (UA) and Director of the Center for Food Safety. He has received numerous awards, including the Poultry Science Association Research Award, American Egg Board Award, UA Food Science Department Outstanding Research Award, and UA Division of Agriculture John White Outstanding Research Award, and was also named a Texas Agricultural Experiment Station Faculty Fellow. He has been a member of the National Academies Standing Committee on Use of Public Health Data in FSIS Food Safety Programs and the United Egg Producers Food Safety Scientific Advisory Council, and is former President of the Arkansas Association of Food Protection, as well as its co-founder. Dr. Ricke has served as an editor for five books and as Editor-in-Chief of two scientific journals. He has co-authored 377 peer refereed research and review articles as well as 46 book chapters, and he has given 126 talks to local, national and international audiences.

## Local invited speakers

### Kristie Wylie, PhD



#### **The Human Virome.**

Washington University School of Medicine, St. Louis, Missouri.

The human virome, defined as the viruses in and on the human body, is an important yet understudied component of the human microbiome. High-throughput nucleic acid sequencing provides a powerful, culture-independent approach for evaluating the virome. We used this method to study the virome in the healthy subjects enrolled in the Human Microbiome Project. We found that healthy adults carry many eukaryotic viruses asymptotically, including herpesviruses and a wide range of divergent papillomaviruses, many of which were carried stably over time. This work led us to recognize that sensitivity was a limitation of virome analysis with high-throughput sequencing. To improve sensitivity, we developed ViroCap, a targeted sequence capture panel, which enriches viral nucleic acid from the complete genomes of comprehensive set of vertebrate viruses prior to sequencing (337 viral species from 190 viral genera and 34 viral families). ViroCap consistently and dramatically increases the percentage of viral sequences, up to 11,000-fold over metagenomic sequencing. It improves overall detection, and it increases the breadth- and depth-of-coverage of the genomes. This technological advance improves our ability to use high-throughput sequencing to study the viruses and viral genomes, and it has been applied to numerous research studies, including analysis of viral outbreaks.

#### **Biographical Sketch:**

### Babu Fathepure, PhD



#### **Delignification of Plant Biomass by Bacteria for Bioethanol Production.**

Oklahoma State University, Stillwater, OK.

The plant cell wall is composed of three structural biopolymers: cellulose, hemicelluloses, and lignin. Of these, lignin perhaps requires the most challenging chemistry for its degradation as it is a highly cross-linked aromatic polymer containing a wide variety of C-C and C-O bonding motifs forming a major obstacle for cost-effectively releasing fermentable sugars for biofuel production. To date much of the work is focused on fungal degradation of lignin and little efforts have been to explore the role of bacteria. We are interested in isolating novel bacteria that degrade lignin and identify genes and enzymes involved in lignin degradation using genomic and proteomic tools. We have enriched a bacterial consortium that degrades lignin from a decaying biomass and isolated several lignin degrading bacteria. Among the isolates, *Rhizobium* sp. strain YS-r and *Pseudomonas* sp. strain YS-p were shown to degrade not only lignin monomers and dimers but also plant lignin in switchgrass and alfalfa. The genomes of these isolates revealed the presence of a variety of lignin degrading genes and cellulose and hemi-cellulose degrading genes. Lately we are exploring the synergistic role of bacteria and fungi in the degradation of plant lignin and production of extracellular lignin-active enzymes.

## Erica Majumder, PhD



### **Role of Thioredoxin in Sulfate and Uranium Reduction in Sulfate-Reducing Bacteria.**

University of Missouri, Columbia, Missouri.

Thioredoxins are small proteins that facilitate reduction of other proteins and small molecules in cells with a dithiol/disulfide exchange mechanism. Thioredoxins are thought to be ubiquitous in nature and have established roles in dealing with oxidative stress and redox signaling. While well characterized in plants and animals, the role(s) of thioredoxins in anaerobes and particularly Sulfate-Reducing Bacteria (SRB) have not been well established. The sulfate reducing bacterium *Desulfovibrio vulgaris* Hildenborough (DvH) is an obligate anaerobe that has the ability to reduce uranium (VI) to uranium (IV). DvH has two thioredoxins and four thioredoxin reductases annotated in the genome and that are expressed *in vivo*. Recent work by Li and Krumholz (Ecotoxicology 2014) has implicated thioredoxin as necessary for the uranium reduction process. In this study we use genetic and biochemical techniques to elucidate the possible roles of thioredoxins in sulfate reduction metabolism and heavy metal metabolism in the SRB DvH.

## David Westenberg, PhD



### **Pairing multimedia with primary literature for active learning**

Missouri University of Science and Technology, Rolla, Missouri

Over the last few decades several species of bumble bees have been declining in the US. A group at the University of Wisconsin-Madison led by Dr. Shawn Steffan discovered that the food source for bumble bee larvae is rich in yeast. Based on this observation, he proposed that the use of fungicides could affect the health of bumble bee colonies. This session will highlight HHMI's "Scientist at Work" video of Steffan's experiment to test this hypothesis by comparing the sizes of bumble bee colonies that forage on flowering plants grown in the presence or absence of fungicides. Coupling the media with relevant primary literature encourages critical thinking, quantitative reasoning, interdisciplinary nature of science and understanding of the relationship between science and society. This activity will encourage students to make observations, read and analyze primary literature and recognize the importance of potential unforeseen consequences of fungicide use.

## Oral Session 1

### I/II. General/Environmental Microbiology (Session 1)

Time	Presentation	Room
9:45-10:00	<b>Linear Avian Beta-Defensin-12 Analogue-3 with Therapeutic Potential Properties.</b> Ming Yang	PSU 308 B/C
10:00-10:15	<b>Endosome to Golgi trafficking requires optimal levels of lipids.</b> Sara Woodman	PSU 308 B/C
10:15-10:30	<b>The role of Gammaproteobacteria in aerobic alkane degradation in oilfield production water from the Barnett Shale.</b> Meredith Thornton	PSU 308 B/C
10:30-10:45	<b>Full-length Vps1 Targets Golgi and Endosomes, Not Endocytic Sites.</b> John Short	PSU 308 B/C

### III. Medical Microbiology / Immunology (Session 1)

Time	Presentation	Room
9:45-10:00	<b>Glutathione: An Essential Mechanism for the Virulence and Vaginal Colonization of Group B Streptococcus in a Murine Model.</b> Elizabeth Walker	PSU 315 A/B
10:00-10:15	<b>The Non-Oxidative Branch of the Pentose Phosphate Pathway Contributes to Resistance Against Oxidative and Nitrosative Stresses in <i>Salmonella enterica</i>.</b> Jeff A. Shaw	PSU 315 A/B
10:15-10:30	<b><i>Chlamydia trachomatis</i> Manipulation of Protein Kinase C.</b> Prakash Sah	PSU 315 A/B
10:30-10:45	<b>Yeast Dynamin and Ypt6 function in parallel pathways during protein recycling from the early endosome to the late Golgi.</b> Pelin Makaraci	PSU 315 A/B

### IV. Undergraduate (Session 1)

Time	Presentation	Room
9:45-10:00	<b>Reducing Hexavalent Chromium to Trivalent Chromium via Halophilic Bacteria.</b> Madison Thomas	PSU 317
10:00-10:15	<b>Antibiotic Resistance of <i>Pseudomonas aeruginosa</i> Recovered From Cystic Fibrosis Patients.</b> William Starr	PSU 317
10:15-10:30	<b>The Golgi Organizer, Vps1, Functions Together with the Tethering GARP.</b> Jared Smothers	PSU 317
10:30-10:45	<b>Improving Inflammation in Bacterial Coinfection by IL-1<math>\beta</math> Regulation.</b> Angeline Rodriguez	PSU 317

## Oral Session 2

### I/II. General/Environmental Microbiology (Session 2)

Time	Presentation	Room
11:00-11:15	<b>Use of Steel Foil in a Semi-Continuous System for Testing Microbially Influenced Corrosion.</b> Mary Eid	PSU 308 B/C
11:15-11:30	<b>Yeast Dynamin Vps1 association with Clathrin: An important interaction for Golgi Homeostasis.</b> Mariel Delgado Cruz	PSU 308 B/C
11:30-11:45	<b>Surface Display for Metabolic Engineering of Industrially Important Acetic Acid Bacteria.</b> Marshal Blank	PSU 308 B/C
11:45-12:00	<b>Analysis of the Impact of Mercury Chloride on the Gut Microbiota of Prairie Voles (<i>Microtus ochrogaster</i>).</b> Kathleen Ahles	PSU 308 B/C

### III. Medical Microbiology / Immunology (Session 2)

Time	Presentation	Room
11:00-11:15	<b>A <math>\beta</math> Propeller Protein, CarP, Plays Role in <i>Pseudomonas aeruginosa</i> Response to Calcium.</b> Michelle King	PSU 315 A/B
11:15-11:30	<b>A Calmodulin-like Calcium Binding Protein, EfhP, Plays Role in Virulence of <i>Pseudomonas aeruginosa</i>.</b> Biraj B. Kayastha	PSU 315 A/B
11:30-11:45	<b><i>Staphylococcus aureus</i> Internalization into Mammalian Host Cells.</b> Rawan G. Eleshly	PSU 315 A/B
11:45-12:00	<b>The <i>bb0168</i>-Encoded DnaK Suppressor Protein is a Stringent Response Associated Protein Required for Infectivity in <i>Borrelia burgdorferi</i>.</b> The <i>bb0168</i> -Encoded DnaK Suppressor Protein is a Stringent Response Associated Protein Required for Infectivity in <i>Borrelia burgdorferi</i> . William K. Boyle	PSU 315 A/B

### IV. Undergraduate (Session 2)

Time	Presentation	Room
11:00-11:15	<b>Expression of the Hypothetical Membrane Protein CBU_1651 from <i>Coxiella burnetii</i>.</b> Keegan McGill	PSU 317
11:15-11:30	<b>Intron Degeneration in the Lichen Fungi <i>Teloschistes chrysophthalmus</i>.</b> Dawson Johnson	PSU 317
11:30-11:45	<b>Investigating the Effects of Nanomaterials in Yeast.</b> Chelsea Campbell	PSU 317
11:45-12:00	<b>Susceptibility of <i>Serratia marcescens</i> to Triclosan after Sensitization using Outer Membrane Permeabilizer Compound 48/80.</b> Kavya Boyina	PSU 317

## Poster Assignments

To improve viewing time and allow people to see posters within their own time slot, please plan to stand by your poster for 45 minutes. Please hang all posters in the morning and leave up for both poster sessions. Posters will be placed on a 4x6 ft board. Please hang your poster on the corresponding poster board listed below.

