Missouri Valley Branch
AMERICAN SOCIETY FOR MICROBIOLOGY

ASM Branches

The Missouri Branch
American Society for Microbiology

Annual Meeting of the Missouri Valley Branch and The Missouri Branch of the American Society of Microbiology

March 4-5, 2016

University of Kansas Medical Center, Kansas City, KS

KU MEDICAL CENTER
The University of Kansas

We would like to welcome you to Kansas City for the 2016 Annual Meeting of the Missouri Valley and Missouri Branches. 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.

Jeffrey L. Bose and Woflram Zückert

Missouri Valley Branch Officers:
Paul Blum, President
Donald Rowen, Secretary-Treasurer
Franklin R. Champlin, Councilor
Earl Blewett, Alternate Councilor

Missouri Branch Officers:
John Steiert, President
Lea Daniel, President-Elect
Gretchen Thornsberry, Secretary-Treasurer
Anna Oller, Councilor
Phillip Lucido, Alternate Councilor

ASM Branch Meeting – KU Medical Center, March 4-5, 2016

Friday, March 4, 2016

5:00 – 5:15P	Welcome by Drs. Zückert and Bose	SoN Auditorium
5:15-5:45P	**Substrate Specificity of a Streptococcal Surface Protease Involved in Quorum Sensing.** Indranil Biswas	SoN Auditorium
5:45-6:15P	**Making friends to make war: quorum sensing, cooperation and interspecies competition.** Josie R. Chandler	SoN Auditorium
6:30-7:30P	Dinner (Zarda's BBQ/d'Bronx Veg Lasagna)	SoN Atrium
7:45-8:45P	**Keynote: Zombies and Infectious Diseases in Popular Culture.** Tara C. Smith	SoN Atrium
9:00P	Adjourn for evening – individual program	

Saturday, March 5, 2016

8:00-9:30A	Coffee & Croissants Poster Session 1 (Undergraduate posters)		Beller Atrium
9:30-11:00A	**Oral Session 1** I. General Microbiology III. Medical Micro/Immun Session 1 IV. Undergraduate Session 1	 Convener: Pam Brown Convener: Christina Krute Convener: Michelle DeGear	 Beller 1003 Beller 1005 Beller 1007
11:15A-12:45P	**Oral Session 2** II. Environmental Microbiology III. Medical Micro/Immun Session 2 IV. Undergraduate Session 2	 Convener: Tom Platt Convener: Erika Lutter Convener: Kelsey Krausz	 Beller 1003 Beller 1005 Beller 1007
12:45-2:30P	Lunch (d'Bronx Pizza) Poster Session 2 (Graduate Students and Postdocs) Branch Business Meetings		Beller Atrium
2:30-3:30P	**Keynote: Streptococcal Social Networking as a Means to Organize Interactions with the Host.** Michael J. Federle		Beller Conf Ctr
3:30-4:00P	**The role of NKG2D in determining intestinal microbiota composition.** Mary A. Markiewicz		Beller Conf Ctr
4:00-4:30P	**Multipolar growth of *A. tumefaciens* is induced by a block in cell division.** Pamela Brown		Beller Conf Ctr
4:45-5:00P	Awards/Closing remarks		Beller Conf Ctr
5:00P	Adjourn		Beller Conf Ctr

ASM Distinguished Lecturers

Tara C. Smith, PhD

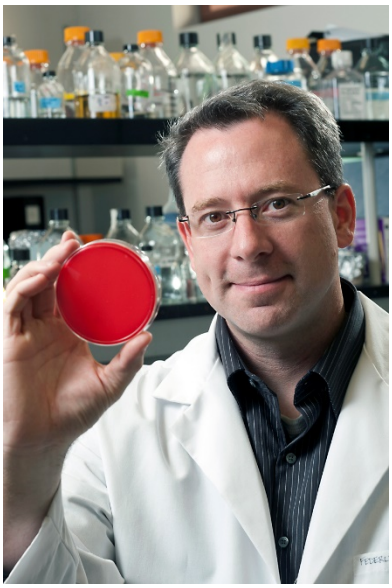
Zombies and Infectious Diseases in Popular Culture

Zombies are the horror movie monster *du jour*, appearing in TV shows and best-selling video games in addition to multiple blockbuster films. This zombie obsession can be harnessed to teach many important concepts in infectious diseases and epidemiology. These concepts will be reviewed and demonstrated in a light-hearted talk.

Biographical Sketch: Dr. Smith joined the faculty of Kent State University College of Public Health in August 2013 following nine years in the Department of Epidemiology at the University of Iowa, where she directed the College's Center for Emerging Infectious Diseases. She completed post-doctoral training at the University of Michigan after obtaining her Ph.D. at the University of Toledo and her B.S. in Biology from Yale University.

Dr. Smith's research focuses on zoonotic infections (infections which are transferred between animals and humans). She was the first to identify livestock-associated strains of methicillin-resistant *Staphylococcus aureus* (MRSA) in the United States, and has pioneered the investigation of this organism in the United States. Dr. Smith has published over 50 peer-reviewed papers and book chapters. She has received over three million dollars in funding from AHRQ, USDA, and NIOSH to carry out her studies. She has presented her research at numerous national and international platforms, including talks on Capitol Hill on the topic of agriculture and antibiotic resistance. Her work has been profiled in many major publications, including *Science*, *Nature,* and *The New York Times*. Dr. Smith is also active in science communication and outreach. She has maintained a science blog since 2005, and has written books on Group A Streptococcus, Group B Streptococcus, and Ebola. She also writes about infectious disease for Slate.com among other sites, and is a member of the advisory board of the Zombie Research Society.

Michael J. Federle, PhD



Streptococcal Social Networking as a Means to Organize Interactions with the Host

The Federle lab has helped to discover a family of proteins and peptide pheromones that are widely-conserved among Gram-positive bacteria. The Rgg family of transcription factors is now known to directly bind short peptide signals that are imported to the cytoplasm and modulate gene expression. Investigations have focused on mechanisms of pheromone maturation, receptor-ligand interactions, transcriptional regulation, and Rgg protein biochemistry. The Rgg family is large and mechanisms of regulation are anticipated to vary with subclasses, but a clear understanding of responses of Rggs to peptides will assist in predicting gene expression responses.

Since pheromone signaling has a clear effect on bacterial gene expression and behavior, the potential to influence behavior may be possible through manipulation of bacterial communication. The Federle lab has initiated high-throughput screening for molecules that block Rgg-pheromone signaling with compounds available in both chemical and genetic libraries. Candidate screening utilizes luciferase and fluorescence reporters together with robotic liquid handling, phage display, and flow cytometry. Initial results have found compounds that specifically and directly bind to Rgg proteins and that compete with pheromone binding.

Biographical Sketch: Dr. Federle obtained his B.S. in Genetics from the University of Wisconsin-Madison (Go Badgers) and this PhD in Microbiology and Molecular Genetics at Emory University. Following a post-doctoral position at Princeton University/Howard Hughes Medical Institute, he became a faculty member in the Department of Medicinal Chemistry and Pharmacognosy at the University of Illinois at Chicago.
Dr. Federle's ongoing research interests are in cell-cell communication among Gram-positive bacteria. His lab has helped identify a new class of quorum-sensing pathways that utilize proteins of the Rgg family and short, imported peptide pheromones. The lab's work focuses on characterization of these pathways, identification of behaviors controlled by them, and development of small molecule probes into novel therapeutics aimed at interfering with bacterial communication to treat disease. Dr. Federle has been awarded an R01 grant from the NIH and was recently named a Burroughs Wellcome Fund Investigator in the Pathogenesis of Infectious Disease. He has been an invited speaker for more than 30 seminars and conferences and has authored 26 articles and book chapters. Dr. Federle serves on the editorial board for *Journal of Bacteriology*, and provides *ad hoc* reviews for *mBio, Infection and Immunity*, and *Applied and Environmental Microbiology*.

Local invited speakers

Indranil Biswas, PhD. Dr. Biswas is a Professor in the Microbiology, Molecular Genetics and Immunology Department at The University of Kansas Medical Center. His lab studies how proteases and two-component regulatory systems affect virulence factor expression and maturation in Streptococci. His lab also studies biofilm formation in *Streptococcus pyogenes* and *Streptococcus mutans*.

Josie Chandler, PhD. Dr. Chandler is an Assistant Professor in the Department of Molecular Biosciences at The University of Kansas. Her lab research is focused on understanding how bacteria communicate and cooperate with each other to carry out complex group behaviors. She primarily studies a cell-cell communication system in bacteria called quorum sensing using *Burkholderia thailandensis* as a model.

Mary Markiewicz, PhD. Dr. Markiewicz is an Assistant Professor in the Microbiology, Molecular Genetics and Immunology Department at The University of Kansas Medical Center. Her lab's research focus is to determine the role of the NK cell activating receptor NKG2D and its ligands in various types of immunity, with a particular interest on the function of this receptor-ligand system in CD8+ T cell responses.

Pam Brown, PhD. Dr. Brown is an Assistant Professor in the Division of Biological Sciences at the University of Missouri. Her lab is interested in understanding the principles that govern bacterial morphology, i.e. how bacteria generate specific shapes. To this end, her studies examine polar growth in the plant pathogen *Agrobacterium tumefaciens*.

Oral Session 1

I. General Microbiology

Time	Presentation	Room
9:30-9:45	**Surface Display for Strain Improvement of the Industrially Important Bacterium *Gluconobacter oxydans*.** Marshal Blank	1003
9:45-10:00	**A New Method for the Characterization of Essential Genes in the Bacterial Plant Pathogen *Agrobacterium tumefaciens.*** Wanda Figueroa-Cuilan	1003
10:00-10:15	**Role of the Min system in *Agrobacterium tumefaciens* cell division.** Sue Ann Flores	1003
10:15-10:30	**Functional Characterization of the Penicillin-Binding Proteins in *A. tumefaciens.*** Michelle Williams	1003
10:30-10:45	**Membrane Lipid Imbalance in *Saccharomyces cerevisiae* Leads to Trafficking Defects toward the Golgi**. Sara E. Woodman	1003

III. Medical Microbiology / Immunology (Session 1)

Time	Presentation	Room
9:30-9:45	***Lactobacillus* administration alters behavior in the prairie vole (*Microtus ochrogaster*).** Kathleen Ahles	1005
9:45-10:00	**The Relation between Calcium Homeostasis and Tobramycin Efflux in a Human Pathogen *Pseudomonas aeruginosa.*** Sharmily S. Khanam	1005
10:00-10:15	**Characterization of Mechanism of Action of DASamP2 against *Pseudomonas aeruginosa* by Isolation of Mutants.** Swapna Medichetti	1005
10:15-10:30	**EfhP, a Putative Calcium Binding Calmodulin like Protein, Plays Role in Calcium Induced Responses of *Pseudomonas aeruginosa.*** Biraj B. Kayastha	1005
10:30-10:45	**CarP, a Putative β Propeller Protein, Plays Role in *Pseudomonas aeruginosa* Response to Calcium.** Michelle King	1005

IV. Undergraduate (Session 1)

Time	Presentation	Room
9:30-9:45	**Isolation of Fermentative Yeasts from Trees.** Matt Gordon	1007
9:45-10:00	**Isolation and Characterization of Novel Halo-Acidophilic Microorganisms Present in Hypersaline Lakes from Western Australia.** Ava L. Hughes	1007
10:00-10:15	**Identification of Novel Antimicrobial Proteins from Extreme Halophilic Archaea.** Yuliya Kunz	1007
10:15-10:30	**Gut Microbiome of the Prairie Lizard, *Sceloporus consobrinus*.** Robby King	1007
10:30-10:45	**Evolution of an rRNA Intron in the Lichen *Teloschistes chrysophthalamus*** Jordanna Glock	1007
10:45-11:00	**Defining the Spectrum of Ultraviolet (UV) Resistance in the UV Resistant Bacteria *Deinococcus radiophilus.*** Diana Hopkins	1007

Oral Session 2

II. Environmental Microbiology

Time	Presentation	Room
11:15-11:30	**Ability of Bacteria to Degrade BTEX, Benzoate and Hexadecane Under Oxic Conditions.** David Adetitun	1003
11:30-11:45	**Narrow-Host Range Lytic Bacteriophage as Antagonists of *Agrobacterium tumefaciens.*** Hedieh Attai	1003
11:45-12:00	**Outer Membrane Exclusion of Hydrophobic Compounds by Environmental Relatives of the Nosocomial Opportunist *Pseudomonas aeruginosa*.** Lauren E. Chambers	1003
12:00-12:15	**Pretreatment of Plant Biomass Using Fungal and Bacterial Co-Culture.** Swechchha Pradhan	1003
12:15-12:30	**Effects of Nutrient Treatments on Leaf-Litter Microbial Communities in a Lowland Tropical Rain Forest.** Brian Bill	1003

III. Medical Microbiology / Immunology (Session 2)

Time	Presentation	Room
11:15-11:30	**Production of monospecific antisera for *Vago* and *virus induced RNA-1*(*vir-1).*** Wilfredo A Lopez	1005
11:30-11:45	**Characterization of Vps1 Domain Interaction with ESCRT Subunits.** Bryan Banh	1005
11:45-12:00	**Link Between Toxin Production and Sporulation in *Clostridium difficile*** Brintha Parasumanna Girinathan	1005
12:00-12:15	**Dynamin Acts Downstream of Ypt6 for Membrane Fusions.** Pelin Makaraci	1005
12:15-12:30	**Functional Connection between Vps1 and GARP Vps51 at the Golgi in Budding Yeast.** Uma Saimani	1005

IV. Undergraduate (Session 2)

Time	Presentation	Room
11:15-11:30	**Hitting the Target: Biochemical Interaction of Vps1 and the Golgi.** John Short	1007
11:30-11:45	**Understanding the Functional Relationship between the Retromer Complex and Vps 1.** Mariel Delgado Cruz	1007
11:45-12:00	**Possible Influence of Outer Membrane Permeability Properties on the General Resistance of *Serratia marcescens* to Hydrophobic Molecules.** Kavya Boyina	1007
12:00-12:15	**The Effect of Environmental Factors on Swarming Motility in a Human Pathogen *Pseudomonas aeruginosa.*** Breeanna C. Russ	1007
12:15-12:30	**Histone Deacetylase HDA5 May Be Involved in *Pseudomonas syringae* Triggered Reduction of Host Histone H3K9 Acetylation.** Victoria Shum	1007
12:30-12:45	**Prevalence of Pathogenic Bacteria in the American Dog Tick (*Dermacentor variabilis*) in Dawson County, Nebraska.** Nathan Harms	1007

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 share a 4x6 ft board. For session 1, please hang on the left side of the board and for session 2 use the right side of the board.

Poster session 1 (Undergraduate)

Poster Board	Poster Title and Presenter
1	**Generation of Improved Oncolytic Poxviruses**. Adam J. Schieferecke
2	**Identification of Transposon insertion sites in Chlamydia** Melissa Berger
3	**Metabolically Engineered *Gluconobacter oxydans* for the Production of Optically Pure Acetoin: a Pharmaceutical Precursor.** Neil Bolduc
4	***Metarhizium* Adhesins and Attachment.** Susie Brown
5	**Identification and Characterization of Essential Genes Involved in the Regulation of Peptidoglycan Synthesis in *Agrobacterium tumefaciens*** Caroline Dunn
6	**The Cra-FruK Complex Alters Regulation of Central Metabolism in γ-proteobacteria.** Max Fairlamb
7	**Yeast-two hybrid analysis of *Chlamydia trachomatis* type III secreted effector proteins.** Jordan Fleming
8	**Microbial Extracellular Enzyme Activity Responses to Long-Term Nitrogen Addition and Annual Burning in Tallgrass Prairie Soil.** Victoria Floyd
9	**Investigations into the Role of MARCKS during *Coxiella burnetii* Infections of THP-1 Cells.** Christa Jackson
10	**Generation of Yeast 2-Hybrid Clones to Examine the Role of Nucleotide Oligomerization and Binding Domain (NOD)-Like Receptors.** Abbi J. Mabary
11	**The Role of Insect Vectors in the Movement of a Plant Virus in Northeastern Kansas**. Matthew A. Ramos
12	**Improving Inflammation in Bacterial Coinfection by IL-1β Regulation.** Angeline Rodriguez
13	**Determining the Interaction Between CT228 and MYPT1 in *Chlamydia trachomatis*** Brooke Romine
14	**The Search for Antibiotic-Producing Bacteria at Henderson State University.** Richard Spencer-Cole and Sarah Johnson
15	**Antibiotic Resistance of *Staphylococcus aureus* Recovered from Cystic Fibrosis Patients.** Wade Arthur
16	**Identification of Novel Antimicrobial Proteins from Extreme Halophilic Archaea.** Yuliya Kunz

Poster session 2 (Graduate Student and Postdoctoral*)

Poster Board	Poster Title and Presenter
1	**Bioavailability Of B20 Biodiesel Fuel Components And Their Relative Contribution To Microbiologically Influenced Corrosion** Oderay C. Andrade
2	**Genetic Manipulation of *Chlamydia trachomatis* Inclusion Membrane Protein CT228 using the Adapted TargeTron System.** Amanda Behar
3	**Antibiotic Resistance in *Staphylococcus aureus* Isolated From Cystic Fibrosis Patients** Rawan Eleshy
4	**Investigation of Microbiologically Influenced Corrosion in B20 Biodiesel and the Development of Accelerated Testing Methods.** James G. Floyd
5	**Prevalence of Non-O157 ShigaToxin-Producing *Escherichia coli* (STEC) in Houseflies (*Musca domestica* L.) from Cattle Feedlots and Dairies.** Anuradha Ghosh
6	**Discovery of Genetic Correlates Encoded by *Chlamydia* that are Important for Mammalian**

	Infection. Kelly Harrison
7	**Quorum Sensing Systems are Maintained by Interspecies Competition.** Kara C Hinshaw
8	**Phenotypic analysis of Transposon mutant strains of *Chlamydia trachomatis***. Scott Labrie
9	**Function of Sigma 54 in *Chlamydia trachomatis.*** Megan McKinney
10	**Functional importance of the N-terminal region of Photoactive yellow protein.** Farzaneh Moghadam
11	**Transcription of T4ASS ORFs in Spotted Fever Group *Rickettsia.*** Chris Richards
12	**Structure and Function Analysis of the Redox-Sensing Transcription Factor Methanogen Specific Vinyl-4-Reductase (MsvR).** Kristen Shelton
13	**Novel synthetic analogs of avian β-defensin-12 with enhanced antimicrobial and immunomodulatory activities**. Ming Yang
14*	***Staphylococcus aureus* Metabolic Adaptations During the Transition to a Vancomycin-intermediate Susceptibility Phenotype.** Stewart G. Gardner
15*	
16	**The Fatty Acid Kinase of *Staphylococcus aureus* Controls Virulence.** Christina N. Krute

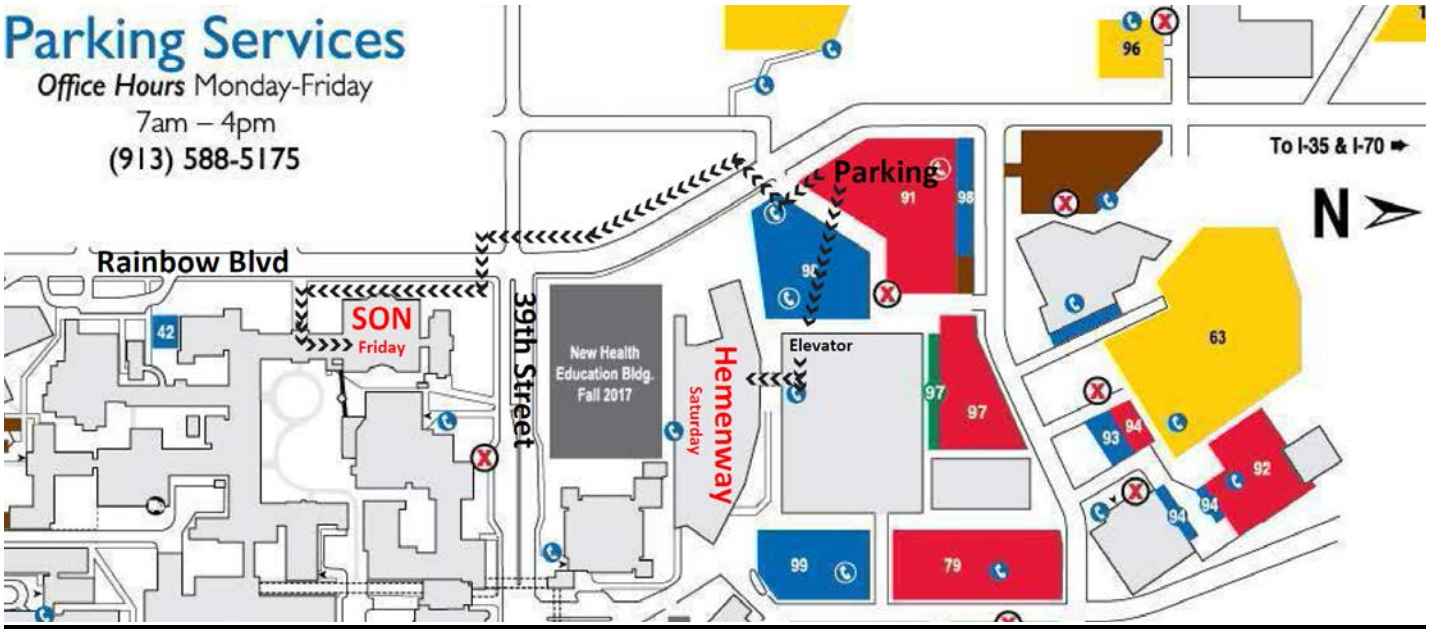
Meeting Participants

Last name	First name	University	Email
Adetitum	David	Oklahoma State University	david.adetitun@okstate.edu
Ahles	Kathleen	Oklahoma State University Center for Health Sciences	kathleen.ahles@okstate.edu
Amburn	Sue Katz	Rogers State University	sue.katz@gmail.com
Andrade	Oderay	University of Oklahoma	Oderay.D.Andrade.Eljuri-1@ou.edu
Attai	Hedieh	University of Missouri-Columbia	hedieh.attai@mail.missouri.edu
Banh	Brian	Missouri State University	btb125@live.missouristate.edu
Behar	Amanda	Oklahoma State University	amanda.behar@okstate.edu
Berger	Melissa	University of Kansas	melissapberger@gmail.com
Bill	Brian	University of Oklahoma	Brian.E.Bill-1@ou.edu
Bishr	Mahmoud	Penn Valley College	mahmoud.bishr@mcckc.edu
Biswas	Indranil	University of Kansas Medical Center	ibiswas@kumc.edu
Blank	Marshal	Missouri State University	marshal444@live.missouristate.edu
Bloomer	Jessie	University of Nebraska at Kearney	bloomerjj@lopers.unk.edu
Blum	Paul	University of Nebraska-Lincoln	pblum1@unl.edu
Bolduc	Neil	Missouri State University	Bolduc11@live.Missouristate.edu
Bose	Jeffrey	University of Kansas Medical Center	jbose@kumc.edu
Boyina	Kavya	Oklahoma State University Center for Health Sciences	boyina.kavya97@gmail.com
Brown	Pam	University of Missouri-Columbia	brownpb@missouri.edu
Brown	Susie	Henderson State University	sb191360@reddies.hsu.edu
Burnett	Christie	North Kansas City Hospital	christie2cats@yahoo.com
Caldas	Martha	Kansas State University	mscaldas@ksu.edu
Chambers	Lauren	Oklahoma State University Center for Health Sciences	lauren.chambers@okstate.edu
Champlin	Franklin	Oklahoma State University Center for Health Sciences	franklin.champlin@okstate.edu
Chandler	Josie	University of Kansas	jrchandler@ku.edu
Cisar	Cindy	Northeastern State University	cisar@nsuok.edu
Clarkston	Cristopher	Kansas State University	chris.clarkston@gmail.com
Daniel	Jeremy	University of Missouri-Columbia	danieljj@missouri.edu
Daniel	Lea	Central Methodist University	ldaniel@centralmethodist.edu
DeGear	Michelle	Oklahoma State University Center for Health Sciences	degear@okstate.edu
Delgado Cruz	Mariel	Missouri State University	Cruz321@live.missouristate.edu
DeMars	Zach	Kansas State University	zrdemars@k-state.edu
Deole	Ratnakar	Northeastern State University	deole@nsuok.edu
Dmitriev	Aidan	University of Kansas	
Dunn	Caroline	University of Missouri-Columbia	cmd6n2@mail.missouri.edu
Eleshy	Rawan	Oklahoma State University	rawan.eleshy@okstate.edu
Ericson	Brad	University of Nebraska at Kearney	erisonb@unk.edu
Fairlamb	Max	University of Kansas Medical Center	mfairlamb@kumc.edu
Federle	Michael	University of Illinois at Chicago	mfederle@uic.edu
Figuero-Cuilan	Wanda	University of Missouri-Columbia	wmffn8@mail.missouri.edu
Fleming	Jordan	Oklahoma State University	jordan.h.fleming@okstate.edu
Flores	Sue Ann	University of Missouri-Columbia	safhh9@mail.missouri.edu
Floyd	James	University of Oklahoma	james.floyd@ou.edu
Floyd	Victoria	Kansas State University	vwfloyd@ksu.edu
Foye	Judy	Stowers Institute for Medical Research	juf@stowers.org
Fuller	Cynthia	Henderson State University	fullerc@hsu.edu