### Poster session 1 (Undergraduate)

Poster Board	Poster Title and Presenter
1-1	<b>Generation of Yeast 2-Hybrid Clones to Examine the Role of Nucleotide Oligomerization and Binding Domain (NOD)-Like Receptors.</b> Abbi Mabary
1-2	<b>Persister Formation in <i>Staphylococcus epidermidis</i> Clinical Isolates.</b> Amber Menard
1-3	<b>Deciphering the Mechanism of Action of the Antimicrobial Peptide DASamP2 by Characterization of Two Resistant Mutants.</b> Christopher Johnson
1-4	<b>Chestnut Hybrids Recruit Different and Distinct Fungal Communities from Shared Nursery Soil.</b> Christopher Reazin
1-5	<b>Effects of Inhibitory Dyes on the Isolation of <i>Myxobacteria</i>.</b> Clayton T. Matthews
1-6	<b>Initial Assessments of Antibiotic Resistance Genes in River Otter Colon Bacteria.</b> Larissa Porter
1-7	<b>UV Adaptation in Halophiles.</b> Haley Green
1-8	<b>Effects of Merkel Cell Polyomavirus Large T Antigen on UV Sensitivity.</b> Jazmine Snow
1-9	<b><i>Staphylococcus aureus</i> Survives Antimicrobial Peptides through Energy Depletion.</b> Kaitlyn Oppliger
1-10	<b>Mitochondrial Dysfunction Associated with Plasmid-Induced Senescence Impacts Nuclear Genome Stability.</b> Natasha Nazir and Kala Chinnaswamy

### Poster session 2 (Graduate Student and Post-Graduate\*)

Poster Board	Poster Title and Presenter
2-1	<b>Sodium Pyruvate Alters the Immune Response to Influenza A Virus Infection in Macrophages.</b> Hazar Abu Salamah
2-2	<b>Quorum Sensing Systems are Maintained by Interspecies Competition.</b> Kara C Hinshaw
2-3	<b>Characterization of a Genus-Specific Unidentified Open Reading Frame Found within the Mitochondrial Genome of <i>Fusarium</i>.</b> Michael MacKillop
2-4	<b>Fine Epitope Mapping of Monoclonal Antibodies to the DNA Repair Protein, RadA.</b> Stephanie N. Nachtrab
2-5	<b>Bacterial Diversity of an Abandoned Mine Land Soil in Southeast Kansas.</b> Rachel Bechtold
2-6	<b>Prevalence and Ribotype Diversity of Toxigenic <i>Clostridium difficile</i> in Community and Healthcare Systems.</b> Anuradha Ghosh*
2-7	<b>Regulation of Protein Synthesis in <i>Trichomonas vaginalis</i> by Tetracycline.</b> Mia E. Hammers*

## Meeting Participants

First Name	Last Name	University	Email
Abysalamah	Hazar	Missouri State University	<a href="mailto:Hazzar1411@live.missouristate.edu">Hazzar1411@live.missouristate.edu</a>
Ahles	Kathleen	Oklahoma State University Center for Health Sciences	<a href="mailto:kathleen.ahles@okstate.edu">kathleen.ahles@okstate.edu</a>
Bechtold	Rachel	Pittsburg State University	<a href="mailto:rbechtold@gus.pittstate.edu">rbechtold@gus.pittstate.edu</a>
Bishr	Mahmoud	Metropolitan Community College-Penn Valle	<a href="mailto:Mahmoud.Bishr@mcckc.edu">Mahmoud.Bishr@mcckc.edu</a>
Blank	Marshal	Missouri State University	<a href="mailto:Marshal444@live.missouristate.edu">Marshal444@live.missouristate.edu</a>
Bourette	Travis	Creighton	<a href="mailto:TravisBourret@creighton.edu">TravisBourret@creighton.edu</a>
Boyina	Kavya	Oklahoma State University	<a href="mailto:boyina.kavya97@gmail.com">boyina.kavya97@gmail.com</a>
Boyle	William	Creighton	<a href="mailto:WilliamBoyle@creighton.edu">WilliamBoyle@creighton.edu</a>
Campbell	Chelsea	Missouri State University	<a href="mailto:Campbell162@live.missouristate.edu">Campbell162@live.missouristate.edu</a>
Champlin	Franklin	Oklahoma State University Center for Health Sciences	<a href="mailto:frankrc@okstate.edu">frankrc@okstate.edu</a>
Deip	Daniel	Northeastern State University	<a href="mailto:cdeip428@gmail.com">cdeip428@gmail.com</a>
Cruz	Mariel Delgado	Missouri State University	<a href="mailto:Cruz321@live.missouristate.edu">Cruz321@live.missouristate.edu</a>
Doele	Ratrar	Northeastern State University	<a href="mailto:Doele@nsuok.edu">Doele@nsuok.edu</a>
Duncan	Kathleen	University of Oklahoma	<a href="mailto:Kathleen.e.duncan-1@ou.edu">Kathleen.e.duncan-1@ou.edu</a>
Eid	Mary	University of Oklahoma	<a href="mailto:mary.eid@ou.edu">mary.eid@ou.edu</a>
Eleshy	Rawan	Oklahoma State University	<a href="mailto:rawan.eleshy@okstate.edu">rawan.eleshy@okstate.edu</a>
Fathepure	Babu	Oklahoma State University	<a href="mailto:babu.fathepure@okstate.edu">babu.fathepure@okstate.edu</a>
Fredrickson	Samantha	Missouri State University	<a href="mailto:Fredrickson221@live.missouristate.edu">Fredrickson221@live.missouristate.edu</a>
Fuller	Cynthia	Henderson State University	<a href="mailto:fullerc@hsu.edu">fullerc@hsu.edu</a>
Ghosh	Anuradha	Pittsburg State University	<a href="mailto:aghosh@pittstate.edu">aghosh@pittstate.edu</a>
Green	Haley	Northeastern State University	<a href="mailto:greenh@nsuok.edu">greenh@nsuok.edu</a>
Grimes	Jay	The University of Southern Mississippi	<a href="mailto:jay.grimes@usm.edu">jay.grimes@usm.edu</a>
Hammers	Mia	A.T. Still University of Health Sciences	<a href="mailto:mhammers@atsu.edu">mhammers@atsu.edu</a>
Harms	Nathan	University of Nebraska - Kearney	<a href="mailto:harmsne@lopers.unk.edu">harmsne@lopers.unk.edu</a>
Hinshaw	Kara	University of Kansas	<a href="mailto:k699h433@ku.edu">k699h433@ku.edu</a>
Johnson	Dawson	University of Nebraska - Kearney	<a href="mailto:johnsondl6@lopers.unk.edu">johnsondl6@lopers.unk.edu</a>
Johnson	Christopher	University of Nebraska - Omaha	<a href="mailto:cpjohnson@unomaha.edu">cpjohnson@unomaha.edu</a>
Kayastha	Biraj	Oklahoma State University	<a href="mailto:biraj.kayastha@okstate.edu">biraj.kayastha@okstate.edu</a>
Kim	Kyoungtae	Missouri State University	<a href="mailto:Kkim@missouristate.edu">Kkim@missouristate.edu</a>
King	Michelle	Oklahoma State University	<a href="mailto:mmking@okstate.edu">mmking@okstate.edu</a>
Lucido	Phillip	Northwest Missouri State University	<a href="mailto:lucido4405@gmail.com">lucido4405@gmail.com</a>
Luedtke	Brandon	University of Nebraska - Kearney	<a href="mailto:luedtkebe@unk.edu">luedtkebe@unk.edu</a>
Lupfer	Christopher	Missouri State University	<a href="mailto:ChristopherLupfer@missouristate.edu">ChristopherLupfer@missouristate.edu</a>
Lutter	Erika	Oklahoma State University	<a href="mailto:erika.lutter@okstate.edu">erika.lutter@okstate.edu</a>
Mabary	Abbigale	Missouri State University	<a href="mailto:ajm200@live.missouristate.edu">ajm200@live.missouristate.edu</a>
MacKillop	Mike	Saint Louis University	<a href="mailto:mackillopmc@slu.edu">mackillopmc@slu.edu</a>
Majumder	Erica	University of Missouri	<a href="mailto:majumdere@missouri.edu">majumdere@missouri.edu</a>
Makaraci	Pelin	Missouri State University	<a href="mailto:Makaraci292@live.missouristate.edu">Makaraci292@live.missouristate.edu</a>
Matthews	Clayton	University of Oklahoma	<a href="mailto:clayton.t.matthews-1@ou.edu">clayton.t.matthews-1@ou.edu</a>
McGill	Keegan	University of Nebraska - Kearney	<a href="mailto:mcgillkt@lopers.unk.edu">mcgillkt@lopers.unk.edu</a>
Menard	Amber	University of Nebraska - Kearney	<a href="mailto:nuxollas@unk.edu">nuxollas@unk.edu</a>
Nachtrab	Stephanie	A.T. Still University of Health Sciences	<a href="mailto:snachtrab@ATSU.edu">snachtrab@ATSU.edu</a>
Nazir	Natasha	Saint Louis University	<a href="mailto:nazirn@slu.edu">nazirn@slu.edu</a>
Nuxoll	Austin	University of Nebraska-Kearney	<a href="mailto:nuxollas@unk.edu">nuxollas@unk.edu</a>
Oppliger	Kaitlyn	University of Nebraska-Kearney	<a href="mailto:oppligerka@lopers.unk.edu">oppligerka@lopers.unk.edu</a>
Paul	John	Missouri State University	<a href="mailto:jp52518@gmail.com">jp52518@gmail.com</a>
Pilkenton	Michael	Missouri State University	<a href="mailto:Pilkenton1@live.missouristate.edu">Pilkenton1@live.missouristate.edu</a>
Porter	Larissa	Henderson State University	<a href="mailto:LP178906@reddies.hsu.edu">LP178906@reddies.hsu.edu</a>
Reazin	Christopher	Kansas State University	<a href="mailto:creaz93@ksu.edu">creaz93@ksu.edu</a>
Ricke	Steven	University of Arkansas	<a href="mailto:sricke@uark.edu">sricke@uark.edu</a>
Rippee	Meagan	Missouri State University	<a href="mailto:rippee417@live.missouristate.edu">rippee417@live.missouristate.edu</a>

Rodriguez	Angeline	Missouri State University	<a href="mailto:Rodriguez281@live.missouristate.edu">Rodriguez281@live.missouristate.edu</a>
Rowen	Donald	University of Nebraska - Omaha	<a href="mailto:drowen@unomaha.edu">drowen@unomaha.edu</a>
Sah	Prakash	Oklahoma State University	<a href="mailto:psah@ostatemail.okstate.edu">psah@ostatemail.okstate.edu</a>
Sallee	Todd	Pantheon	<a href="mailto:todd.sallee@patheon.com">todd.sallee@patheon.com</a>
Schweiger	Paul	Missouri State University	<a href="mailto:pschweiger@missouristate.edu">pschweiger@missouristate.edu</a>
Shaffer	Julie	University of Nebraska - Kearney	<a href="mailto:shafferjj@unk.edu">shafferjj@unk.edu</a>
Shaw	Jeff	Creighton	<a href="mailto:JeffShaw@creighton.edu">JeffShaw@creighton.edu</a>
Short	John	Missouri State University	<a href="mailto:Short7814@live.missouristate.edu">Short7814@live.missouristate.edu</a>
Smothers	Jared	Missouri State University	<a href="mailto:Smothers88@live.missouristate.edu">Smothers88@live.missouristate.edu</a>
Snow	Jazmine	Kansas State University	<a href="mailto:jazasn timer@KSU.edu">jazasn timer@KSU.edu</a>
Starr	William	Oklahoma State University	<a href="mailto:starrwc@ostatemail.okstate.edu">starrwc@ostatemail.okstate.edu</a>
Steiert	Jack	Missouri State University	<a href="mailto:Johnsteiert@missouristate.edu">Johnsteiert@missouristate.edu</a>
Tanner	Ralph	University of Oklahoma	<a href="mailto:rtanner@ou.edu">rtanner@ou.edu</a>
Thomas	Madison	Northeastern State University	<a href="mailto:thoma122@nsuok.edu">thoma122@nsuok.edu</a>
Thornton	Meredith	University of Oklahoma	<a href="mailto:mthornton217@ou.edu">mthornton217@ou.edu</a>
Walker	Elizabeth	St. Louis University	<a href="mailto:keyea@slu.edu">keyea@slu.edu</a>
Wells	Dick	Ozarks Technical Community College	<a href="mailto:wellsr@otc.edu">wellsr@otc.edu</a>
Westenberg	David	Missouri University of Science and Technology	<a href="mailto:djwesten@mst.edu">djwesten@mst.edu</a>
Woodman	Sara	Missouri State University	<a href="mailto:woodman1@live.missouristate.edu">woodman1@live.missouristate.edu</a>
Wylie	Kristine	Washington University in St. Louis	<a href="mailto:kwylie@wustl.edu">kwylie@wustl.edu</a>
Yang	Ming	University of Missouri	<a href="mailto:mywb9@mail.missouri.edu">mywb9@mail.missouri.edu</a>

## Directions



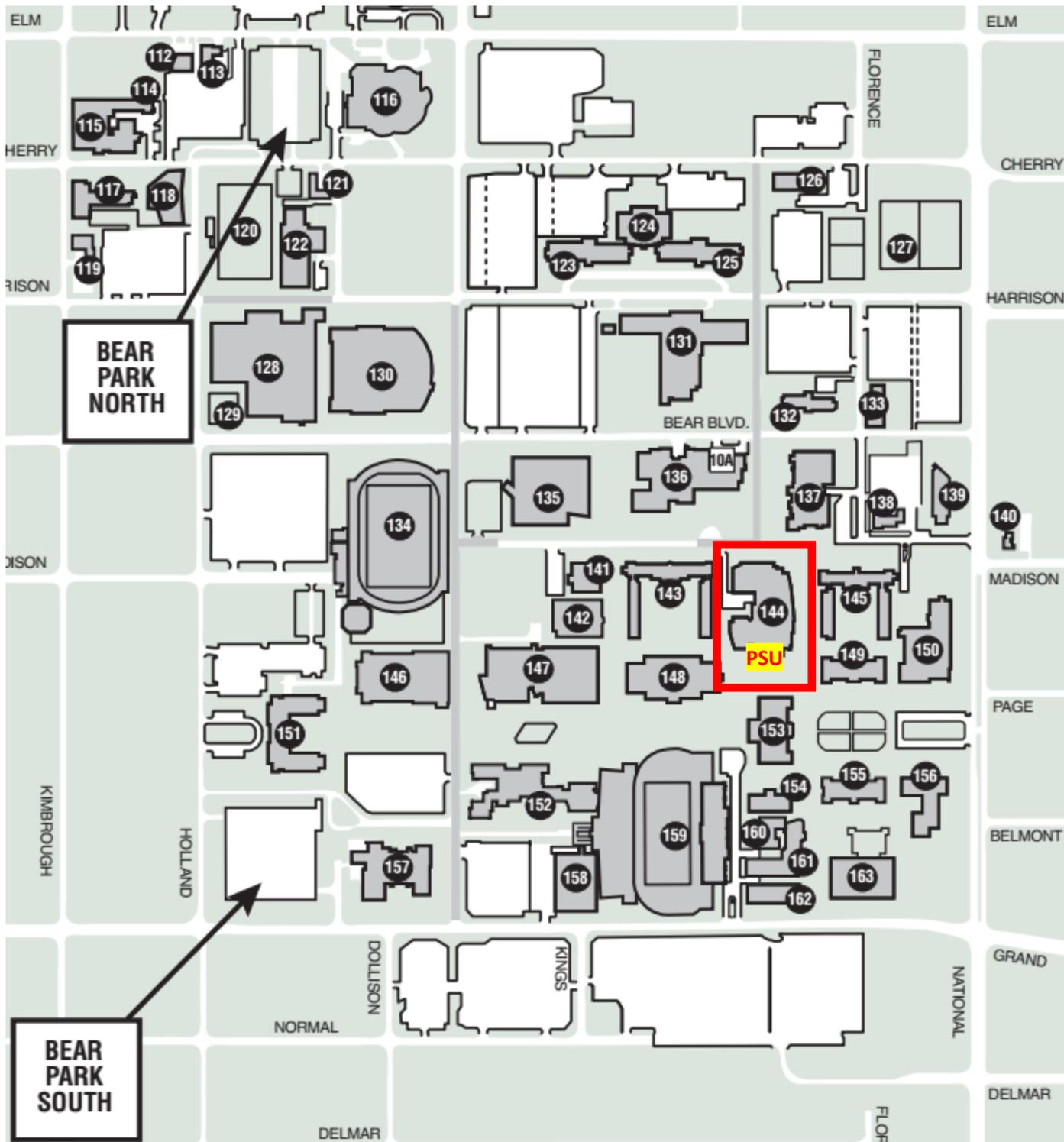
The meeting will take place at the Plaster Student Union (PSU, pictured above) located on the campus of Missouri State University.

[Click Here For Parking Map](#). Note that the map does not label the Plaster Student Union (PSU). However, it is located where Chick-fil-A, Starbucks, and Subway appear.

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## **Student Oral Presentation Abstracts (Session 1)**

### ***I/II. General/Environmental Microbiology Graduate Student Oral Presentation (session 1)***

#### **Linear Avian Beta-Defensin-12 Analogue-3 with Therapeutic Potential Properties**

Ming Yang (Doctoral)\*, Chunye Zhang, Michael Z. Zhang, and Shuping Zhang.

College of Veterinary Medicine, University of Missouri, Columbia, Missouri.

Function-structure study of avian  $\beta$ -defensin-12 (AvBD-12) indicated that it had broad-spectrum antimicrobial, neutralizing LPS and chemotactic properties. The chemotactic activity was to both avian immune cells and mouse immature dendritic JAWSII cells, a unique feature indicating its potential application as chemotherapeutic agents in both avian and mammalian hosts. To simplify the structure, the three conserved disulfide bridges were eliminated by replacing cysteines with alanine and serine residues. The peptide charge was increased by changing negatively charged amino acid residues to positively charged residues. AvBD-12A3, with a net positive charge of +9, hydrophobicity of 40% and a predicted CCR2-binding domain, showed 8 to 16-fold decrease in minimal inhibition concentration (MIC) at low nutrient condition against *E. coli*, *S. Typhimurium*, and *P. aeruginosa* and 2-fold decrease in MIC against *S. aureus*, and retained more than 50% of wild-type chemotactic activity to JAWSII cells, compared to AvBD-12. In addition, AvBD-12A3 remained approximately 80% of killing potency against *E. coli* and *P. aeruginosa* in 150 mM NaCl condition. The results suggests that linear analogue with the high net positive charge and CCR2-binding domain can increase antimicrobial activity, keep the wild-type chemotactic activity, and conquer most of salt-sensitivity. In conclusion, analogue AvBD-12A3 can serve as a template for the design of novel antimicrobial peptides with chemotactic property and salt resistance.

#### **Endosome to Golgi trafficking requires optimal levels of lipids.**

Sara E. Woodman (Master's)\*, Justin Conover, Chris Trousdale, and Kyoungtae Kim.

Missouri State University, Springfield, Missouri.

Protein recycling is an essential cellular process that involves endocytosis, retrograde trafficking, and exocytosis. In mammalian systems, membrane lipids, including cholesterol, sphingolipids, and phospholipids, play a pivotal role in protein recycling. To address this role in budding yeast, *Saccharomyces cerevisiae*, we utilized GFP-Snc1, a v-SNARE protein serving as a fluorescent marker for faithfully reporting the recycling pathway. We observed moderate to significant GFP-Snc1 recycling defects upon overexpression or depletion of phospholipids, ergosterol, and sphingolipids, indicating that the homeostasis of membrane lipid levels is prerequisite for proper protein recycling. Membrane lipid imbalance also resulted in an accumulation of the late endosome marker Vps10-GFP, indicating retrograde trafficking from endosomes to the Golgi may be defective. To elucidate the possible cause for the defect, we stained the actin cytoskeleton, then quantified the percentage of cells with visible actin cables. Compared to wild-type cells, membrane lipid mutant cells exhibited lower levels of actin cables, indicating the actin cytoskeleton is disrupted upon membrane lipid imbalance. Taken together, these results show that impairment of proper recycling may be due to disruption of the actin cytoskeleton, which causes trafficking defects between endosomes and Golgi. The potential mechanisms of actin cytoskeleton disruption are currently being studied.

## **The role of Gammaproteobacteria in aerobic alkane degradation in oilfield production water from the Barnett Shale.**

Meredith M Thornton (Master's)\* & Kathleen E. Duncan.

University of Oklahoma, Norman, Oklahoma

Petroleum hydrocarbon components, e.g. *n*-alkanes and BTEX, can be partially degraded aerobically and feed anaerobic microbes present in oilfield production water. Biogenic sulfide production by anaerobic sulfate-reducing bacteria leads to biocorrosion of petroleum production machinery and can result in the accidental release of petroleum pollutants. We hypothesized that Gammaproteobacteria are responsible for aerobic degradation of *n*-alkanes stimulating downstream biocorrosion. Sequence analysis of the 16S rRNA ribosomal gene sequence libraries showed both aerobic and anaerobic genera in the original tank water. Cultures were set up under aerobic conditions for growth on *n*-alkanes, fatty acids, BTEX, and oxidized BTEX compounds. Enriched cultures degraded *n*-alkanes and fatty acids but not BTEX or oxidized BTEX compounds. The 16S libraries following enrichment with *n*-alkanes showed dominantly Gammaproteobacteria with sequence identities most similar to *Halomonas*, *Marinobacter*, *Shewanella* and to *Roseovarius* (Epsilonproteobacteria). Six strains of *Halomonas* were isolated from the enrichments. These results suggest Gammaproteobacteria may initiate the cascade of hydrocarbon degradation and contribute to downstream biocorrosion by supplying sulfate-reducing bacteria with partially oxidized metabolites as a carbon source. Characterizing aerobic *n*-alkane degrading organisms provides insight into the beginning steps of hydrocarbon degradation and downstream biocorrosion promoting the optimization of mitigation strategies related to petroleum pollution.