Funk	Joel	John Brown University	jofunk@jbu.edu
Gardner	Stewart	University of Nebraska-Lincoln	stewartgardner@gmail.com
Ghosh	Anuradha	Pittsburg State University	aghosh@pittstate.edu
Glock	Jordanna	University of Nebraska at Kearney	glockje@lopers.unk.edu
Gordon	Matt	University of Central Missouri	mjg27540@ucmo.edu
Greene	Andrew	Metropolitan Community College-Penn Valley	andrew.greene@mcckc.edu
Grubb	Lauren	William Jewell College	grubbl@william.jewell.edu
Harms	Nathan	University of Nebraska at Kearney	harmsne@lopers.unk.edu
Harrison	Kelly	University of Kansas	mmking@ostatemail.okstate.edu
Hefty	Scott	University of Kansas	pshefty@ku.edu
Hinkle	Cole	Kansas State University	cdhinkle@ksu.edu
Hinshaw	Kara	University of Kansas	kara.hinshaw@gmail.com
Hopkins	Diana	Rogers State University	dianahpkns@gmail.com
Hughes	Ava	Missouri University of Science & Technology	alh8r7@mst.edu
Jackson	Christa	John Brown University	JacksonCD@jbu.edu
Johnson	Sarah	Henderson State University	SJ172147@reddies.hsu.edu
Kayastha	Biraj	Oklahoma State University	biraj@ostatemail.okstate.edu
Khanam	Sharmily	Oklahoma State University	khanam@okstate.edu
Kim	Kyoungtae	Missouri State University	kkim@missouristate.edu
King	Michelle	Oklahoma State University	mmking@ostatemail.okstate.edu
King	Robby	Northeastern State University	king46@nsuok.edu
Krausz	Kelsey	University of Kansas Medical Center	kkrausz@kumc.edu
Krute	Christina	University of Kansas Medical Center	ckrute@kumc.edu
Kunz	Yuliya	Northeastern State University	kuskova@nsuok.edu
Labrie	Scott	University of Kansas	scottlabrie@gmail.com
Lonergan	Katlyn	Missouri University of Science and Technology	kml5hd@mst.edu
Lopez	Wilfredo	University of Nebraska at Kearney	lopezwa@lopers.unk.edu
Lucido	Phillip	Northwest Missouri State University	lucido4405@gmail.com
Luedtke	Brandon	University of Nebraska at Kearney	luedtkebe@unk.edu
Lupfer	Christopher	Missouri State University	CristopherLupfer@missouristate.edu
Lutter	Erika	Oklahoma State University	erika.lutter@okstate.edu
Mabary	Abbi	Missouri State University	ajm200@live.missouristate.edu
Makaraci	Pelin	Missouri State University	makaraci292@live.missouristate.edu
Markiewicz	Mary	University of Kansas Medical Center	mmarkiewicz@kumc.edu
McDonald	Allison	Oklahoma State University Center for Health Sciences	allison.mcdonald@okstate. Edu
McKinney	Megan	University of Kansas	mem2015@ku.edu
Medichetti	Swapna	University of Nebraska-Omaha	smedichetti@unomaha.edu
Moghadam	Farzaneh	Oklahoma State University	farzaneh.moghadam@okstate.edu
Morgan	Daniel	Henderson State University	DM180186@reddies.hsu.edu
Nguyen	Linda	University of Nebraska-Lincoln	lnguyen26@live.com
Nicks	Trevor	William Jewell College	trevornicks@gmail.com
Oller	Anna	University of Central Missouri	oller@ucmo.edu
Paper	Janet	Kansas State University	paperjan@ksu.edu
Parasumanna Girinathan	Brintha	Kansas State University	brintha@ksu.edu
Peterson	Gregory	University of Kansas	greg_peterson@ku.edu
Platt	Thomas	Kansas State University	tgplatt@ksu.edu
Pradhan	Swechchha	Oklahoma State University	swechch@ostatemail.okstate.edu
Rahn-Li	Lilah	William Jewell College	rahn-leel@william.jewell.edu
Ramos	Matthew	Kansas State University	matt39@ksu.edu

Richards	Chris	Oklahoma State University	ricch@ostatemail.okstate.edu
Rodriguez	Angeline	Missouri State University	Rodriguez281@live.missouriState.edu
Romine	Brooke	Oklahoma State University	brookdr@okstate.edu
Rowen	Donald	University of Nebraska-Omaha	drowen@unomaha.edu
Russ	Breeanna	Oklahoma State University	breeanr@ostatemail.okstate.edu
Sah	Prakash	Oklahoma State University	prakash.sah@okstate.edu
Saimani	Uma	Missouri State University	Uma26580@live.missouristate.edu
Schieferecke	Adam	Kansas State University	ajschief@ksu.edu
Schremmer	Stephanie	University of Kansas	
Shaffer	Julie	University of Nebraska at Kearney	shafferjj@unk.edu
Shelton	Kristen	University of Oklahoma	kristen.k.shelton-1@ou.edu
Short	John	Missouri State University	Short7814@live.missouristate.edu
Shum	Victoria	University of Nebraska-Lincoln	vshum2473@gmail.com
Smith	Tara	Kent State University	tsmit176@kent.edu
Snow	Jazmine	Kansas State University	jazasnow@ksu.edu
Soules	Katelyn	University of Kansas	ksoules@ku.edu
Spencer-Cole	Richard	Henderson State University	RS173672@reddies.hsu.edu
Steiert	Brianna	William Jewell College	steiertb@william.jewell.edu
Steiert	Jack	Missouri State University	johnsteiert@missouristate.edu
Sway	Pradhan	Oklahoma State University	swechchha.pradhan@okstate.edu
Swint-Kruse	Liskin	University of Kansas Medical Center	lswint-kruse@kumc.edu
Thornsberry	Gretchen	Northwest Missouri State University	gthorns@nwmissouri.edu
van Dijk	Karin	University of Nebraska-Lincoln	kvandijk2@unl.edu
Vogel	Erich	University of Nebraska-Lincoln	ewvogel@gmail.com
Wade	Arthur	Oklahoma State University	wade.arthur@okstate.edu
Westenberg	David	Missouri University of Science and Technology	djwesten@mst.edu
Whitlock	Sara	John Brown University	WhitlockS@jbu.edu
Williams	Michelle	University of Missouri-Columbia	maw2q3@mail.missouri.edu
Woodman	Sara	Missouri State University	woodman1@live.missouristate.edu
Yang	Ming	University of Missouri	mywb9@mail.missouri.edu
Zhang	Chunye	University of Missouri	mywb9@mail.missouri.edu
Zuckert	Wolf	University of Kansas Medical Center	wzueckert@kumc.edu

Directions



On Fri night, park as indicated. Walk up Rainbow Blvd and enter Murphy Hall (picture below) on your left, it is across the street from Five Guys. After entering, take an immediate left and continue until you see the School of Nursing and registration table.

On Saturday, park as indicated. Take the elevator in the parking garage to the 5th floor and walk across the short bridge to the Hemenway Building. Enter building, walk forward and take stairs to the second floor.

Student Oral Presentation Abstracts

I. General Microbiology Graduate Student Oral Presentation

Surface Display for Strain Improvement of the Industrially Important Bacterium *Gluconobacter oxydans*.

Marshal Blank (Masters)* and Dr. Paul Schweiger.

Missouri State University, Springfield, Missouri.

Acetic acid bacteria are invaluable in biotechnology due to their natural ability to incompletely oxidize carbon substrates to produce enantiopure chiral molecules without the need for complex and expensive chemistries. However, few molecular tools exist for strain improvement. One such tool is surface display, where recombinant enzymes are localized to the outer membrane via anchor proteins. This direct access to substrates results in simplified product extraction and increased yields. To this end, we developed a surface display system to deliver alkaline phosphatase PhoA to the membrane of *Gluconobacter oxydans* using OprF188 as the surface anchor as a proof-of-concept. Phosphatase activity was quantified in whole-cell reactions, and expression of a fluorescent Halotag in lieu of PhoA will further confirm correct surface localization by OprF. This system will be employed for novel and improved production of value-added products by this industrially important bacterium. For example, expression of enzymes such as α-amylase and/or glucoamylase will allow *Gluconobacter* to grow on inexpensive and renewable substrates such as starch, greatly reducing production costs. Molecular tools like this system will broaden the industrial potential of acetic acid bacteria.

A New Method for the Characterization of Essential Genes in the Bacterial Plant Pathogen *Agrobacterium tumefaciens.*

Wanda Figueroa-Cuilan* (Doctoral), Jeremy J. Daniel, and Pamela J.B. Brown.

University of Missouri, Columbia, Missouri.

The bacterial plant pathogen, *A. tumefaciens*, exhibits an atypical mode of polar growth. Efforts to study the mechanism of polar growth have been hampered by a lack of genetic tools for the characterization of essential genes in *A. tumefaciens*. To overcome this challenge, we have engineered a *tn*7-based method for inducible control of transcription from an engineered site on the chromosome. To test for precise regulation, we monitored the restoration of biofilm phenotypes during complementation of null mutants. Our results suggest that restoration of adhesion in a biofilm mutant requires the presence of inducer when a complementing gene is inserted on the chromosome under the control of the *lac* promoter. In order to further characterize our system, a free GFP strain was constructed. Western blotting analyses have shown that the chromosome-based system exhibits rapid protein depletion and tighter regulation when compared to the pSRK inducible plasmid system. Finally, our optimized system has enabled us to delete and deplete essential genes including *ctrA,* which encodes a cell cycle regulator, and *ftsZ*, which encodes a cell division protein. We conclude that our optimized *tn*7-based system is a useful for the characterization of essential genes in *Agrobacterium*.

Role of the Min system in *Agrobacterium tumefaciens* cell division.

Sue Ann Flores* (Masters), Jeremy J. Daniel, and Pamela J.B. Brown.

University of Missouri, Columbia, Missouri.

In *A. tumefaciens*, the essential FtsZ protein is located at the growth pole before shifting to the mid-cell right before division. Here, we conduct a systematic characterization of the Min system in *A. tumefaciens* to better understand the regulation of FtsZ ring placement. When the *minCDE* locus is deleted most cells grow normally; however some cells become elongated or branched. Deletion of individual *min* genes results in morphological changes, due to misplacement of the FtsZ rings. We find that the *minE* deletion causes the most pronounced phenotype. The absence of MinE causes asymmetric placement of FtsZ rings leading to the production of daughter cells of variable cell lengths. Remarkably, small cells contain DNA and continue to divide until they are no longer viable. Taken together, our data suggest that the Min system contributes to the regulation of FtsZ placement. We hypothesize that *A. tumefaciens* must contain additional regulators of FtsZ placement. Thus, we are conducting a saturating transposon mutagenesis screen to identify proteins that become essential for cell survival in the absence of the Min system. We expect these results will provide a better understanding of the regulation of FtsZ placement during cell division of *A. tumefaciens*.

Functional Characterization of the Penicillin-Binding Proteins in *A. tumefaciens.*

Michelle Williams (Doctoral)*, Jeremy Daniel and Pamela Brown.

University of Missouri, Columbia, Missouri.

In contrast to the dispersed cell wall growth patterning of *E. coli* and other well-studied bacilli, *Agrobacterium tumefaciens* peptidoglycan (PG) biosynthesis is constrained to a single pole during elongation. We hypothesize that the activity of penicillin binding proteins (PBPs), which mediate PG biosynthesis, must be precisely targeted to the growth pole during elongation. Based on bioinformatic analysis, we find that *A. tumefaciens* PBP homologs have diverged from their well-studied counterparts in other rod-shaped bacteria, including examples of gene loss and gene duplication. Here we begin to systematically characterize the PBPs to determine if they function in polar elongation, cell division, or both. Remarkably, we observe that most PBP-GFP fusions display both polar and midcell localization. In addition, we observe that PBP overexpression leads to aberrant morphology, which is indicative of altered peptidoglycan metabolism due to the presence of the PBPs. A systematic characterization of the function of the PBPs in *A. tumefaciens* is underway and we expect this will help elucidate the mechanisms of cell wall synthesis in polar-growing bacteria.

Membrane Lipid Imbalance in *Saccharomyces cerevisiae* Leads to Trafficking Defects toward the Golgi.

Sara E. Woodman (Masters)*, Justin Conover, Chris Trousdale, and Kyoungtae Kim.

Missouri State University, Springfield, Missouri.

Protein recycling is an essential cellular process that involves endocytosis, intracellular trafficking, and exocytosis. It has been shown in mammalian systems that membrane lipids, including cholesterol, sphingolipids, and phospholipids, play a pivotal role in protein recycling. In order to address the roles of plasma membrane lipid components on protein recycling in budding yeast, *Saccharomyces cerevisiae*, we utilized GFP-Snc1, a v-SNARE protein serving as a fluorescent marker for faithfully reporting the recycling pathway. Here we show results that display moderate to significant GFP-Snc1 recycling defects upon overexpression or inactivation of phospholipid, ergosterol, and sphingolipid biosynthesis enzymes, indicating that the homeostasis of membrane lipid levels is prerequisite for proper protein recycling. Through using a truncated version of GFP-Snc1 that cannot be recycled from the plasma membrane, we determined that abnormalities in Snc1 localization in membrane lipid overexpression or underexpression mutants are not due to defects in the synthetic/secretory pathway, but rather in the recycling pathway. The location of mistargeted GFP-Snc1 and potential mistargeting mechanisms are currently being studied.

II Environmental Microbiology Graduate Student Oral Presentation

Effects of Nutrient Treatments on Leaf-Litter Microbial Communities in a Lowland Tropical Rain Forest.

Brian Bill* (Doctoral), Michael Kaspari, and Bradley Stevenson.

University of Oklahoma, Norman, OK

The mineralization rate of nutrients sequestered in tropical rain forest leaf litter is predicted to increase with global temperatures. Because microorganisms specialize along gradients of substrates, disruption of these gradients through increased mineralization may, in turn, affect the diversity and structure of microbial communities. Therefore we hypothesized that nutrient additions will suppress microbial diversity and shift the structure of leaf litter microbial communities. To test this hypothesis, leaf litter microbial communities were characterized using high throughput sequencing of 16S rRNA gene libraries across a factorial treatment of nitrogen, phosphorous, and potassium fertilizations as part of the Smithsonian Tropical Research Institutes' Gigante Fertilization Experiment. The addition of nitrogen reduced the diversity of the leaf litter community by ~10% and significantly shifted community composition and structure, suggesting that microbial communities responded to changes in nutrient pools. These results supported the prediction that increased mineralization of limited nutrients in leaf litter will reduce the diversity and change the structure of microbial communities, leading to changes in function (i.e. decomposition of detritus), and resistance to disturbances.

Ability of Bacteria to Degrade BTEX, Benzoate and Hexadecane Under Oxic Conditions

David Adetitun[1,2], Olayemi, A. B.[2], Kolawole O. M[2]. and Babu Fathepure[1]

[1]Department of Microbiology and Molecular Genetics, Oklahoma State University
[2]Department of Microbiology, University of Ilorin, Nigeria

Petroleum contamination is caused accidently but keep recurring due to the need for oil and gas in all segments of society and everyday life. Chemical degradation of these pollutants leaves cancer-causingcompounds behind but microbial degradation does not. 21 bacteria were isolated from kerosene and gasoline artificially contaminated soil. This was done to determine the bacterium that will be able to thrive in the presence of the hydrocarbons. The ultimate goal of this work is to use bacteria to clean up kerosene and gasoline-contaminated soil for agricultural purposes. The 21 bacteria were grown on kerosene and gasoline as carbon sources and they grew well when optical density, pH and total viable count were used as indicators. 5 bacterial species that showed better growth on kerosene and gasoline were selected for further studies. These 5 isolates were tested for their ability to degrade aromatic compounds (BTEX). These isolates were also tested for their ability to grow on hexadecane and benzoate as the sole carbon sources. Degradation of BTEX was monitored using gas chromatograph, use of benzoate was examined using spectrophotometer while utilization of hexadecane was measured by colony counts on plates. Molecular tools are currently being used to identify these isolates.

Narrow-Host Range Lytic Bacteriophage as Antagonists of *Agrobacterium tumefaciens.*

Hedieh Attai (Doctoral)*, George Smith, and Pamela Brown.

University of Missouri, Columbia, Missouri.

Agrobacterium tumefaciens causes crown gall disease, resulting in crop damage. Bacteriophages can be used as biocontrol agents to protect plants from such pathogens. Thus, we have isolated five lytic bacteriophages from environmental sources (Agrophages (AP 1-5)). Specificity testing against select bacteria reveals that all phages have a narrow-host range, primarily specific to *A. tumefaciens* C58-derived strains. Potato disc assay results indicate that coinoculation with a phage cocktail limits *A. tumefaciens* infection. We have sequenced the genomes of three Agrophages in order to identify putative endolysins, which may be responsible for muralytic activity on the *Agrobacterium* cell wall enabling these phages to lyse their hosts. We have shown that a putative endolysin in AP2 and AP3, Phage Peptidoglycan Hydrolase (PPH), has unique properties, including the presence of a transmembrane domain on its C terminus. Zymography using peptidoglycan as a substrate suggests the PPH is catalytically active. Endogenous expression of PPH causes aberrant cell division—branching morphology in *A. tumefaciens* cells and filamentation in *E. coli*—followed by cell lysis. These observations imply that PPH is capable of gaining access to the periplasm, without a holin or spanin, where it may interact with cell division machinery and cleave the peptidoglycan.

Outer Membrane Exclusion of Hydrophobic Compounds by Environmental Relatives of the Nosocomial Opportunist *Pseudomonas aeruginosa*.

Lauren E. Chambers (Masters)*, Michelle A. DeGear, and Franklin R. Champlin

Oklahoma State University Center for Health Sciences, Tulsa, OK

Outer membrane exclusionary properties underlie the intrinsic resistance of *Pseudomonas aeruginosa* to the hydrophobic biocide triclosan, but these properties have not been investigated for environmental organisms. Representative members of the microbial community were obtained by directly plating surface waters collected from five locations throughout Oklahoma onto R2A medium without and with triclosan. Based on 16S rDNA gene sequence similarity with *P. aeruginosa* and the relationship to hydrophobic substances, two susceptible and two resistant isolates were selected for further study. Outer membrane permeability properties with regard to hydrophobic compounds were initially investigated using a macrobroth dilution bioassay to determine isolate susceptibility to triclosan and the mechanistically- disparate hydrophobic antibiotic, novobiocin. The nonpolar fluorescent probe 1-N-phenylnapthylamine (NPN) was used to confirm the accessibility of the outer membrane. Batch cultural kinetics revealed growth similar to the control at all concentrations of triclosan for resistant isolates, but the susceptible isolates were inhibited in a manner commensurate with triclosan concentrations. These results support the notion that outer membrane properties limit permeation by hydrophobic substances for environmental *Pseudomonas* species in a manner similar to that of *P. aeruginosa*.

Pretreatment of Plant Biomass Using Fungal and Bacterial Co-Culture.

Swechchha Pradhan (Masters)* and Babu Fathepure.

Oklahoma State University, Stillwater, Oklahoma

Delignification is a critical step in the bioconversion of lignocellulosic biomass into useful monomers. Fungi and bacteria both have evolved pathways to degrade lignin. However, only little efforts have been made to study them together as an integrated microbial system. The major focus of this research is to study lignin degradation using *Pseudomonas* sp. YS-1p and *Phanerochaete chrysosporium* when inoculated as individual cultures as well as in co-cultures with varying ratios of fungi to bacteria on mineral salts medium supplemented with plant biomass. Two different plant biomass were used, including sugarcane bagasse and switchgrass. Flasks were inoculated with *P. chrysosporium* (F) and strain YS-1p (B) at various ratios including 1:0 (F:B), 1:1 (F:B), 1:10 (F:B). 1:50 (F:B) and 0:1 (F:B). Culture samples were withdrawn periodically to assay for lignin degrading enzymes including lignin peroxidase (Lip), dye decolorizing-peroxidase (DyP), laccase and also to monitor population dynamics of the microorganisms used. Our preliminary results show that lignin degrading enzyme activity depended on the type of plant biomass used. Monitoring microbial population dynamics during the course of plant biomass degradation showed that strain YS-1p grew best in the presence of *P. chrysosporium* suggesting that bacteria might have benefitted from degradation products of fungus.

Category III Medical Microbiology / Immunology

Lactobacillus administration alters behavior in the prairie vole (*Microtus ochrogaster*)

Kathleen Ahles (doctoral)*, Senait Assefa, J. Thomas Curtis, and Gerwald A. Koehler

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

The human gut is the home to billions of bacteria which vary greatly from person to person. Dietary changes and exposure to antibiotics or toxins are known to alter gut microbial composition, often leading to intestinal maladies such as irritable bowel syndrome. In addition to the intestinal effects of microbial imbalances, such shifts may also incite changes in behavior through the gut-brain axis. The gut-brain axis is a bidirectional pathway of communication between the brain and the digestive system. Changes in the gut microbiota have been associated with behaviors such as social aversion, increased anxiety, and depression.

The purpose of this study was to explore microbial modulation of the gut-brain axis in the prairie vole (*Microtus ochrogaster*). Though typically a highly social animal, prairie voles exhibit withdrawal following chronic low-level mercury exposure, and this behavioral change correlates with subtle shifts in the gut microbial environment. One strain of probiotic *Lactobacillus* was introduced to the vole via drinking water and its impact on social behaviors, exploratory behaviors, and anxiety was investigated. Significant behavioral effects on all measures were seen not only following mercury exposure, but also after probiotic administration.

Characterization of Vps1 Domain Interaction with ESCRT Subunits

Bryan Banh (Masters)[1]*, Hyoeun McDermott[1], Shiva Kumar Goud Gadila[1], and Dr. Kyoungtae Kim[1]

[1]Department of Biology, Missouri State University, Springfield, Missouri 65897

Vacuolar protein sorting 1 (Vps1) is a dynamin-like GTPase involved in multiple cellular trafficking pathways. Vps1 is a membrane peripheral protein that interacts with an array of intracellular organelles, including peroxisomes, endosomes, vacuoles, and the late Golgi. It appears that Vps1 functions together with a selective group of proteins that reside at each organelle. Though Vps1's implication in membrane remodeling has been well recognized, the mechanisms by which it interacts with its functional partners remain poorly understood. Our yeast two-hybrid assay revealed that Vps1 interacts with at least 4 endosomal proteins, including subunits of ESCRT II and III complexes; Vps22, Vps36, Vps24, and Vps2. Interestingly, Vps1 interacts primarily with the helical domain (HD) at the N-terminal region of Vps22 and Vps36. The investigation of precise domains of Vps24 and Vps2 that bind to Vps1 is in progress. ESCRT II and III complexes are directly involved in the sorting of Cps1 at the endosome for its final delivery to the vacuolar lumen. Upon loss of each subunit of these ESCRT complexes, Cps1 was found to be located at the rim of vacuole or at the E compartment. *vps1*Δ cells displayed a similar defect, albeit less significant compared with ESCRT mutants, suggesting Vps1 being a potential member for Cps1 sorting, possibly functioning together with the above-mentioned binding partners at the endosome. In addition, we have performed a screen for novel Vps1 binding partners in *Saccharomyces cerevisiae* using a Yeast two-hybrid library system. Our results show seventeen as-yet-unidentified Vps1 binding proteins. To validate our results, we have selected two proteins to undergo more stringent assays. Our research may provide more insight into Vps1's diverse roles by understanding its partners at each organelle.

EfhP, a Putative Calcium Binding Calmodulin like Protein, Plays Role in Calcium Induced Responses of *Pseudomonas aeruginosa*

Biraj B. Kayastha (Doctoral) and Marianna A. Patrauchan.

Oklahoma State University Stillwater, OK.

Pseudomonas aeruginosa, an opportunistic pathogen, is the main cause of chronic lung infection and mortality in individuals with cystic fibrosis. We have shown that its virulence and antibiotic resistance is induced by calcium (Ca^{2+}). Earlier, we identified a putative Ca^{2+}-binding calmodulin-like protein EfhP containing two EF-hand motifs. We characterized its role in Ca^{2+}-induced plant infectivity, production of pyocyanin, and oxidative stress resistance. Based on bioinformatic analysis, we predicted that EfhP is anchored into the inner membrane facing its EF-hands into the periplasm and that it preferentially binds Ca^{2+}. To confirm that EfhP binds Ca^{2+} and test whether it undergoes conformational changes upon binding, we have purified the soluble EfhP without the transmembrane region. SDS-PAGE confirmed the purity of the protein and showed that it likely forms dimers. Next, it will be subjected to isothermal titration calorimetry for measuring Ca^{2+} binding affinity. We also plan to study the transcriptional profile of *efhP* in response to Ca^{2+} and other host factors. For this, we are constructing a reporter with *efhP* promoter cloned upstream of the *lux* operon. These are the milestones in studying the role of EfhP in Ca^{2+} signaling and virulence regulation in *P. aeruginosa.*

The Relation between Calcium Homeostasis and Tobramycin Efflux in a Human Pathogen *Pseudomonas aeruginosa*

Sharmily S. Khanam*, Manita Guragain, Afom Bokre, and Marianna A. Patrauchan.

Department of Microbiology and Molecular Genetics, Oklahoma State University, OK.

Pseudomonas aeruginosa is an opportunistic multidrug resistant pathogen causing severe infections. Our group discovered that elevated Ca^{2+} increases the pathogen's tobramycin resistance at least tenfold, and identified six contributing multidrug efflux transporters of Resistance Nodulation Division (RND) family (MexAB-OprM, MuxABC-OpmB, MexEF-OprN, MexJK-OprM, MexXY-OprM and CzcCBA). We also determined that *P. aeruginosa* PAO1 maintains intracellular Ca^{2+} homeostasis, and identified five putative Ca^{2+} transporters involved in this process (PA3920, PA2435, PA2092, PA4614, and PA2604). Interestingly, four of these transporters also contributed to Ca^{2+}-induced tobramycin resistance. Thus, we hypothesized there is a regulatory link between intracellular Ca^{2+} homeostasis and RND-mediated tobramycin efflux in *P. aeruginosa*. By monitoring intracellular Ca^{2+}, we have determined that one of the RND systems, MexEF-OprN, is involved in Ca^{2+} efflux. On the other hand, promoter activity assay showed that Ca^{2+}-induced transcription of another RND transporter, *mexAB-oprM,* is reduced in the PA2435 disrupted mutant with disturbed Ca^{2+} homeostasis, suggesting the role of the latter in transcriptional regulation of the RND system. This provides additional evidence that intracellular Ca^{2+} is regulating physiology of the pathogen, contributing to enhanced protection against antimicrobials and host defenses in response to elevated Ca^{2+}.