## **Full-length Vps1 Targets Golgi and Endosomes, Not Endocytic Sites.**

John Short (Master's)\*, Kyoungtae Kim.

Missouri State University, Springfield, Missouri.

Intracellular protein sorting requires dynamin-like proteins; who's dysfunctions in humans are linked to Alzheimer's disease. Yeast Vps1 defects are likewise associated with dysregulation of multiple pathways of vesicle trafficking. Vps1 has been shown *in vitro* to interact with membrane lipids of Golgi bodies and endosomes, but had not been tested *in vivo*. The biochemical mechanisms of Vps1 targeting to organelle membranes also remain poorly understood. To investigate, truncated Vps1 domain fragments: GTPase, Middle, GED, a presumed PRD, GTPase+Mid, Mid+GED, and full-length Vps1 were N-terminally tagged with mRFP and expressed in *vps1Δ* background cells. Biomarkers for endocytic sites, late Golgi, and late endosomes were tagged with GFP. Colocalization of GFP-fused markers with mRFP-fused Vps1 and its fragments was quantified using fluorescence microscopy. Contrary to widely accepted models of Vps1 as an endocytic factor, we found that mRFP-Vps1 did not colocalize with endocytic sites. Only full-length mRFP-Vps1 colocalized with endosomes and Golgi as expected, while no domain fragments recovered the normal phenotype. Furthermore, the truncated Vps1 fragments were not functional for CPY sorting at the Golgi or the recycling of the CPY receptor Vps10 toward the Golgi. These suggest that full-length Vps1 protein is essential for its proper targeting and function.

### **III. Medical Microbiology / Immunology Graduate Student Oral Presentation (session 1)**

#### **Glutathione: An Essential Mechanism for the Virulence and Vaginal Colonization of Group B Streptococcus in a Murine Model.**

Elizabeth A. Walker (Doctoral)\*, Rachel M. Treat, Bayleigh A. White, and Blythe E. Janowiak.

Saint Louis University, Department of Biology, St. Louis, Missouri.

Group B Streptococcus (GBS) is a common commensal bacterium of the human vaginal microbiome with 25% of women being naturally colonized. In individuals with healthy immune systems GBS does no harm. However, GBS can be transferred to infants during vaginal birth where it can cause meningitis, making GBS a leading cause of infant mortality in the U.S. Currently, GBS-positive mothers are treated with broad spectrum antibiotics to avoid the transfer of GBS to their infants, but with the rise of antibiotic resistant bacteria, new methods of treatment are necessary. We hypothesize that glutathione, an antioxidant produced in large quantities by GBS, may be that viable drug target. Using a murine model of sepsis and vaginal colonization, we have shown that GBS which lack glutathione synthesis are unable to cause a septic infection or continuously colonize the vaginal microbiome. These results suggest that glutathione synthesis is indeed a viable drug target.

#### **The Non-Oxidative Branch of the Pentose Phosphate Pathway Contributes to Resistance Against Oxidative and Nitrosative Stresses in *Salmonella enterica*.**

Jeff A. Shaw (Doctoral)\* and Travis J. Bourret.

Creighton University School of Medicine, Omaha, Nebraska.

*Salmonella enterica* serovar Typhimurium is a facultative intracellular pathogen that is a major cause of food-borne illness worldwide. During infection of its host, *S. Typhimurium* encounters numerous host defenses, including reactive oxygen species (ROS) and reactive nitrogen species (RNS). One way *S. Typhimurium* defends itself against ROS and RNS is by maintaining cellular reducing power through the oxidative branch of the pentose phosphate pathway (oxPPP). Downstream of the oxPPP, intermediate products from the non-oxidative branch (non-oxPPP) can be channeled to glycolysis or utilized for biosynthetic reactions. Despite its importance for cell physiology, a role for the non-oxPPP in defense against ROS and RNS has yet to be described. This study investigated the contribution of transketolases of the non-oxPPP to ROS/RNS resistance. The *S. Typhimurium* genome encodes three transketolases (TktA, TktB, and STM2340-41), each of which produced detectable enzymatic activity. Mutant strains lacking all three transketolases showed increased sensitivity to ROS and RNS *in vitro*. However, the contribution of each transketolase differed between oxidative and nitrosative stresses. This study reveals an essential role for the non-oxPPP in resisting ROS and RNS, and suggests that it may contribute to the intracellular survival of *S. Typhimurium*.

## ***Chlamydia trachomatis* Manipulation of Protein Kinase C**

Prakash Sah<sup>1</sup> (Doctoral)\*, Ted Hackstadt<sup>2</sup>, Erika Lutter<sup>1</sup>

<sup>1</sup>Department of Microbiology and Molecular Genetics, Oklahoma State University, Stillwater, OK, USA;

<sup>2</sup>Laboratory of Intracellular Parasites, NIAID, NIH Rocky Mountain Laboratories, Hamilton, USA

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

## **Yeast Dynamin and Ypt6 function in parallel pathways during protein recycling from the early endosome to the late Golgi.**

Pelin Makaraci (Master's)\*, Kyoungtae Kim, Aria H. McDermot.

Missouri State University, Springfield, Missouri.

Protein recycling is an important cellular process required for cell homeostasis. Lines of evidence demonstrated that Vps1, a dynamin homologue in yeast, is implicated in protein recycling from the endosome-to-Golgi, however, the function of Vps1 in this pathway remains elusive. The present study reveals that Vps1 genetically and physically interacts with Ypt6, a master GTPase in the recycling pathway. GTPase inactive (Ypt6 G139E and Ypt6 T24N) and a constitutively active mutant (Ypt6 Q69L) of Ypt6 interact with Vps1, indicating that the interaction of Vps1 with Ypt6 does not depend on the status of the Ypt6 activity. Cells lacking Ypt6 displayed a severe defect in Snc1 recycling, and the abnormal phenotype was rescued by overexpression of Vps1, and vice versa. Therefore, it is most likely that Vps1 functions in a parallel pathway with Ypt6 during protein recycling. Additionally, overexpressing GTPase point mutants of Vps1 was not sufficient enough to rescue the abnormal phenotype in *ypt6Δ* cells, suggesting an essential role of GTP binding and hydrolysis for Vps1 function in Snc1 retrieval toward the Golgi. Additionally, the studies showed that Vps1 interacts with Vti1 and Snc2 SNAREs, essential for endosome-derived vesicle fusion with the late Golgi, pointing to a novel role of Vps1 in the late stage of the endosome-to-Golgi traffic.

#### **IV. Undergraduate or High School Oral Presentation (session 1)**

##### **Reducing Hexavalent Chromium to Trivalent Chromium via Halophilic Bacteria**

Nayeli Moreno-Lopez, Madison Thomas (Undergraduate)\*, Ratnakar Deole

Northeastern State University, Department of Natural Sciences, Broken Arrow, Oklahoma

Hexavalent chromium is a recognized human carcinogen that has been found in drinking water. Local water sources can become contaminated from natural chromium deposits or industrial pollution. In July 2011, a Public Health Goal was set to 0.2 ppb of hexavalent chromium in drinking water. EPA is currently reviewing its recommended limits. The current methods to convert hexavalent chromium to the safe form, trivalent chromium involve chemical techniques that are moderately effective, cause further pollution, and are expensive. A more environmentally friendly and cost effective way to convert it is bioremediation using the enzyme chromium reductase, which can be found in some halophilic bacteria. Chromium reductase has the potential to reduce the toxicity of hexavalent chromium to trivalent chromium. The overall goal for this study is to isolate, identify, and characterize halophilic bacteria that possess this enzyme. The first objective of this research was to isolate halophilic bacteria that was able to grow in the presence of potassium chromate, a hexavalent chromium compound, in different concentrations. Currently we are conducting analysis of an isolated bacteria's enzyme activity to investigate its ability to reduce chromium.

##### **Antibiotic Resistance of *Pseudomonas aeruginosa* Recovered From Cystic Fibrosis Patients**

William Starr (Undergraduate)\*, Rawan Eleshly, Nighat Mehdi, and Erika Lutter

<sup>1</sup>Department of Microbiology and Molecular Genetics, Oklahoma State University, Stillwater, OK

<sup>2</sup>Oklahoma Cystic Fibrosis Center, University of Oklahoma Health Sciences Center, Oklahoma City, OK

Cystic Fibrosis (CF) patients produce dehydrated thick mucus in their lungs and lack the ability to clear this mucus due to mutations in the cystic fibrosis transmembrane conductance regulator gene (CFTR gene). Once the infection has been acquired, eradication of *P. aeruginosa* from the CF lung is rare. This study aims to determine resistance profiles of *P. aeruginosa* clinical isolates. Kirby-Bauer tests were performed on 52 isolates using nine different antibiotics which represent multiple antibiotic classes. In addition, DNA was extracted from the CF isolates and PCR was performed to verify the presence of eight prominent antibiotic resistance genes. The results showed that all of the isolates had resistance to at least one of the nine antibiotics; however, not all of isolates showed the presence antibiotic resistance genes by PCR. Results indicated that higher dosing of antibiotics is needed for CF patients due to infections being able to survive the immune system, smaller antibiotic treatments, and swapping of genetic material between bacterial species. By understanding antibiotic resistance of *P. aeruginosa* from CF patients in regards to the mechanisms in which this resistance is acquired, treatment options for CF patients can be more specialized.

## **The Golgi Organizer, Vps1, Functions Together with the Tethering GARP**

Jared Smothers (Undergraduate)\*, Uma Saimani, and Kyoungtae Kim

Missouri State University, Biology Department, Springfield Missouri

Retrograde traffic from the endosome toward the *trans*-Golgi Network (TGN) requires the sorting of cargo at the endosome, and the generation of cargo-laden transport carriers. Transport carriers are trafficked via the cytoskeletal tracks for subsequent tethering and fusion at the TGN. Vacuolar Protein Sorting 1 (Vps1) is a yeast dynamin-like protein, which has been implicated in the retrograde recycling pathway. In yeast, the GARP tethering complex is responsible for anchoring vesicles at the late Golgi membrane. Previous research found genetic interaction between Vps1 and all components of the GARP tethering complex, leading us to hypothesize that Vps1 functions together with the GARP complex for endosome-to-the-TGN trafficking. Using a yeast-2-hybrid system we identified a 33 amino acid region near the C-terminal of Vps51 (a subunit of the GARP complex) that interacts with Vps1. Our sequence homology analysis revealed two conserved residues, E127 and Y129, in the 33 amino acid segment of yeast Vps51. Vps51 mutants harboring these point mutations did not interact with Vps1, and cells expressing these point mutations displayed severe defects in the transport of Snc1 from the endosome to the TGN. This suggests a physiological relevance of the binding of Vps1 with the conserved amino acid residues in Vps51 for the traffic. Our functional analysis revealed Vps1 acts upstream of Vps51. In addition, we found that Vps1 is required for proper targeting of Vps51 and its binding partner Tlg1 to the late Golgi. These findings suggest that Vps1 may play an essential role in the organization of a local protein network, including tethering and fusion machinery required for the later stage of the endosome to Golgi traffic.