Production of monospecific antisera for *Vago* and *virus induced RNA-1*(*vir-1).*

Wilfredo A Lopez (Masters)*, Brad L. Ericson, Darby Carlson, and Kimberly A. Carlson.

University of Nebraska at Kearney, Kearney, Nebraska.

Monospecific antisera production is used for experimental analysis of a protein of interest because it allows functional annotation. In *Drosophila melanogaster*, the genes *Vago* and *virus induced RNA-1* (*vir-1)* are involved in innate immunity during Nora virus infection. However, the antiviral mechanism that *Vago* is involved in is not fully understood and the role of *vir-1* within this mechanism has not been determined. For further experimental analysis, codon optimized proteins were constructed for Vago and vir-1. CD-1 Swiss outbred female mice were injected with either the Vago or vir-1 codon optimized protein for monospecific antisera production. Western Blot analysis demonstrated positive products for both antisera (Vago – 18.1 KDa; vir-1 – 47.4 KDa). The antisera will be used to determine the location of expression of Vago and vir-1, in conjunction with Nora virus infection, and to determine tissue-specificity. This research is novel because previous production of Vago protein was unsuccessful due to poor stability during production and the monospecific antisera for Vago did not exist until now. Ultimately, by creating monospecific antisera for Vago and vir–1 proteins it will allow for future work in determining the antiviral mechanism of action.

Characterization of Mechanism of Action of DASamP2 against *Pseudomonas aeruginosa* by Isolation of Mutants.

Swapna Medichetti(Masters)*, Aniket Sawant[1], Michelle Paulson[1], Christopher Johnson[1], Guangshun Wang[2] and Donald Rowen[1]

[1]Department of Biology University of Nebraska Omaha

[2]Department of Microbiology and Pathology, University of Nebraska Medical Center

Human bacterial pathogens are evolving resistance to antibiotics currently used for therapy. There is a need to develop new strategies to combat resistant microbes such as *Staphylococcus aureus* and *Pseudomonas aeruginosa* which exhibit resistance to many drugs available today. Antimicrobial peptides (AMP) are a diverse group of molecules that possess antimicrobial properties and are being studied for use as antibiotics. Recently a new antimicrobial peptide(DASamP2) designed by Dr. Wang (UNMC) was observed to be effective against both *S. aureus* and *P. aeruginosa* and holds great promise as a potential antibiotic agent. The goal of my project is to determine the mechanism of action and potential targets of DASamP2 by isolating mutants of *P.aeruginosa* with altered resistance to DASamP2. We have performed transposon mutagenesis of a sensitive wild type P. aeruginosa strain and screened for increased resistance to DASamP2. Out of 3600 mutants screened, we isolated 10 mutants that showed increased resistance to DASamp2. We are performing Inverse Polymerase Chain Reaction (iPCR) to identify the gene mutated by transposon insertion. Location of the transposon and the gene disrupted by its insertion will be determined by sequencing. Characterizing the genes will help in elucidating the target and mechanism of action of DASamp2.

CarP, a Putative β Propeller Protein, Plays Role in *Pseudomonas aeruginosa* Response to Calcium

Michelle King (Doctoral)* 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 (Ca^{2+}) induces virulence and antibiotic resistance in *P. aeruginosa*. Earlier we identified a Ca^{2+}-regulated protein, CarP, which was predicted to form a 5 bladed β-propeller structure with a Ca^{2+} binding site in the center. We characterized its role in several virulence-related Ca^{2+}-dependent phenotypes and cell tolerance to high Ca^{2+}. We also showed that the expression of *carP* is regulated by a Ca^{2+} induced two-component system. To further characterize the role of CarP in Ca^{2+}-modulated virulence and adaptation to host, we aim to identify the host factors that regulate the expression of *carP.* For this, we constructed a reporter with *carP* promoter cloned upstream of the *lux* operon, which allows measuring the promoter activity based on the luminescence produced by the *lux* system. In addition to elevated Ca^{2+}, CO_2 and oxidative stressors, H_2O_2, we will test the effect of CF sputum and antibiotics used to treat *Pseudomonas* infections. These data will enlighten us on the potential role CarP plays in regulating Ca^{2+}-induced modulations of *P. aeruginosa* virulence and fitness in response to host environment.

Link Between Toxin Production and Sporulation in *Clostridium difficile*

Brintha Parasumanna Girinathan (Doctoral)* and Revathi Govind.

Kansas State University, Manhattan, Kansas.

Clostridium difficile is the major cause of nosocomial diarrhea and life threatening complication such as pseudomembranous colitis. It produces highly resistant dormant spores during adverse conditions as a form of cell differentiation. The spores serve as infectious vehicle, responsible for transmission of the disease and persistence of the organism in the environment. In *Bacillus subtilis*, pleiotropic regulator SinR inhibits sporulation. A homolog of *sinR* encoding gene present in *Clostridium difficile* genome 630 was predicted to have a similar role. Our transciptome analysis of *tcdR* mutant in R20291 strain indicated low levels of *sinR* and sporulation-specific sigma factors, σ(F), σ(E), σ(G), and σ(K). To understand the role of s*inR* in *Clostridium difficile*, we constructed s*inR* mutant in R20291. However, we noticed significant reduction in sporulation efficiency by *sinR* mutant when compared to wild-type. This study indicate that despite high gene conservation, the function of *sinR* gene alter *significantly in Clostridium difficile*. Our previous study showed that tcdR had a major role to play in sporulation and this data suggest that *sinR* is a positive regulator of sporulation and *tcdR* controls sporulation through *sinR*.

Dynamin Acts Downstream of Ypt6 for Membrane Fusions

Pelin Makaraci (Masters)*, Kyoungtae Kim (Ph.D.), Aria H. McDermot (M.S.).

Missouri State University, Springfield, Missouri.

Membrane recycling is an important cellular process required for cell homeostasis. Lines of evidence showed that Vps1, a dynamin homologue in yeast, is implicated in protein recycling from the early endosome to the late Golgi. The detailed function of Vps1 in this pathway remains elusive. By using the yeast-two hybrid assay, the present research reveals that Vps1 physically interacts with Ypt6, a master GTPase protein in this pathway, and that Ypt6 binds to the GTPase activity domain and the middle domain of Vps1. Moreover, we found that the full-length Vps1 binds to the N-terminal and the C-terminal third of Ypt6. In an attempt to map the domains required for Vps1-Ypt6 interaction, we observed that the full-length version of Vps1 is required for its binding to the domains of Ypt6. In addition, we found that presumable constitutively active Ypt6 mutants (Ypt6 G139E and Ypt6 T24N) and inactive mutant (Ypt6 Q69L) interact with Vps1 with a similar binding affinity when compared with wild type Ypt6. In light of our observation that the overexpression of Vps1 rescued the abnormal recycling phenotype caused by loss of Ypt6, but not vice versa, it is likely that Vps1 functions downstream of Ypt6. Furthermore, the overexpression Vps1 GTPase domain was sufficient enough to rescue the abnormal phenotype in *ypt6*Δ, suggesting that Vps1's active role in the recycling pathway relies on its intrinsic ability to hydrolyze GTP.

Functional Connection between Vps1 and GARP Vps51 at the Golgi in Budding Yeast.

Uma Saimani*(Masters), Ashley Smock and Kyoungtae Kim

Missouri State University, Springfield, Missouri – 65897

Vacuolar Protein Sorting 1 (Vps1), a yeast homolog to human Dynamin, has been implicated to play a prominent role in recycling cellular traffic from the early endosome to the Golgi network. Previous research showed a genetic interaction of Vps1 with Vps51, a component of the GARP tethering complex, which mediates vesicular fusion at the late Golgi. Vps1 consists of an N-terminal GTPase domain, a Middle domain, and a C-terminal GED domain. For elucidating the functional relationship between Vps1 and Vps51, we utilized the yeast two-hybrid system and found that a 33a.a peptide between 99^{th} and 132^{nd} residues near the C-terminal of Vps51 interacts with the GTPase and middle domain. A tentative interdependency between these proteins in their Golgi targeting was assessed by introducing GFP tagged Vps51 into a *vps1*Δ cell, and a threefold increase of the GFP-puncta in number was observed, when compared with wild type cell. For determining if the defect was due to the direct effect of loss of Vps1, we expressed Vps1 using a yeast expression vector in the *vps1*Δ cells, and this rescued the abnormal GFP puncta number. Currently, we are testing a potential *in-vitro* interaction of Vps1 and Vps51 using a GST pulldown assay and working on the physiological significance of the Vps1-Vps51 interaction.

Category IV. Undergraduate or High School Oral Presentation.

Possible Influence of Outer Membrane Permeability Properties on the General Resistance of *Serratia marcescens* to Hydrophobic Molecules.

K. Boyina[2] (Undergraduate)*, M. Hawkins[1, 2], and F. R. Champlin[2].

Union High School[1], Tulsa, OK. Oklahoma State University

Center for Health Sciences, Tulsa, OK.

Serratia marcescens is a gram-negative, opportunistic pathogen that causes certain infections in immunocompromised patients and neonates. The purpose of this project was to determine the susceptibility of *S. marcescens* ATCC 8100 to ten disparate hydrophobic or lipophilic antibacterial agents in comparison with *Escherichia coli* ATCC 25922, *Pasteurella multocida* ATCC 11039, and *Pseudomonas aeruginosa* PA01, gram-negative bacteria having known outer membrane permeability properties for hydrophobic substances. A standardized disc agar diffusion bioassay was employed to determine baseline susceptibility levels for each organism. . Minimum inhibitory and bactericidal concentrations of triclosan and novobiocin were determined for each using a conventional macrobroth dilution bioassay. *S. marcescens* was found to be generally resistant to hydrophobic antibiotics in a manner consistent with the refractory organisms, *P. aeruginosa* and *E. coli*. It was also found to be resistant to the hydrophobic biocide triclosan like *P. aeruginosa,* but unlike *E. coli* and *P. multocida*. . These results support the notion that as with *P. aeruginosa* the outer membrane of *S. marcescens* may be responsible for its resistance to hydrophobic substances in general and triclosan specifically. This is in contrast with the negative control organism *P. multocida* and is presently is being confirmed with further experimentation.

Understanding the Functional Relationship between the Retromer Complex and Vps 1

Mariel Delgado Cruz (Undergraduate)*, Chris Trousdale.

Missouri State University, Springfield Missouri

Membrane trafficking is the process by which cells excrete waste and other elements as well as ingest extracellular substances in order to maintain homeostasis. Specifically, retrograde trafficking follows the movement from the endosome to the Golgi complex. When retrograde transport is disturbed, it has been shown to associate with diseases such as Alzheimer's, Parkinson's, Huntington's and Amyotrophic Lateral Sclerosis. In yeast, retrograde transport is aided by the Retromer, a multi-subunit protein complex which coats the endosome. Here, we present the functional relationship with the Retromer complex and Vps 1, the yeast homologue to mammalian dynamin. Our data revealed that the Vps 1 and Retromer subunits genetically and physically interact. Future studies will look into the physiological significance of the interaction between Vps1 and Retromer in the context of retrograde trafficking.

Isolation of Fermentative Yeasts from Trees.

Matt Gordon (Undergraduate) and Anna Oller.

University of Central Missouri, Warrensburg, MO.

Identification of new yeast strains provides new opportunities important to the flavor profiles of fermented beverage and food industry. This experiment investigated environmental yeast growth on trees, leaves and dehydrated fruit for fermentative properties. Plum, blackberry, apple, cherry and white grape leaves and some with the dried fruit were aseptically collected and weighed between .1 and .16 grams. Each sample was placed into a 100 mL flask of Yeast Extract Broth with chloramphenicol added. Yeasts were grown at 25 C for 48-72 hours at 80 rpm on a shaker incubator. Matte colonies displaying yeast like characteristics were isolated on new Acidic Yeast Malt, Acidic Yeast Malt with Methyl d-glucopyranoside, and TSA plates by adding 10 microliters of broth culture and spreading over plate with sterile spreader. Slides were gram stained for yeast verification. All samples thus far grew yeasts, with some containing unique aromas. Colonies appearing as yeasts were then reisolated onto a second plate for purity and further experiments. Afterwards, isolated yeasts were inoculated into Mannitol Broth tubes containing either a 45% or 50% concentration to biochemically test for fermentation. The tubes, with parafin sealing the top of solution isolating it from the open air, then produced either small to medium amount of bubbles indicating the fermentation possibilities of some of the yeasts. The cultured yeasts displaying fermentative properties will be identified via DNA sequencing.

Prevalence of Pathogenic Bacteria in the American Dog Tick (*Dermacentor variabilis*) in Dawson County, Nebraska.

Nathan Harms (Undergraduate)*, Parth Chaudhari, Dr. Julie Shaffer, and Dr. Brandon Luedtke,

Department of Biology, University of Nebraska at Kearney, NE

The American dog tick (*Dermacentor variabilis*) has a wide distribution that spans the eastern United States and extends into central Nebraska. *D. variabilis* has been known to carry the pathogenic bacteria *Rickettsia rickettsii*, *Ehrlichia chaffeensis*, *Anaplasma phagocytophilum*, and *Francisella tularensis*, which can cause Rocky Mountain spotted fever (RMSF), ehrlichiosis, anaplasmosis, and tularemia, respectively. Very little is known about the risk of contracting these diseases in central Nebraska. To investigate this, 93 male and 64 female ticks were collected from Dawson County, Nebraska to be tested. Total DNA was extracted and subjected to PCR using primers specific for *Rickettsia spp.*, *Ehrlichia spp., Anaplasma spp.,* and *Francisella spp.* followed by gel electrophoresis. Positive PCR reactions for *Ehrlichia spp*. were found in 30 females (47%) and 21 males (23%). No *Francisella spp.* or *Anaplasma spp.* were found in either our male or female samples. *Rickettsia spp.* was found in 4 females (7%) and 2 males (3%) from our samples. Five of the *Rickettsia spp.* positive PCR samples were sequenced, and were all identified as *R. montanensis*, a nonpathogenic relative of *R. rickettsii*. These results suggest that the risk of contracting tick-borne RMSF in central Nebraska is not as high as previously assumed.

Defining the Spectrum of Ultraviolet (UV) Resistance in the UV Resistant Bacteria *Deinococcus radiophilus.*

Diana Hopkins (undergraduate)*.

Rogers State University, Claremore, Oklahoma.

Deinococcus radiophilus is a non-motile, aerobic mesophilic bacteria. It is also ultraviolet (UV) resistant, able to repair its broken genome after exposure to radiation levels which would kill a majority of organisms. This species contains superoxide dismutase (SOD) enzymes which enable UV resistance along with the thick cell wall surrounding the bacteria. It was postulated that *D. radiophilus* would demonstrate inhibited growth, a result of DNA damage, after exposure to long UV light wavelengths as the related species *D*. *radiodurans* does. Long UV wavelengths, UV-A, are 320 to 400 nanometers (nm); UV-B, the medium wavelengths, are 290 to 320 nm; and short wavelengths, UV-C, are 200 to 290 nm. The purpose of this study was to determine which wavelength(s) *D. radiophilus* was most sensitive to. The bacteria were exposed to 4 UV wavelengths (254 nm, 302 nm, 312 nm, and 360 nm) and aliquots were diluted and plated after 1, 3 and 7 minutes exposure. Colony counts were used to determine growth inhibition. It was found that *D. radiophilus* was most affected by 254 nm, in the UV-C range, disproving the original hypothesis and demonstrating that this organism responds differently to UV irradiation from *D. radiodurans*.

Isolation and Characterization of Novel Halo-Acidophilic Microorganisms Present in Hypersaline Lakes from Western Australia.

Ava L. Hughes, Katlyn M. Lonergan, and Melanie R. Mormile.

Department of Biological Sciences, Missouri University of Science & Technology, Rolla, Missouri.

The microbial communities in the acidic hypersaline environments in Lake Magic, Lake Gounter, Lake Gneiss, and Lake Aerodrome in Western Australia are currently unknown. These lakes are of interest due to their pH and salt concentrations, recorded with ranges between 1.4-3.5 pH and 13-32% salt concentration. Halite and gypsum evaporites form a crustal layer within the sediment. Previously, microorganisms have been found to be acidophilic and halo-tolerant, but not halo-acidophilic. With this combination of extreme conditions, we expect novel halo-acidophilic microorganisms to be isolated. Matrices characterizing pH and salt concentration limits have been developed to determine the extent and preferential growth and to help with isolating novel bacteria. Growth results from matrices have led us to believe there are different communities in evaporite and sediment samples. Other analysis, scanning electron microscopy, indicates that diatoms are also present in these environments. Our results will lead to an understanding of this new category of extremophiles.

Gut Microbiome of the Prairie Lizard, *Sceloporus consobrinus*.

Robby King (Undergraduate)*, Mark Paulissen, Cindy R. Cisar.

Department of Natural Sciences, Northeastern State University, Tahlequah, OK.

Few studies have been performed on the gut microbiome of reptiles. In this study, we investigated the gut microbiota of the prairie lizard, *Sceloporus consobrinus*. Cloacal swab samples were collected from 18 prairie lizards captured in northeastern Oklahoma. High throughput 16S rRNA gene sequencing of fecal DNA samples was used to identify bacteria. Bacteria from eleven different phyla were identified in the samples: Acidobacteria, Actinobacteria, Bacteroidetes, Cholorflexi, Cyanobacteria, Deinococcus-Thermus, Firmicutes, Fusobacteria, Gemmatimonadetes, Proteobacteria, and Tenericutes. Only Proteobacteria, Bacteroidetes, Firmicutes, and Actinobacteria were present in all samples. The most abundant phylum was Proteobacteria constituting 60.82 ± 32.64% of total bacteria, followed by Bacteroidetes (17.15 ± 22.27%) and Firmicutes (12.73 ± 14.66%). The remaining phyla were present in low abundance (average < 6% of total bacteria). Comparison of gut bacterial communities in male versus female lizards showed no statistically significant differences. The high level of variability in bacterial community composition among individual lizards contrasts with data obtained from gut microbiome studies of mammals and birds. Further studies into the ecology of *S. consobrinus* could lead to insights on the cause of variability in the gut microbiome of this lizard species.

The Effect of Environmental Factors on Swarming Motility in a Human Pathogen *Pseudomonas aeruginosa*

Breeanna C. Russ (Undergraduate)*, Amber E. Price, Michelle M. King, Marianna A. Patrauchan

Oklahoma State University, Stillwater, OK

Pseudomonas aeruginosa is a ubiquitous bacterium that can be found in a variety of environments such as soil, water, plants, and animals. It is also a human pathogen causing severe infections, and a leading cause of death in Cystic Fibrosis (CF) patients. *P. aeruginosa* possesses many virulence factors, one of which is motility. There are three types of motility in this organism: swimming, twitching, and swarming. Swarming motility is required for biofilm formation, which is another important determinant of *P. aeruginosa* virulence. Here we characterized the effect of several conditions, commonly associated with human lung environment, on *P. aeruginosa* swarming. We have determined that elevated Ca^{2+} increased swarming distance and induced pyocyanin production in the swarming cells. Changing Mg^{2+} concentrations and humidity did not impact swarming distance, but the swarming pattern showed unique concentric circles at low Mg^{2+} and elevated Ca^{2+}. Lowering phosphate levels had no effect in the presence of $MgSO_4$, but significantly reduced swarming in the presence of $MgCl_2$. Low Fe^{2+} showed no effect at ambient levels of CO_2, but significantly increased swarming at 5 % CO_2. These results demonstrate that host environment regulates swarming motility of *P. aeruginosa* and thus defines its ability to spread and cause a disease.

Hitting the Target: Biochemical Interaction of Vps1 and the Golgi.

John Short (Undergraduate)*, Shiva Kumar Goud Gadila, Uma Saimani, Kyoungtae Kim.

Missouri State University, Springfield, Missouri.

Protein trafficking is tightly choreographed and requires proteins to be targeted to their proper destinations. Vps1 is a member of the vacuolar protein sorting family with diverse membrane remodeling roles in protein trafficking and secretion, including endocytosis, Golgi body cargo sorting, and vesicle formation at the Golgi. Though interaction of Vps1 with Golgi lipids was previously reported, the biochemical targeting mechanism of Vps1 to the Golgi remains elusive. To map the domain(s) required for Vps1's targeting to the Golgi, we expressed Vps1 fragments, which were N-terminally fused with mRFP; GTPase, Middle, GED, GTPase-Middle, GTPase-GED, and Middle-GED. The extent of Golgi recruitment of these Vps1 fragments in *vps1*Δ cells was studied, and we found that only the Middle and GED domains targeted to the late Golgi, which was marked by Gga1-GFP. To study the significance of fragment targeting we tested the function of Vps1 sorting of CPY at the late Golgi. Only full-length Vps1 was able to rescue the abnormal CPY sorting defect caused by Vps1 knockout, indicating that Middle and GED domains are required for targeting of Vps1, and that the N-terminal GTPase plays an essential catalytic function for CPY sorting.

Histone Deacetylase HDA5 May Be Involved in Pseudomonas Syringae Triggered Reduction of Host Histone H3K9 Acetylation

Victoria Shum(undergraduate)*, Jennifer Wies[1], Linda Nguyen[1], Michael Visenio[2], and Karin van Dijk[1],

[1]Department of Biochemistry, University of Nebraska – Lincoln, Beadle Center 1901 Vine Street, Lincoln, NE 68588

[2]Department of Biology, Creighton University, 2500 California Plaza, Omaha, NE 68178

Pseudomonas syringae DC3000 employs a type III secretion system (T3SS) to inject effector proteins (T3Es) into plant cells and cause disease. We are interested in determining the extent that *P. syringae* T3Es are involved in modulating gene expression of immunity-related genes via host chromatin to favor pathogenesis. We have found rapid deacetylation of host histone H3 lysine 9 (H3K9) in response to DC3000 but not to a T3SS defective D*hrcC* mutant. Using Chromatin immunoprecipitation we found reduced H3K9 acetylation along a subset of immunity-related genes in the DC3000 infected plants, which correlated with decreased expression. To determine which T3Es are involved in the deacetylation, we analyzed plants infiltrated with poly-effector mutants lacking different combinations of T3E genes. Immunoblot analysis showed no deacetylation in plants infiltrated with a mutant lacking most of the T3Es, affirming a T3E role in deacetylation. We also focused on determining which histone acetyltransferases (HATs) or histone deacetylases (HDACs) participate in deacetylation of H3K9 by analyzing transcriptional changes of HACs and HDACs in *P. syringae*-exposed plants. We found histone deacetylase, *HDA5*, to be upregulated in plants exposed to DC3000 compared to those exposed to *hrcC*. We are currently determining if HDA5 possesses any role in deacetylating H3K9 along immunity-related genes.

Identification of Novel Antimicrobial Proteins from Extreme Halophilic Archaea

Yuliya Kunz (undergraduate)*, Dr. Ratnakar Deole. Northeastern State University, Broken Arrow, Oklahoma.

Hypersaline environments are inhospitable to life due to their high salt concentration, which is hygroscopic, and causes cell desiccation and death. However, such harsh environments provide a dwelling for certain microorganisms, which have acquired the ability to survive in these conditions. Archaea, Bacteria and Eukarya have evolved to proliferate in hypersaline environments. Studies on biodiversity of hypersaline ecosystems have determined archaea as the most prevalent denizens. Extreme halophilic archaea (haloarchaea) belong to the single *Halobacteriaceae* family, which thrive in environments with NaCl concentration as high as 1.5-5.0 M. Selective advantage for halophilic archaea in such harsh environment could be due to production of proteinaceous antimicrobials, called halocins. Unlike antimicrobial compounds of bacteria and eukaryotes, halocins have not been well characterized yet. Their antimicrobial properties against the pathogenic microorganisms are still unknown. Therefore, extreme halophilic archaea is an untapped source of novel antimicrobial compounds which could be developed into potential antibiotic medications, possibly aiding in conquering some drug-resistant pathogens. In this project soil samples from the Great Salt Plains, Oklahoma were screened for halocin producing haloarchaea. Isolates showing antimicrobial properties and their halocins were identified and tested using biochemical techniques and molecular biology analyses such as ribotyping and PCR.

Undergraduate or High School Poster Presentation

Antibiotic Resistance of *Staphylococcus aureus* Recovered from Cystic Fibrosis Patients

Wade Arthur* (Undergraduate)[1], Rawan Eleshy (PhD)[1], Night Mehdi[2], and Erika Lutter[1]

[1] Department of Microbiology and Molecular Genetics, Oklahoma State University, Stillwater, OK [2] Oklahoma Cystic Fibrosis Center, University of Oklahoma Health Sciences Center, Oklahoma City, OK

Cystic Fibrosis (CF) is an autosomal recessive condition which is caused by a mutated CFTR. The CFTR protein is located on epithelial cells throughout the human body causing CF to occur in multiple organs. However, symptoms of CF are most commonly seen in the lungs. A mutated CFTR gene leads to dehydration of the lung's airways and also traps mucus inside the lungs. Many recent studies that look at the complexity of these infections have suggested that many different types of bacteria persist in the CF lung and acquire antibiotic resistance, which significantly hinders treatment options and impacts patient mortality. Our laboratory has collected many CF patient samples from which the pathogens have been recovered and stored. To better understand how the antibiotics affect the pathogens from CF patients, Kirby-Bauer disc diffusion tests were performed using the recovered CF pathogens. The Kirby-Bauer discs used the *S. aureus* isolates and different antibiotics look for antibiotic resistance. The results demonstrate that there is a high degree of resistance to multiple antibiotics by *S. aureus* isolates and that the resistance phenotypes vary between patients. Understanding the level of antibiotic resistance of these isolates will give insights into future treatment options for these patients.