## **Improving Inflammation in Bacterial Coinfection by IL-1 $\beta$ Regulation.**

Angeline Rodriguez (Undergraduate)\*, Andrea Taylor, Hazar Abusalamah, Abbi Mabary, Hanna Ingram and Christopher Lupfer.

Missouri State University, Biology Department, Springfield Missouri

Viral bacterial coinfections are known to cause severe pneumonia, especially in the elderly and in pediatric patients. Antibiotics like  $\beta$ -Lactams kill the bacteria but fail to improve symptoms suggesting a faulty immune system may play an important role in the disease. Interleukin-1 $\beta$  (IL-1 $\beta$ ) is an important immune signaling cytokine responsible for inflammation. It exists as an inactive precursor that can be activated by Caspase-1 containing inflammasomes (multi-protein complex). Influenza A virus and *Streptococcus pneumoniae* activate the inflammasome through the NOD-like receptor protein NLRP3. Previous reports indicate that IL-1 $\beta$  levels are dramatically elevated during coinfection with Influenza A virus and *Streptococcus pneumoniae*. However, how IL-1 $\beta$  levels increase and their importance in coinfection is not known. We have discovered that IL-1 $\beta$  expression and secretion is increased during coinfection as a result of activation of multiple signaling pathways simultaneously. This was concluded in experiments where macrophages or transgenic mice deficient in the *Myd88*<sup>-/-</sup>, *Aim2*<sup>-/-</sup> or *Nlrp3*<sup>-/-</sup> genes were examined for their effects on IL-1 $\beta$  augmentation. In addition, we are also examining treatment options using a combination of drugs including IL-1 $\beta$  inhibitors.

## **Student Oral Presentation Abstracts (Session 2)**

### ***I/II. General/Environmental Microbiology Graduate Student Oral Presentation (session 2)***

#### **Use of Steel Foil in a Semi-Continuous System for Testing Microbially Influenced Corrosion.**

Mary M. Eid (Master's)\* and Ralph S. Tanner.

University of Oklahoma, Norman, Oklahoma

Biocorrosion (Microbially Influenced Corrosion) affects many industries across the world. The cost and prevention of corrosion is estimated at \$276 billion per year in the United States. Current systems for testing biocorrosion are either dynamic or static. Dynamic systems are materials intensive, expensive and require specialized equipment and training, while static systems require long incubations to obtain significant results. The purpose of this study is to create a technique for detecting and analyzing biocorrosion that would address all the aforementioned caveats. It is hypothesized that stainless steel foil provides a suitable support for biofilm/biocorrosion studies in a semi-continuous system. A biocorrosion test cell was developed using low carbon steel foil in a semi-continuous system. This system maximizes test surface area and surface area to weight ratio in the cell compared to other methods. Additionally, regularly scheduled medium replacement leads to shorter incubation times. This biocorrosion test system was successful, just requiring a three week minimum incubation to yield significant data compared to the control values. It has also been used to successfully document and determine the efficacy of corrosion control agents.

#### **Yeast Dynamin Vps1 association with Clathrin: An important interaction for Golgi Homeostasis.**

Mariel Delgado Cruz (Master's)\*, Shiva Kumar Goud Gadilia, Uma Saimani, John C.W. Short, Hyoeun McDermott, Kyougtae Kim.

Missouri State University, Springfield, Missouri.

Vps1 in yeast has been implicated in a variety of cellular functions ranging from endocytosis to protein sorting. Our research seeks to further characterize Vps1 function by studying its implication at the Golgi. Colocalization studies indicated that Vps1 colocalized with Clathrin at the Trans Golgi Network. Furthermore, yeast two-hybrid experimentation provides evidence that full-length Vps1 and its truncated versions bind to the C-terminal region of the Clathrin Heavy Chain 1 (Chc1). Further reinforcing the idea that Vps1 and Chc1 work together, Carboxypeptidase Y (CPY) assays indicated that cells lacking both Vps1 and Chc1 displayed more severe (CPY) sorting defects at the Golgi compared to strains lacking only Vps1. Additionally, these Vps1 fragments became dominant-negative for CPY sorting upon overexpression. These results suggest that Vps1 binds to Chc1 in order for efficient Golgi-to-endosome membrane trafficking to occur. In addition, we found that Vps1, without the aid of clathrin, is an important regulator in late Golgi turnover.

## **Surface Display for Metabolic Engineering of Industrially Important Acetic Acid Bacteria**

Marshal A. Blank (Master's)\* and Paul Schweiger.

Missouri State University, Biology Department, Springfield, Missouri.

Acetic acid bacteria (AAB) have the unique ability to partially oxidize sugars, alcohols, and polyols in the periplasm as part of their normal metabolism. The resulting products are often enantiopure and are excreted fully into the medium, thus these microorganisms are suited for a variety of applications, such as the production of pharmaceuticals, food additives, consumer products, and industrial compounds. Unfortunately, few molecular tools exist for metabolic engineering of AAB. To this end, a surface display system was developed to express recombinant enzymes at the cell surface of the industrially important bacterium, *Gluconobacter oxydans*. As proof of concept, outer membrane protein OprF was fused to bacterial alkaline phosphatase PhoA, and activity of the reporter enzyme was quantified in whole-cell assays. Furthermore, a linker library was generated to optimize this original surface display system for AAB. Indeed, modification of the OprF-PhoA fusion protein with variable linker sequences had significant effects on biocatalysis. Surface display could be used both to extend the capabilities of acetic acid bacteria in current biotechnological processes, and to broaden the potential of these microbes in the production of value-added products.

## **Analysis of the Impact of Mercury Chloride on the Gut Microbiota of Prairie Voles (*Microtus ochrogaster*).**

Kathleen Ahles (Doctoral)\*, Senait Assefa, J. Tom Curtis, Gerwald Koehler.

Oklahoma State University Center for Health Sciences, Tulsa, Oklahoma.

The gastrointestinal tract is home to a dynamic mixture of bacteria, fungi, and viruses collectively known as the gut microbiota. The composition of the gut microbiota is dictated by a variety of host-specific factors including sex, age, and diet, but exposure to exogenous substances such as antibiotics and heavy metals have also been shown to impact its community structure. The purpose of the present study was to examine the impact of mercury chloride on the gut microbiota of male and female prairie voles (*Microtus ochrogaster*). Voles were exposed to *ad libitum* mercury chloride (60 ppm) for a period of 10 weeks, at which time fecal samples were collected and processed for 16S rRNA gene sequencing. QIIME analysis revealed significant changes in the alpha diversity of the gut microbial population in female voles following mercury exposure, but this trend failed to be significant in their male counterparts. These data highlight the importance of host gender in modulating the impact of heavy metal exposure on the gut microbial community.

### **III. Medical Microbiology / Immunology Graduate Student Oral Presentation (session 2)**

#### **A $\beta$ Propeller Protein, CarP, Plays Role in *Pseudomonas aeruginosa* Response to Calcium**

Michelle King (Doctoral)\*, Mariette Barbier, and Marianna A. Patrauchan.

Oklahoma State University, Stillwater, OK

*Pseudomonas aeruginosa* is an opportunistic pathogen that causes severe acute and chronic infections in humans, particularly, in cystic fibrosis (CF) patients. Our group has shown that calcium ( $\text{Ca}^{2+}$ ) induces virulence and antibiotic resistance in *P. aeruginosa*. Earlier we identified a  $\text{Ca}^{2+}$ -regulated protein, CarP, which was predicted to form a 5 bladed  $\beta$ -propeller structure with a putative  $\text{Ca}^{2+}$  binding site in the center of the propeller. We characterized its role in several  $\text{Ca}^{2+}$ -regulated production of virulence factors and cell tolerance to high  $\text{Ca}^{2+}$ . To further characterize the role of CarP in  $\text{Ca}^{2+}$ -regulated virulence and adaptation to host, we aim to identify the environmental nutritional cues and the host factors that control the expression of *carP*. Furthermore, we investigated the role of CarP in virulence by using *Galleria mellonella* and mouse virulence models. Disruption of *carP* reduced worm killing by 60% and decreased survival of *P. aeruginosa* in mice by 30%. We also determined that deletion of *carP* abolished  $\text{Ca}^{2+}$ -induced production of pyoverdine, iron chelator, required for the pathogen's virulence. Further studies aim to characterize  $\text{Ca}^{2+}$ -binding capabilities of CarP and advance our knowledge on the molecular mechanisms of  $\text{Ca}^{2+}$  regulation of *P. aeruginosa* virulence and fitness in response to host environment.

#### **A Calmodulin-like Calcium Binding Protein, EfhP, Plays Role in Virulence of *Pseudomonas aeruginosa*.**

Biraj B. Kayastha<sup>1</sup> (Doctoral)\*, Rendi Rogers<sup>1</sup>, Mariette Barbier<sup>2</sup> and Marianna Patrauchan<sup>1</sup>

<sup>1</sup>Oklahoma State University Stillwater, OK

<sup>2</sup>West Virginia University School of Medicine

*Pseudomonas aeruginosa* is an opportunistic pathogen causing severe chronic infections in cystic fibrosis patients. Earlier, we have shown that its virulence is induced by  $\text{Ca}^{2+}$ . We also reported a putative  $\text{Ca}^{2+}$ -binding protein, EfhP, containing two predicted EF-hand motifs. We showed that EfhP mediates  $\text{Ca}^{2+}$  regulation of the pathogen's infectivity and virulence factor production. Here, by using wax worm and murine macrophage infection model, we show that EfhP contributes to the pathogen's virulence and intracellular survival. To confirm the ability of EfhP to bind  $\text{Ca}^{2+}$ , we His-tag purified the soluble portion of the protein, and after removing the tag with TEV protease, subjected to Dynamic Light Scattering (DLS) and Isothermal Titration calorimetry (ITC). DLS indicated that EfhP forms dimers and tetramers. ITC confirmed that EfhP binds  $\text{Ca}^{2+}$  but not  $\text{Mg}^{2+}$ . Currently, we aim to solve the multimeric state of the protein and calculate the  $K_d$  of  $\text{Ca}^{2+}$  binding. Further we aim to identify the residues involved in  $\text{Ca}^{2+}$  binding, by generating point mutations within the EF hands, and measuring  $\text{Ca}^{2+}$ -binding. Future studies will aim to detect whether EfhP undergoes conformational changes upon  $\text{Ca}^{2+}$ -binding, and identify its protein partners. Once confirmed, EfhP will be the first structurally characterized  $\text{Ca}^{2+}$  sensor in prokaryotes.