Identification of Transposon insertion sites in Chlamydia

Melissa Berger (Undergraduate)*, Kelly Harrison, Greg Peterson, Jason Wickstrum, Scott Labrie,

P. Scott Hefty

Department of Molecular Biosciences, University of Kansas, Lawrence, KS, USA

Chlamydia trachomatis is a gram-negative, obligate intracellular pathogen and the most commonly reported bacterial STI in the United States and worldwide. *Chlamydia* infections result in a range of health issues from pelvic inflammatory disease and sterility, to blindness or pneumonia. Despite the significant public health impact, many aspects of *C. trachomatis* pathogenesis are poorly understood, which is largely due to the limited amount of genetic tools currently available. Transposon mutagenesis offers a strategy for discovering specific genetic components that are associated with a given phenotype. This genetic tool has been utilized in many pathogens, resulting in an enhanced understanding of basic biological processes, and the discovery of gene products associated with host infection and pathogenesis. However, a transposon mutagenesis system has not been developed for *C. trachomatis*. In this study, a library of transposon mutants was created, and insertion sites were identified using an arbitrary PCR technique. Insertions were confirmed using flanking primer PCR. As a result of this study, 13 different transposon mutants were identified, with insertions in a several genes, including 4 hypothetical proteins and an intragenic insertion. This and future studies will introduce new insight into chlamydial biology, potentially leading to innovative pathogen-specific antibiotic or vaccine developments.

Metabolically Engineered *Gluconobacter oxydans* for the Production of Optically Pure Acetoin: a Pharmaceutical Precursor.

Neil Bolduc (Undergraduate)*, Dr. Paul Schweiger.

Missouri State University Department of Biology, Springfield, Missouri.

Gluconobacter oxydans belongs to a distinct group of acetic acid bacteria known for their unique ability to incompletely oxidize substrates under normal growth conditions, releasing products into the medium. This feature is biotechnologically relevant as the incompletely oxidized products are often stereo- and regio-selective. This unique metabolism is dependent on membrane-bound dehydrogenases that channel electrons from substrates into the respiratory chain. These dehydrogenases are natural biocatalysts that simplify the production and recovery of enantiopure chemicals, which normally require expensive and troublesome organic chemistry to produce, providing a route to sustainable green chemical biomanufacturing. One aim is to metabolically engineer *G. oxydans* for the production of enantiopure acetoin. Acetoin was designated a top 30 platform chemical by the US DOE and is used to produce pharmaceuticals, cosmetics, food flavorings, and liquid composites. Two *G. oxydans* enzymes are predicted to be important for acetoin production: 1) a known PQQ-dependent polyol dehydrogenase (SldBA), and 2) an uncharacterized FAD-dependent sorbitol dehydrogenase (mSDH). To rationally design *G. oxydans* for enantiopure acetoin production we are investigating the role of mSDH in biomanufacturing by analyzing deletion mutants and expression strains. This information will be used to produce strains for improved sustainable green chemical biomanufacturing of enantiopure acetoin.

Metarhizium Adhesins and Attachment.

Susie Brown[1] (Undergraduate)*, Kesha Pilot[1], Stefan Jaronski[2] and Cynthia Fuller[1].

[1]Henderson State University, Arkadelphia AR 71923; [2]USDA ARS Northern Plains Research Laboratory, Sidney, MT, 59270

Metarhizium species are insect pathogenic fungi that inhabit soil, thought to also reside in the rhizosphere of plants and engage in endophytic nitrogen transfer from insects to plants. Several species of *Metarhizium* are currently used as microbial insecticides. Root colonization of crops with fungal insecticides could provide safe and effective protection against insect pests attacking those roots. Successful rhizosphere colonization has been inconsistent. *Metarhizium* adhesin protein two (MAD2) allows for attachment to plants. While a functional *mad2* gene has been confirmed in strains of *Metarhizium* that colonized roots, it is unknown whether additional strains being developed as microbial insecticides possess a functional copy of the *mad2* gene. This study assessed whether *mad1* and *mad2* genes are present in *Metarhizium* being developed as insecticides. Students designed primers for *mad1* and *mad2*, extracted fungal DNA and performed polymerase chain reaction (PCR). Both *mad1* and *mad2* genes are present in four of five strains tested. Functional expression of the MAD2 protein is currently being assessed using onion skin attachment assays. The commercial *M. brunneum* strain Strain F52 appears to attach to both yellow and sweet onion epithelium but conidia did not germinate at 24 hours. Future experiments will be discussed.

Identification and Characterization of Essential Genes Involved in the Regulation of Peptidoglycan Synthesis in *Agrobacterium tumefaciens*

Caroline Dunn (Undergraduate)*, Wanda Figueroa-Cuilan, Jeremy J. Daniel, and Pamela Brown.

University of Missouri, Columbia, Missouri.

The bacterial plant pathogen, *Agrobacterium tumefaciens*, restricts new cell wall biogenesis to the cell pole during elongation and to the mid-cell during cell division. We hypothesis that some of the genes involved in the regulation of cell wall biogenesis will be essential for cell survival. In this study, we focus on 26 proteins encoded by essential genes with domains predicted to interact with cell wall or which are annotated as hypothetical proteins. Phenotypic characterization of the overexpression strains in the presence and absence of inducer includes growth curve data analysis and viable plate counts. These experiments are conducted in varying NaCl concentrations to determine if mutant strains are sensitive to osmotic pressure. Epifluorescence microscopy is used to monitor cell morphology, DNA structure, and sites of cell wall biogenesis in the mutants. Thus far, 5 overexpression mutants have been constructed and subject to preliminary characterization. Two possible candidates for genes involved in the regulation of cell wall biogenesis have been identified thus far based on salt sensitivity observed in growth curves and unusual cell morphologies. Future work will involve the construction of depletion strains to observe the impact of removing these essential genes on cell wall biogenesis in *A. tumefaciens*.

The Cra-FruK Complex Alters Regulation of Central Metabolism in γ-proteobacteria.

Max Fairlamb (Undergraduate)*, Dipika Singh, Chamitha Weeramange, Benjamin Rau, Sarah Meinhardt, Sudheer Tungtur, Dr. Aron Fenton, Dr. Liskin Swint-Kruse.

University of Kansas Medical Center, Kansas City, Kansas.

For pathogenesis, several γ–proteobacteria require the central transcription regulator Cra (catabolite repressor activator). In *E.coli*, Cra binding to DNA operators directly alters transcription of over 100 genes; those encoding glycolytic enzymes are repressed, whereas those for gluconeogenesis and the Krebs cycle are activated. Cra-DNA binding is allosterically diminished when Cra binds fructose metabolites: either micromolar levels of fructose-1 phosphate (F-1-P), which is known to be generated upon fructose import; or millimolar levels of fructose-1,6-bisphosphate (F-1,6-BP). F-1,6-BP is generated from F-1-P by the enzyme fructose-1-kinase (FruK) or from metabolism of other sugars and is a key intermediate in glycolysis. Here, we present several new aspects of Cra regulation: First, Cra directly interacts with FruK to form a tight protein-protein complex. Second, F-1,6-BP enhances either the Cra/FruK interaction and/or CRA/FruK complex binding to DNA. Third, FruK catalyzes the reverse reaction of F-1,6-BP to F-1-P, providing a pathway for the synthesis of F-1-P in the absence of fructose. Finally, since FruK itself is repressed by Cra, these newly-reported events add layers to the dynamic regulation of central metabolism in response to changing nutrients. We anticipate that all of these steps must be carefully balanced to allow pathogenesis of many γ–proteobacteria.

Yeast-two hybrid analysis of *Chlamydia trachomatis* type III secreted effector proteins

Jordan Fleming, Amanda Behar, and Erika Lutter

Department of Microbiology and Molecular Genetics, Oklahoma State University, Stillwater, OK, USA

Chlamydia trachomatis is a very well-known sexually transmitted infection that affects millions of people on an annual basis. As an obligate intracellular pathogen it requires the host cell for survival and usurps host cell resources. After *Chlamydia* gains entry into the host cell it begins to secrete proteins, called effector proteins, through a type III secretion system that interacts with yet undetermined host cell proteins. Some of these proteins will insert themselves into the inclusion membrane, while others will be secreted into the host cell milieu. To date, little is known about what these secreted effectors do and what host proteins they interact with during an infection. To help determine the role of these secreted effectors, the corresponding Chlamydial genes were cloned into Yeast-two-hybrid bait vectors, transformed into yeast and screened against a HeLa cDNA library for interacting partners. Positive clones that interact with the bait proteins have been identified. Current efforts focus on sequencing the interacting prey to identify potential host proteins that are interacting with these Chlamydial effector proteins.

Microbial Extracellular Enzyme Activity Responses to Long-Term Nitrogen Addition and Annual Burning in Tallgrass Prairie Soil

Victoria Floyd (undergraduate)*, Lydia Zeglin.

Kansas State University, Division of Biology, Manhattan, KS

Land management affects availability of nutrients to microbial cells living in the soil. In tallgrass prairie, annual burning reduces soil nitrogen (N) availability, while lack of fire allows organic matter to accumulate. Because both N and carbon (C, from organic matter) can limit growth and activity of microbial cells, we predicted that microbial extracellular enzyme activity (EEA) would differ with contrasting land management history. We predicted that the activity of N-acquiring enzymes would be lower, and the activity of C-acquiring and phosphorus (P)-acquiring enzymes would be higher, in both unburned soils and fertilized soils, due to increased cellular demand for C and P under less N-limiting conditions, and that EEA would change seasonally. To evaluate these predictions, we measured a suite of EEAs in soils from a long-term N fertilization and annual burning experiment at the Konza Prairie Biological Station. Results include lower N-acquiring N-acetylglucosaminidase activity in fertilized soils, and higher C-acquiring cellobiohydrolase activity in unburned soils, supporting our predictions. EEA changed over time, but less dramatically than expected, suggesting that microbial enzymatic responses to short-term changes in temperature and nutrients are relatively well buffered. Soil microbial enzyme production responds to land management practices that affect overall nutrient availability.

Investigations into the Role of MARCKS during *Coxiella burnetii* Infections of THP-1 Cells.

Christa Jackson (undergraduate)*, Sara Whitlock (undergraduate), and C. Joel Funk

Dept. of Biology, John Brown University, Siloam Springs, AR

Coxiella burnetii is a zoonotic intracellular bacterium that causes human Q-fever. When inhaled, *C. burnetii* is phagocytized by alveolar macrophages, inducing the host cell phagolysosome to develop into a parasitophorous vacuole (PV), a lysosome-like compartment. As *C. burnetii* replicates within the PV, it secretes effectors into the host cell using a Type IVB secretion system (T4BSS). Previous research has shown that disruption of host cell signaling by *C. burnetii* results in activation of PKC and that phosphorylation of MARCKS, a PKC substrate, is significantly increased during the infection. The objective of this study was to examine the role of MARCKS using specific inhibitors. The level of MARCKS protein was knocked-down using siRNA treatment of infected HeLa cells. These infected cells contained *C. burnetii*, but many PV's were larger in size and contained fewer bacteria than those of infected cells treated with control siRNA. A peptide inhibitor of MARCKS appeared to have a similar effect on infected THP-1 cells, causing enlarged PV's compared to control peptide treatments. These results indicate that MARCKS may have a role in controlling the size of *C. burnetii*-induced PV's through manipulation of the host cell PKC signaling system.

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

Abbi J. Mabary (Undergraduate)*, Angeline E. Rodriguez, Sohnya Kleiman, and Christopher Lupfer.

Department of Biology, Missouri State University, Springfield, MO.

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, like NLRP3, have the ability to activate Caspase-1 and form the "Inflammasome", which is a multiprotein complex responsible for cleaving the potent inflammatory cytokine interleukine-1β (IL-1β). Another NLR, NLRP12, functions as a regulator of inflammation, thus serving as a negative feedback mechanism. Although the general function of these two proteins is known, how they are activated is not known. We are, therefore, embarking on a journey to find novel proteins that interact with NLR proteins in an effort to decipher the mechanisms by which they function. We are generating a yeast 2-hybrid system to examine the interaction of NLR proteins with a human cDNA library. Novel interactions discovered through this 2-hybrid screen should provide novel insight into the function of these NLR proteins and help us understand the immune response to infectious and non-infectious diseases.

The Role of Insect Vectors in the Movement of a Plant Virus in Northeastern Kansas.

Matthew A. Ramos (Undergraduate)*, Dorith Rotenberg, Alma Laney, Alexandria Kieffaber, and Anna Whitfield.

Kansas State University Department of Plant Pathology, Manhattan, Kansas.

Barley yellow dwarf is a plant disease caused by yellow dwarf viruses (YDVs) in the family *Luteoviridae*. YDVs are named for the symptoms displayed by infected plants: yellowing and stunting. The project goals are to determine the incidence of virus and of the aphids that function as vectors in agricultural and native grass systems in Northeast Kansas. Native grasses are hypothesized to function as a viral reservoir. Aphid populations were monitored using yellow sticky cards and the most common species identified via morphological features. The most numerous vector was bird cherry oat aphid, followed by rice root aphid, English grain aphid, and greenbug. We found many of the same aphid species overlapping between crops and native grasses. Multiplex reverse transcriptase PCR (RT-PCR) was used for virus detection in plant and aphid samples collected from fields. Samples of both types tested positive for YDV viruses, with barley yellow dwarf virus (BYDV) PAV and BYDV-PAS being the most prevalent. PCR results indicated aphids and plants were infected with either a single virus, or a coinfection of multiple viruses. We will continue monitoring aphid abundance and colonization of agricultural and native grass fields, correlating this with the presence of virus in Northeastern Kansas.