## ***Staphylococcus aureus* Internalization into Mammalian Host Cells.**

Rawan G. Eleshly (Doctoral)\*, and Erika I. Lutter.

Oklahoma State University, Stillwater, Oklahoma.

*Staphylococcus aureus* is associated with chronic lung infections that are difficult to treat with antibiotics such as cystic fibrosis (CF). *S. aureus* is one of the very first pathogens to colonize and infect CF patients and it remains prevalent throughout the lives of these patients. Internalization into host cells is a strategy that *S. aureus* uses to survive in infected patients. In this study, we hypothesize that *S. aureus* isolated from CF patients can be internalized into epithelial lung cells *in vitro* as a strategy to promote survival. To test this hypothesis, we examined the ability of CF *S. aureus* isolates to be internalized into human epithelial lung cells (A-549) in tissue culture. Using differential staining and fluorescent microscopy, we were able to show that *S. aureus* has the ability to enter mammalian cells. Next, we aim to investigate what eukaryotic cellular components are required for *S. aureus* to gain entry into these cells. Understanding this adaptation strategy is important for current treatment failures as well as future therapeutic options.

## **The *bb0168*-Encoded DnaK Suppressor Protein is a Stringent Response Associated Protein Required for Infectivity in *Borrelia burgdorferi*.**

William K. Boyle (doctoral)<sup>1\*</sup>, Jeff A. Shaw<sup>1</sup>, Ashley Groshong<sup>2</sup>, Jon S. Blevins<sup>3</sup>, Frank C. Gherardini<sup>4</sup> and Travis J. Bourret<sup>1</sup>.

<sup>1</sup>Creighton University School of Medicine, Omaha, Nebraska

<sup>2</sup>UConn Health, Farmington, Connecticut

<sup>3</sup>University of Arkansas for Medical Sciences, Little Rock, Arkansas

<sup>4</sup>National Institute of Allergy and Infectious Diseases, Hamilton, Montana

*Borrelia burgdorferi*, the causative agent of Lyme disease, requires the stringent response for survival in *Ixodes* spp. ticks. In this study, we set out to characterize the role of the *bb0168*-encoded DnaK suppressor protein (DksA) on *B. burgdorferi* gene expression and infectivity. Wild-type and *dksA*-deficient *B. burgdorferi* strains were subjected to nutrient limitation by shifting cultures grown under microaerobic conditions (5% CO<sub>2</sub>, 3% O<sub>2</sub>), in BSKII medium to RPMI medium lacking serum. Microarray analysis comparing the transcriptomes of a wild-type *B. burgdorferi* strain and a *dksA*-deficient mutant revealed that DksA impacts the transcriptional response under nutrient limitation, including the expression of plasmid encoded genes. Moreover, the induction of plasmid encoded infectivity genes by shifting cultures from pH 7.6 to pH 6.8, and the subsequent immunoblotting revealed the inability of the *dksA*-deficient strain to express OspC and DbpA at wild-type levels. The reduced expression of virulence-associated genes corresponds to the inability of the *dksA*-deficient *B. burgdorferi* strain to infect Swiss Webster mice following subcutaneous injection with inoculums of 10<sup>3</sup> or 10<sup>5</sup> spirochetes/mouse. Collectively, the results indicate that DksA is a global regulator of gene expression in *B. burgdorferi*, and is required for infectivity in a murine model of infection.

#### **IV. Undergraduate or High School Oral Presentation (session 2)**

##### **Expression of the Hypothetical Membrane Protein CBU\_1651 from *Coxiella burnetii*.**

Keegan McGill (Undergraduate)\* Brandon Luedtke,

University of Nebraska at Kearney, Department of Biology, Kearney, Nebraska

*Coxiella burnetii* is an obligate intracellular pathogen and etiological agent of query fever. To cause disease, *C. burnetii* uses a Type IVB secretion system (T4BSS) to create a parasitophorous vacuole and release effector proteins to control the host cell functions. A gene unique to *C. burnetii* is CBU\_1651, which is predicted to encode a membrane protein that might interact with the T4BSS since the gene is located between the T4BSS genes *icmW* and *icmX*. The overall goal of this study is to characterize the localization of CBU\_1651 during axenic growth and in an infectious setting. To determine localization, the development of antibodies against CBU\_1651 is essential and is the current goal of this project. To raise antibodies, recombinant CBU\_1651 was expressed with an N-terminal green fluorescent protein (GFP) fusion using the pEXP1 expression vector in BL 21 AI *Escherichia coli*. After the expression of CBU\_1651 was confirmed by SDS-PAGE, it was purified using a poly-histidine tag and Ni<sup>2+</sup> metal affinity column. After purification, the GFP was be cleaved from CBU\_1651 and sent for mass spectrometry analysis prior to the commercial development of primary antibodies.

##### **Intron Degeneration in the Lichen Fungi *Teloschistes chrysophthalmus*.**

Dawson Johnson (Undergraduate)\*, Derek Kleier and Dawn M. Simon.

University of Nebraska at Kearney, Kearney, NE.

Introns are ubiquitous in eukaryotes, yet they likely initially arose as purely selfish elements. We are interested in understanding this process. In particular, we are focused on spliceosomal introns that are found in nuclear ribosomal RNA (nrRNA). These introns are largely restricted to lichen-forming fungi, suggesting a recent origin which makes their evolutionary history much more tractable than most spliceosomal introns. We specifically hypothesize that nrRNA spliceosomal introns arise from degeneration of group I ribozymes, which are also common in nrRNA genes in lichen-forming fungi. Here we focus on one position in the small subunit (SSU) in the lichen-forming fungi *Teloschistes chrysophthalmus*. This position has introns of varying lengths, all of which contain sequences typical of spliceosomal introns and many also have potential secondary structures typical of group I ribozymes. The overall objective of the study is to discover additional introns that represent intermediate steps in the transition from group I ribozymes to spliceosomal introns. Here, we use an expanded set of introns, collected from samples across much of the North American geographic range of *T. chrysophthalmus* to provide evidence of degeneration both in secondary structure and *in vivo* splicing.

## Investigating the Effects of Nanomaterials in Yeast

Chelsea Campbell (Undergraduate)\*, Julie Curless, Daniel Kim, and Kyoungtae Kim.

Missouri State University, Biology Department, Springfield, Missouri.

Nanoparticles are commercially used in everyday products including zinc sunscreen and water resistant fabrics and surfaces, but in the future they may be used in targeted treatment of cancer, printable monitoring systems, and foldable phones. The Environmental Protection Agency in 2005 stated an interest in assessing the risks associated with nanotechnologies, but as of 2016 they had made no progress towards gathering data. During this time the commercial use of nanoparticles increased fivefold. Understanding the effects of nanoparticles is crucial for the responsible use of these technologies. The aim of this project is to investigate the effect of nanomaterials on budding yeast (*Saccharomyces cerevisiae*) using growth assays, FUN-1 staining for metabolic activity and RNA sequencing. Almost every gene in the yeast genome has a human homolog, making yeast a great model for understanding the potential effects of nanoparticles on human health. In this study we specifically look at silver (Ag) nanoparticles. When exposed to silver, growth curves suggest an inhibitory effect of the treatment in concentrations above 5ug/ml. In addition, FUN-1 staining results indicate little metabolic effect from the same concentrations of exposure. RNA sequence analysis is currently in progress. We are also investigating the effects of Cadmium (CdSe/ZnS) nanomaterials.

## Susceptibility of *Serratia marcescens* to Triclosan after Sensitization using Outer Membrane Permeabilizer Compound 48/80.

Kavya Boyina (Associates)<sup>a,\*</sup>, Jennifer Yang<sup>a</sup>, Allison A. McDonald<sup>b</sup>, and Franklin R. Champlin<sup>b</sup>.

Oklahoma State University College of Agricultural Sciences and Natural Resources <sup>a</sup>,  
Stillwater, OK and Center for Health Sciences <sup>b</sup>, Tulsa, OK.

Triclosan is a hydrophobic antibacterial biocide commonly used in health care settings to which the nosocomial opportunists *Pseudomonas aeruginosa* and *Serratia marcescens* are intrinsically resistant. We have hypothesized that the impermeability properties of the *S. marcescens* outer membrane for hydrophobic substances underlies its intrinsic resistance to triclosan. A model system of disparate *S. marcescens* strains was constructed. Disk agar diffusion and batch culture triclosan titration bioassays were conducted to determine triclosan susceptibility levels. An outer membrane permeabilization assay was employed using compound 48/80 in efforts to sensitize the refractory organism to triclosan. Uptake of hydrophobic fluorescent probe 1-N-phenyl naphthylamine (NPN) was assessed for confirmation. Antibigrams and titrations revealed test organisms to be generally resistant to hydrophobic substances, including triclosan. Compound 48/80 synergistically sensitized all *S. marcescens* strains to low triclosan levels to different degrees, all less than seen for *P. aeruginosa*. NPN data revealed only one *S. marcescens* strain to be made accessible to NPN by outer membrane permeabilization, in a manner consistent with one control *P. aeruginosa* strains. Data support that as with *P. aeruginosa* intrinsic resistance to triclosan in *S. marcescens* is due at least in part to the exclusionary properties of its outer membrane for hydrophobic substances.

## Undergraduate or High School Poster Presentation

### **1-1. Generation of Yeast 2-Hybrid Clones to Examine the Role of Nucleotide Oligomerization and Binding Domain (NOD)-Like Receptors**

Abbi Mabary (Undergraduate)\*, Angeline Rodriguez, Hazar Abysalamah and Christopher Lupfer PhD.

Missouri State University, Biology Department, Springfield, Missouri.

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

### **1-2. Persister Formation in *Staphylococcus epidermidis* Clinical Isolates.**

Amber Menard (Undergraduate)\*, Seoyoung Song, Sierra Kline, and Austin Nuxoll.

Department of Biology, University of Nebraska at Kearney, Kearney, NE

*Staphylococcus epidermidis* is a commensal organism, normally found on the skin of mammals. As an opportunistic pathogen, *S. epidermidis* causes disease in immunocompromised individuals, mediated through indwelling medical devices. Antibiotic treatment of these infections is often unsuccessful, leading to chronic, relapsing infections with poor patient prognosis. A likely explanation for these observations is the presence of persister cells (a subpopulation of dormant cells). High persister isolates have been shown to occur in other microbial pathogens such as *Pseudomonas aeruginosa* and *Candida albicans*. Recent work in the related pathogen, *S. aureus*, demonstrates persister formation is dependent on energy depletion through the TCA cycle. Therefore, we examined whether high persister isolates occurred among *S. epidermidis* clinical isolates through an energy dependent mechanism. We found *S. epidermidis* clinical isolates frequently have a high persister phenotype when challenged with vancomycin. To determine if this phenotype occurred through an energy dependent mechanism, we measured ATP concentrations in these isolates. Indeed, the majority of isolates with a high persister phenotype also showed lower ATP concentrations, suggestive of a TCA cycle dependent mechanism. Antibiotic treatment frequently fails, even among antibiotic susceptible pathogens - these preliminary results indicate persister cells are an important component in this process.

### **1-3. Deciphering the Mechanism of Action of the Antimicrobial Peptide DASamP2 by Characterization of Two Resistant Mutants.**

Christopher Johnson (Undergraduate)\*, Swapna Medichetti, Aniket Sawant, Adrienne Werner and Donald Rowen.

Department of Biology, University of Nebraska, Omaha, NE

Due to the proliferation of drug-resistant strains of pathogenic bacteria, there is an urgent need for alternatives to traditional antibiotics. Antimicrobial peptides (AMPs) are a promising alternative to traditional small molecule therapies. DASamP2, an AMP developed by the Wang lab at the University of Nebraska Medical Center, is effective against *Pseudomonas aeruginosa* and *Staphylococcus aureus*, but the mechanism of action is not currently understood. In order to identify genetic changes that affect susceptibility to this AMP, our lab has previously created eleven mutant strains of *P. aeruginosa* which demonstrated increased resistance to DASamP2 using transposon mutagenesis. We think that the genetic basis for the resistant phenotype of two of these mutants has now been identified, using inverse PCR and DNA sequencing. One of these mutants, resistant to greater than 8x the MIC of DASamP2, appears to have a mutation in PA1588, which encodes a 3-hydroxyacyl-CoA dehydrogenase. Disruption of *mexF*, a gene encoding a protein which serves as the inner-membrane portion of a resistance-nodulation-cell division (RND) multidrug efflux transporter complex, appears to be responsible for producing resistance to 3x the MIC in a second mutant.

### **1-4. Chestnut Hybrids Recruit Different and Distinct Fungal Communities from Shared Nursery Soil.**

Christopher Reazin<sup>1</sup> (Undergraduate)\*, Ari Jumpponen<sup>1</sup>, Richard Baird<sup>2</sup>.

<sup>1</sup>Kansas State University, <sup>2</sup>Mississippi State University

In the early twentieth century, the pathogenic fungus, *Cryphonectria parasitica*, responsible for chestnut blight was introduced from Asia to North America and subsequently to Europe. Chestnut blight devastated the American chestnut tree (*Castanea dentata*) throughout all of its historical range in North America. Since then, breeding programs that hybridize the blight-susceptible American chestnut with blight-resistant Chinese chestnuts have been employed to develop blight resistant chestnut lines. In this study, we compared how chestnut seedlings differ in the composition of their rhizobiome fungal communities across two American and one Chinese parental lines as well as six distinct hybrid lines. To dissect the fungal rhizobiomes, we extracted the total genomic DNA from root and soil samples, PCR-amplified the fungal DNA barcode, the highly variable Internal Transcribed Spacer region 2 (ITS2) of the ribosomal RNA gene, and sequenced the ITS2 amplicons on the Illumina MiSeq platform. Our data show that the hybrids have compositionally distinct fungal rhizobiomes when compared to their American and Chinese parents and many hybrids also differ in their rhizobiome fungal communities. Our data suggest that breeding American and Chinese chestnuts for fungal pathogen resistance may lead to unintended consequences on the recruitment of fungal symbionts from shared soil inoculum.

### **1-5. Effects of Inhibitory Dyes on the Isolation of *Myxobacteria*.**

Clayton T. Matthews (Undergraduate)\*,

University of Oklahoma, Norman, Oklahoma.

*Myxobacteria* are a group of Gram-negative soil bacteria that are nearly universal in the environment and known to produce a diversity of secondary metabolites such as antimicrobial compounds. The *Myxobacteria* that have been cultivated represent only a fraction of this varied group. This study's objective is to improve the cultivation and isolation of *Myxobacteria* by inhibiting the growth of other bacteria utilizing Crystal Violet, Brilliant Green, and Acriflavine dyes. It is hypothesized that the inhibitor dyes will impede other bacteria, while *Myxobacteria* remain unaffected. The concentration of these dyes in media was optimized for their ability to inhibit cultivable bacteria but not *Myxobacteria*. The project's methodology involved discovering dye concentrations, testing soil samples, developing on media cultures using the dyes, and observing a difference in the amount of colonies with and without the dyes. None of the three dyes inhibited the growth of six representative *Myxobacteria* at concentrations greater than 1 mg/L, 1 mg/L, and 0.1 mg/L, respectively. Testing resulted in the three-way combination of these dyes inhibiting some bacteria with no effect on the growth of *Myxobacteria*.

### **1-6. Initial Assessments of Antibiotic Resistance Genes in River Otter Colon Bacteria.**

Larissa Porter\*, Allison Surf, Cindy Fuller and Renn Tumilson.

Henderson State University, Arkadelphia, AR

Antibiotic resistance is both a local and global public health problem. Arkansas ranks among the top seven states in antibiotics prescribed. Between 2011 and 2014, 1004 antibiotic resistant bacteria were found among 7051 isolates Hospital-Acquired Infections. While the non-judicious use of antibiotics has clearly been linked to the selection of antibiotic resistant bacteria, attention has recently turned to environmental reservoirs of antibiotic resistant bacteria/genes. Investigators have shown high levels of antibiotics, phosphorous, organic chemicals and heavy metals in Northwest Arkansas waterways but have not investigated antibiotic resistant bacteria or genes. Our research aims to evaluate resistance genes in Arkansas waterways, using otters as the indicator organisms. We recovered DNA from material in the lumens of frozen otter colons collected in all areas of Arkansas. Bacterial DNA was confirmed by the use of PCR with 16s forward and reverse primers. Subsequently, PCR was performed using primers that amplified an internal region of the bla-TEM resistance gene. PCR products were sequenced to confirm their identity. Future research will focus on confirmation of the bla-TEM gene in other otters and assessment of the presence of other antibiotic resistance genes in otters from across the state.

### **1-7. UV Adaptation in Halophiles.**

Haley Green (undergraduate)\*, Daniel Diep (Undergraduate), Ratnakar Deole

Northeastern State University, Broken Arrow, OK.

The increasing ozone depletion is a rising problem, and with skin cancer being the most diagnosed form of cancer and still escalating, a solution needs to be sought after. The current sunscreens on the market are not extremely effective or environmentally friendly, while some sunscreens even have shown to cause skin cancer itself. One solution to this is finding what halophiles do when exposed to ultra violet light. Halophiles are extremophiles that can with stand high salt environments and thus, can adapt under punishing conditions. One adaptation is under UV exposure they can survive by producing a protein to protect themselves. What we think this protein might be is a mycosporine or a mycosporine like amino acid. These halophiles may produce mycosporine or mycosporine like amino acid, which protect the bacteria from harsh ultra violet light through a photo stabilizing action. Using what proteins halophiles produce could give an environmentally friendly and non-cancer causing effective sunscreen.

### **1-8. Effects of Merkel Cell Polyomavirus Large T Antigen on UV Sensitivity.**

Jazmine Snow<sup>1</sup> (Undergraduate) \*, Tristan McCallister<sup>1</sup>, Adelina Parral<sup>2</sup>, Nicholas A. Wallace<sup>1</sup>

<sup>1</sup>Division of Biology, Kansas State University, Manhattan, KS

<sup>2</sup>Seward County Community College, Liberal, KS

Merkel Cell Carcinoma (MCC) is a rare, but aggressive cancer with around a 60% 5-year survival rate. Merkel cell polyomavirus (MCPyV) infections cause about 80% of MCC. MCPyV+ MCC cells express a large-T antigen (MCPyV LT) with homology to the simian virus 40 large-T oncogene. MCPyV LT inhibits repair of UV induced intra- and inter-strand DNA crosslinks (ICLs), likely by aberrantly activating the ATR signaling pathway that ICL repair. We hypothesize that these defects in ICL repair will be exacerbated by mutations in ICL repair proteins. To test this hypothesis, we used lentiviral transduction to stably express either LXS<sub>N</sub> (Vector control) or LXS<sub>N</sub> MCPyV LT in fibroblast cells that have an ICL repair gene (XPA) deleted (XPA<sup>-</sup> fibroblasts). Next, we adapted a cell viability assay to measure sensitivity to a UV gradient (0-4.0 mJ). In parallel, we measured UV sensitivity by MTT assay. We saw similar results with both assays, where compared to vector control MCPyV LT decreased cell viability by 47.9% and 55.1% after 1.0 mJ of UV, in cell viability and MTT assays, respectively. We are continuing to measure UV sensitivity in XPA<sup>-</sup> fibroblasts and will compare this data to XPA complimented fibroblasts.

### **1-9. *Staphylococcus aureus* Survives Antimicrobial Peptides through Energy Depletion.**

Kaitlyn Oppliger (Undergraduate)\* and Austin Nuxoll.

Department of Biology, University of Nebraska at Kearney, Kearney, NE.

*Staphylococcus aureus* causes a number of infections, varying from minor skin and soft tissue infections to more complicated illnesses such as bacteremia or endocarditis. Antibiotic therapy is often unsuccessful against chronic infections, which results in chronic, relapsing infections. Antibiotic treatment often fails because of two reasons - resistance and tolerance. Much is known about the former, while tolerance remains poorly understood. Tolerance is mediated by a subpopulation of dormant cells called persister cells. Recent work revealed antibiotic tolerance in *S. aureus* is energy dependent. Specifically, we found reduced TCA cycle activity is required for persister formation. While deletion of TCA cycle genes increased survival to antibiotics it remained unclear whether this deletion would increase survival to innate immunity associated components. One such component, antimicrobial peptides (AMPs), have garnered much attention as an alternative to treat antibiotic resistant organisms but little is known in regards to whether the same mechanism of antibiotic tolerance also confers tolerance to AMPs. Treatment with the AMP, LL-37 revealed TCA cycle mutants had increased survival compared to wild type *S. aureus*. These findings suggest persisters not only are problematic in terms of antibiotics, but also present a challenge for the immune system when combating these infections.

### **1-10. Mitochondrial Dysfunction Associated with Plasmid-Induced Senescence Impacts Nuclear Genome Stability.**

Natasha Nazir (Undergraduate)\*, Kala Chinnaswamy (Undergraduate)\*, Amani Alharthi, Charles D. Baudo, and John. C. Kennell.

Saint Louis University, Saint Louis, Missouri.

Strains of *Neurospora crassa* containing the Mauriceville mitochondrial plasmid are prone to senesce due to plasmid integration into the mitochondrial genome or as a result of plasmid over-replication. RNA-sequencing analysis of pre-senescent cultures revealed an unexpectedly high number of single nucleotide polymorphisms (SNPs) in the nuclear genome. The number of SNPs in senescent cultures was found to be significantly greater than those associated with plasmid-free strains, suggesting that mitochondrial dysfunction associated with Mauriceville plasmid leads to increased rates of nuclear mutation. The reversion rates of auxotrophic Mauriceville strains were examined to assess the frequency of nuclear mutations. Plasmid-containing strains were found to have up to a 40-fold increase in reversion rates in transfers close to senescence, suggesting that mitochondrial dysfunction influences nuclear mutation rates. To further test this hypothesis, the viability of multi-nucleate macroconidia versus uni-nucleate microconidia was compared. The results showed that as pre-senescent cultures were transferred, the viability of microconidia decreased much sooner than macroconidia, which may reflect that the presence of additional nuclei in macroconidia are able to complement mutations in essential genes. Together, these findings suggest that mitochondrial dysfunction associated with plasmid induced senescence impacts nuclear genome stability.

## **Graduate Student Poster Presentation Abstracts**

### **2-1. Sodium Pyruvate Alters the Immune Response to Influenza A Virus Infection in Macrophages.**

Hazar Abu Salamah (Master's)\*, Christopher Lupfer.

Department of Biology, Missouri State University, Springfield, MO

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

### **2-2. Quorum Sensing Systems are Maintained by Interspecies Competition.**

Kara C Hinshaw (Doctoral)\*, Ellen B Nessari, and Josephine R. Chandler.

University of Kansas, Lawrence, Kansas.

Many bacteria use quorum sensing (QS) to coordinate expression of genes in a population-wide manner. These gene products can be classified as public goods which are shared with all members of the population (secreted proteases and antibiotics) or private goods which only benefit the producing individual (cellular factors). We use a laboratory competition model between *Burkholderia thailandensis* and *Chromobacterium violaceum* to study the connection between QS and interspecies competition. Previously, we showed that both species produce antibiotics that inhibit growth of the other species. Here we demonstrate that *Chromobacterium* uses QS to control production of a multidrug efflux pump to promote survival in the presence of several antibiotics. Through our competition studies, we found that the QS-intact *Chromobacterium* increased in frequency in the presence of *Burkholderia* producing antibiotic bactobolin while QS-deficient are killed. Because QS also controls production of antimicrobials, this results in a net increase in the competitiveness of the *Chromobacterium* population during co-culture. Our results also demonstrate a mechanism where interspecies competition serves to stabilize and protect QS systems, which in turn promotes the competitive ability of the population. This has important implications for how QS systems evolve and are maintained during growth in mixed microbial communities.

### **2-3. Characterization of a Genus-Specific Unidentified Open Reading Frame Found within the Mitochondrial Genome of *Fusarium*.**

Michael MacKillop (Doctoral)\*, Hadaer Hamzah, Mauricio Diazgranados, and John C. Kennell.

Saint Louis University St. louis, MO.

WGS Sequencing of the mitochondrial DNA (mtDNA) in the filamentous fungal genus *Fusarium* revealed a highly variable region (HVR) in the genome located between the *rnl* and *nad2* genes. Prior characterization of this region in species of *Fusarium* has revealed a large unidentified open reading frame (LV-uORF), within all species characterized to date. The LV-uORFs are variable in amino acid size and content between species, but highly conserved within certain species complexes, with the exception of the *Fusarium oxysporum* Species Complex. The LV-uORF ORF1931, encoding a putative polypeptide of 1931 amino acids, was found in the HVR of 32 isolates from four species within the *Fusarium graminearum* Species Complex (FGSC). This LV-uORF is actively transcribed, but the putative polypeptide (1931p) has yet to be detected. Current research aims to identify and localize 1931p within *F. graminearum* PH-1 mitochondria via cellular fractionation and immunoblotting. Unlike the high conservation of the LV-uORFs within the FGSC, isolates from the FOSC exhibit greater variability, ranging from 2200 to 2500 amino acids. The FOSC is an ideal lineage for both bioinformatics analysis and functional assays. Here we examine phylogenetic evidence of the allelic diversity within the LV-uORF of 47 strains from *F. oxysporum f.sp. cubense*.

### **2-4. Fine Epitope Mapping of Monoclonal Antibodies to the DNA Repair Protein, RadA.**

Stephanie N. Nachtrab (Master's)\*, Deborah A. Hudman, Jacob L. Hiatt, Jin Seo, Samuel J. Pullen, Neil J. Sargentini, and Melissa K. Stuart.

A.T. Still University of Health Sciences, Kirksville, MO.

RadA is well-conserved across many species. In *Escherichia coli*, RadA participates in recombinational repair of radiation-damaged DNA. This process uses an undamaged DNA strand in one DNA duplex to fill a gap in a homologous DNA duplex. In this study, we identified the epitopes in RadA recognized by monoclonal antibodies 6F5 and 2A2 created in our lab. RadA was altered by protein truncation, alanine scanning mutagenesis, and limited V8 protease digestion. Recognition of altered RadA by MAb 6F5 and 2A2 was evaluated by western blotting. Additionally, synthetic peptides comprising RadA sequences that blocked MAb recognition of wild-type RadA were identified by competitive ELISA. The binding site recognized by MAb 6F5 mapped to the extreme C-terminus of RadA, while the MAb 2A2 binding site was found to be contiguous with the KNRXG motif conserved in eubacterial and plant RadA. Certain single alanine substitutions led to shifts in RadA electrophoretic mobility or the apparent absence of RadA from *E. coli* lysates, changes that correlated with enhanced UV radiation sensitivity. These data support the hypothesis that the MAbs are useful tools for detecting changes in RadA structure not predicted by DNA sequencing, but which have an impact on RadA function.

## 2-5. Bacterial Diversity of an Abandoned Mine Land Soil in Southeast Kansas

Rachel Bechtold (Master's)\* and Anuradha Ghosh

Dept. of Biology, Pittsburg State University, Pittsburg, KS

Acid mine drainage (AMD) occurs near abandoned coal mines in southeast Kansas leading to low soil pH. Soil bacteria are useful indicators of ecosystem health in these perturbed areas. The goal of the present study was to assess microbial diversity of an AMD site. In fall (2015) and summer (2016), soil samples were aseptically collected from five topographically diverse sites and physico-chemical characteristics were evaluated. Fifty eight morphologically different colonies were characterized using physiological and biochemical tests and were identified at the species level using 16S rRNA gene sequencing. Additionally, acidophilic bacterial strains were screened using selective media. Soil pH ranged from 2.5-6.8 and varied concentrations of arsenic, manganese, and iron were detected. Biochemical tests revealed a diverse metabolic potential of the bacterial population. The majority of bacterial species belonged to common soil inhabitant phyla *Firmicutes* and *Actinobacteria*. A total of 17 acidophilic bacterial isolates were identified and would be subjected to small-scale bioremediation process using lyophilization complemented with other physico-chemical techniques. A baseline measurement of bacterial diversity and soil chemistry of AMD sites in this region is novel in its kind. The findings have potential use in AMD remediation and restoration of natural habitat for plants and animals.

## **Post-Graduate Poster Presentation Abstracts**

### **2-6. Prevalence and Ribotype Diversity of Toxigenic *Clostridium difficile* in Community and Healthcare Systems.**

M Jahangir Alam<sup>1</sup>, Jacob McPherson<sup>1</sup>, Julie Miranda<sup>1</sup>, Feroz Hossain<sup>1</sup>, Khurshida Begum<sup>1</sup>, Kelley Poblete<sup>1</sup>, Mohammed Khaleduzzaman<sup>1</sup>, Wasimul Q. Chowdhury<sup>2</sup>, Anuradha Ghosh<sup>3</sup> (Assistant Professor)\*, Todd Lasco<sup>4</sup>, Kevin W Garey<sup>1</sup>

<sup>1</sup>University of Houston College of Pharmacy, Houston, TX

<sup>2</sup>Georgia Southern University Statesboro, GA

<sup>3</sup>Pittsburg State University, Pittsburg, KS

<sup>4</sup>Baylor St. Luke's Medical Center, Houston, TX

Toxigenic *Clostridium difficile* is the most common hospital-acquired infectious agent in the U.S. and is an emerging community-acquired pathogen. Spores of *C. difficile* can survive and transmit in any environ and can be a source of human infections. The present study aimed to isolate and characterize toxigenic *C. difficile* from various sources to better understand the ecology and epidemiology of the pathogen. As part of an ongoing surveillance effort, 3,109 clinical stool samples (CS) from hospitalized patients, 1,697 environmental surface swab (ES) from hospital environs, and 400 community environmental samples from the soles of shoes (SS) from non-healthcare workers were collected. Samples were subjected to anaerobic enrichment and characteristic *C. difficile* colonies from selective media were confirmed toxigenic by PCR and using fluorescent PCR ribotyping. The majority of CS (43.6%), 13.3% of high touch ES, and 26% of SS were *C. difficile* positive. Among the three sources 92.9%, 65.8% and 64.1% were toxigenic strains, respectively. Most of the ribotypes were common among all three source isolates. A high prevalence of toxigenic *C. difficile* was detected from SS that were similar ribotypes to CS and ES isolates. Our findings suggest a large reservoir of community environmental contamination of toxigenic *C. difficile*.

### **2-7. Regulation of Protein Synthesis in *Trichomonas vaginalis* by Tetracycline.**

Mia E. Hammers (Post-Master's)\* and Melissa K. Stuart.

A.T. Still University of Health Sciences, Kirksville, Missouri.

Resistance of *Trichomonas vaginalis* to metronidazole has led to evaluation of tetracycline (TET) as a potential treatment for trichomoniasis. Transcriptome analysis performed by others has identified *T. vaginalis* genes that were up- or down-regulated by exposure to TET. The goal of this study was to determine whether TET-induced changes at the RNA level correlate with changes occurring at the protein level. Parasites cultured for 4 h in TYM medium containing 0 and 500 µg/mL TET were subjected to SDS-PAGE through 20-cm polyacrylamide gels. Proteins were stained with colloidal Coomassie Blue, and those displaying prominent up- or down-regulation were identified by MALDI-ToF/MS. In the presence of TET, synthesis of enolase and glycogen phosphorylase was up-regulated, demonstrating a positive correlation with TET-induced transcription and suggesting a role for TET in alteration of carbohydrate metabolism. Production of inositol-1-phosphate synthase was down-regulated by TET, in direct opposition to results of transcriptome analysis. TET increased synthesis of  $\alpha$ -actinin, but the effect of TET on  $\alpha$ -actinin gene transcription has not been reported. These results will facilitate understanding of how transcriptomic alterations caused by TET translate into proteomic expression, and may aid in identifying the antibiotic's mechanism of action against *T. vaginalis*.