Improving Inflammation in Bacterial Coinfection by IL-1β Regulation.

Angeline Rodriguez (undergraduate)* and Abbi Mabary, Christopher Lupfer PhD.

Department of Biology, Missouri State University, Springfield, MO.

Viral bacterial coinfections have been known to cause severe pneumonia. Antibiotics like β-Lactams kill the bacteria, yet cause inflammation in the process. Interlukine-1β (IL-1β) is an important immune signaling molecule responsible for inflammation. It exists as an inactive precursor that can be activated by the Caspase-1 containing inflammasome (multi-protein complex). Influenza A virus and *Streptococcus pneumoniae* are common coinfecting pathogens and previous reports indicate that IL-1β levels are dramatically elevated during this coinfection. However, how IL-1β levels increase and their importance in coinfection is not known. We have discovered that coinfection results in the activation of multiple immune signaling pathways simultaneously, which leads to dramatically elevated levels of IL-1β. In the future, we will examine additional pathways and test inhibitors of IL-1β as therapies to control inflammation during viral bacterial coinfections.

Determining the Interaction Between CT228 and MYPT1 in *Chlamydia trachomatis*

Brooke Romine* (Undergraduate), Amanda Behar and Erika Lutter.

Oklahoma State University, Stillwater, Oklahoma.

Chlamydia trachomatis is a human pathogen responsible for an array of diseases. *C. trachomatis* infections are the most commonly reported bacterial sexually transmitted infections, with 3 million cases estimated annually in the United States. Despite the longevity of health concerns, there are still fundamental gaps in our understanding of *Chlamydia* pathogenesis, specifically in regard to the mechanisms used to manipulate host proteins for intracellular survival and growth. One of the host proteins identified to be recruited during infection is myosin phosphatase. This interaction is significant since changes in myosin regulation are critical for the development and proliferation of cancer cells. *Chlamydia* is the first bacterial pathogen identified to manipulate myosin phosphatase during infection, and does so via the chlamydial protein CT228. Our central hypothesis is that truncations in CT228 will be deficient in interacting with MYPT1 in a yeast-two-hybrid system. Three CT228 truncations were generated with each truncation removing 10 amino acids from the end of CT228. Using a yeast-two-hybrid system, the CT228 truncations (bait) and MYPT1 (prey) were assessed for interaction by using restrictive media. Understanding the interaction between CT228 and MYPT1 may shed significant insights into how a *Chlamydia* infection may lead to increased rates of cervical cancer.

Generation of Improved Oncolytic Poxviruses.

Adam J. Schieferecke (Undergraduate)*, Chen Peng, Sherry L. Haller, and Stefan Rothenburg.

Division of Biology, Kansas State University, Manhattan, Kansas.

A promising new weapon in the fight against cancer is the use of oncolytic viruses that possess the ability to selectively kill cancer cells and thus have great theraputic potential. Myxoma virus (MYXV) is a poxvirus that cannot establish an active infection in humans but possesses the ability to infect and lyse some human cancer cells. However, some other human cancer cells are resistant to MYXV infection, which might be explained by increased expression and activation of the antiviral protein kinase R (PKR). We show that the MYXV protein M156 inhibited rabbit PKR but did not inhibit human PKR, whereas M156 orthologs from other poxviruses, such as deerpox virus (DPXV) and raccoonpox virus (RCPV), showed strong inhibition of human PKR. The lack of inhibition of human PKR by M156 might explain why MYXV has limited oncolytic activity in some human cancers. In order to improve the oncolytic potential of MYXV, we incorporated either the DPXV or RCPV orthologs in place of M156 in MYXV and obtained two recombinant viruses. Our preliminary data suggests that the recombinant viruses replicated better in some human breast cancer cells than wild-type MYXV, which indicates improved oncolytic potential.

The Search for Antibiotic-Producing Bacteria at Henderson State University.

Richard Spencer-Cole (Undergraduate)*, Sarah Johnson (Undergraduate)*, Daniel Morgan, Payten Frunzi, and Cynthia Fuller.

Henderson State University, Arkadelphia, AR 71923

The rising threat of antimicrobial resistance is a worldwide health and economic challenge. The United States Centers for Disease Control (CDC) and the Executive Branch, as well as the World Health Organization (WHO), have released reports outlining the serious threat of antimicrobial resistance and proposing strategies to combat antibiotic resistant bacteria, including development of new antibiotics. Henderson State University's Introduction to Microbiology Classes participate in the Small World Initiative, a program that seeks to crowd-source discovery of novel antibiotics in local soil samples, and involve undergraduates in research activities aimed to increase retention and interest in pursuing careers in science. Students screened soil samples for antibiotic producing bacteria using safe relatives of common pathogenic bacteria. Numerous antibiotic producing bacteria have been obtained. Several isolates have been identified to the genus level using 16s rRNA sequences and represent a diverse spectrum of gram negative and gram positive bacteria. Research is still in progress to identify additional antibiotic producers, as well as to characterize potential antibiotics. Potential antibiotics from bacterial isolates were extracted with water or ethyl acetate, dried, and showed activity against several safe relatives.

Graduate Student Poster Presentation Abstracts

Bioavailability Of B20 Biodiesel Fuel Components And Their Relative Contribution To Microbiologically Influenced Corrosion

Oderay C. Andrade (Masters)*, James Floyd, Blake W. Stamps, Bradley S. Stevenson. University of Oklahoma, Department of Microbiology and Plant Biology.

Richard C. Striebich, Oscar N. Ruiz. Fuels and Energy Branch, Aerospace Systems Directorate, Air Force Research Laboratory, Wright-Patterson AFB**.** Wendy J. Crookes-Goodson. Soft Matter Materials Branch, Materials and Manufacturing Directorate, Air Force Research Laboratory, Wright-Patterson AFB.

B20 fuel is a blend of diesel and single chain fatty acid methyl esters (FAME) derived from different animal or plant sources. Depending on the raw material (soy, canola, palm, tallow, among others), biodiesel feedstocks vary in their FAME profile. This research focused in understanding how microbial activity and biodegradation are influenced by nutrient availability in B20 fuel systems. To assess the susceptibility of current B20 feedstocks, we obtained a number of biological isolates from fouled B20 storage tanks. Then, we designed a series of biodegradation experiments to provide a quantitative measurement of the specific components of the fuel that are consumed by the isolates. Also, the chemical composition of B20 samples obtained from the tanks were analyzed, and patterns were find in the fuel that can potentially be linked to different rates of contamination. A correlation between laboratory experiments and field observation was possible using linear discriminant analysis. The data suggest that the fungal isolates are capable of degrading B20 and might have a preferential consumption for fuel components like Palmitic Acid Methyl Ester. Our final goal is to provide a blueprint for the optimal blend of FAME in biodiesel that potentially limit microbial degradation associated with B20.

Genetic Manipulation of *Chlamydia trachomatis* Inclusion Membrane Protein CT228 using the Adapted TargeTron System

Amanda Behar (PhD)*[1], Cayla Johnson[2], Derek Fisher[2] and Erika Lutter[1 1]

Department of Microbiology and Molecular Genetics, Oklahoma State University, Stillwater, OK, USA; [2] Department of Microbiology, Southern Illinois University Carbondale, Carbondale, IL, USA

Chlamydia trachomatis is the most frequently reported bacterial sexually transmitted infection. Even after a *C. trachomatis* infection is treated, there is an increased risk for the development of pelvic inflammatory disease and cervical cancer, but the mechanisms are poorly understood. As an obligate intracellular pathogen, *C. trachomatis* usurps many host cell-signaling pathways from within a membrane bound vacuole, called an inclusion. *C. trachomatis* is also known to synthesize and secrete via the type III secretion system, inclusion membrane proteins (Incs) that insert into the inclusion membrane and serve as the interface between *Chlamydia* and the host. *C. trachomatis* is the first bacterial pathogen observed to recruit myosin phosphatase (MYPT1) for means of host cell exit, and does so through the chlamydial protein, CT228. The chlamydial TargeTron system was used to genetically inactivate *CT228* in the *C. trachomatis* genome. TargeTron insertion was confirmed by PCR and expression of the *CT229-CT224* operon of the mutant was verified to rule out polar effects. The mutant was verified to be deficient in CT228 production and MYPT1 recruitment by immunofluorescence. This study demonstrates successful gene inactivation of the chlamydial protein CT228 and confirms the role of CT228 in MYPT1 recruitment.

Antibiotic Resistance in *Staphylococcus aureus* Isolated From Cystic Fibrosis Patients

Rawan Eleshy (PhD)*[1], Night Mehdi[2,] and Erika Lutter[1]

[1] Department of Microbiology and Molecular Genetics, Oklahoma State University, Stillwater, OK, USA; [2] Oklahoma Cystic Fibrosis Center, University of Oklahoma Health Sciences Center, Oklahoma City, OK

Cystic fibrosis (CF) is a common genetic disease caused by a mutation in the CFTR gene. The mutation leads to dehydrated thick mucus and impaired mucocillary clearance. Thus, the environment within the CF lung airways becomes ideal for bacterial colonization. *Staphylococcus aureus* is one of the first colonizers in the CF lung and is prevalent throughout the life of CF patients. It is very adept at developing resistance to antibiotics; therefore, it is of great concern to the medical community. This study investigates the prevalence and presence of antibiotic resistance genes within *S. aureus* isolates from CF patients of various ages. Prior studies identified nine genes that are correlated with antibiotic resistance in CF patients. Isolates were tested for the presence of any of these resistance genes by PCR. Susceptibility tests were performed to determine if these isolates show a resistance phenotype. Surprisingly, some isolates contained the resistance genes, but did not show resistance in the susceptibility tests. Other isolates showed resistance on Kirby-bauer plates without the presence of genes. Understanding the antibiotic resistance mechanisms of *Staph aureus* isolates from CF patients will provide significant insights into the complexity of CF infections and may help in future patient treatments.

Investigation of Microbiologically Influenced Corrosion in B20 Biodiesel and the Development of Accelerated Testing Methods.

James G. Floyd (Doctoral)*, Blake W. Stamps[1], Oderay C. Andrade[1], and Bradley S. Stevenson[1]. Wendy J. Goodson[2].

The University of Oklahoma[1]. Air Force Research Laboratory, Materials and Manufacturing Directorate[2].

As part of its 2013 Strategic Energy Plan, the US Air Force has been increasing the use of alternative fuels in ground vehicles and equipment. In compliance with this plan, many military bases are now using B20 biodiesel and have infrastructure dedicated to its storage and dispensing. This fuel is an 80:20 blend of petroleum-derived ultra low sulfur diesel and single chain fatty acid methyl esters (FAME) derived from plant or animal fats. FAME and petroleum diesel have similar physical and chemical properties; however, biodiesel contains more oxygen, is more hygroscopic, and is more oxidatively unstable. This potentially increases the susceptibility to microbial contamination and degradation when compared to 100% petroleum diesel. Investigation of B20 fuels at two AFBs showed that microbial contamination was the root cause of reported issues with fuel quality (color, clarity, particulates). Molecular characterization of the microbial assemblages showed that these fuels harbored a high concentration of a few fungal and/or bacterial taxa. The research described here focuses on investigating the potential increase in corrosion risk as a consequence of microbial contamination of B20 and development of an accelerated MIC test for materials associated with the use, storage, and dispensing of fuels.

Prevalence of Non-O157 ShigaToxin-Producing *Escherichia coli* (STEC) in Houseflies (*Musca domestica* L.) from Cattle Feedlots and Dairies

Rukmini Puri Giri[1], Anuradha Ghosh[2#*], Jessica Thomson[1], and Ludek Zurek[1, 2]

[1]Department of Entomology, [2]Department of Diagnostic Medicine and Pathobiology,
Kansas State University, Manhattan, KS
#Present address: Department of Biology, Pittsburg State University, Pittsburg, KS

Cattle are asymptomatic reservoirs of Shiga toxin-producing *Escherichia coli* (STEC), a major foodborne pathogen. These bacteria are released to the environment in animal feces. The present study aimed to assess the prevalence of seven non-O157 STEC serogroups (O104, O26, O45, O145, O103, O121, and O111), in houseflies from confined cattle environments. Houseflies were collected in summer and fall from a total of nine cattle feedlots and three dairy farms. They were surface sterilized, and individually tested for STEC by: 1) direct plating and 2) enrichment followed by immunomagnetic separation. Individual serogroups and virulence traits were confirmed using multiplex PCR. Concentration of enterics ranged from 4log–7log CFU/fly. Out of 463 houseflies, 159 (34.3%) carried *E. coli* serogroups of interest (O104, O103, O45, O121, O26, and O145) and 34 (7.3%) houseflies were positive for >1 serogroups. STEC was found in 7 (1.5%) flies. *Escherichia coli* O103 and O104 harbored *stx1* and *ehxA* while O45 carried *stx1*, *eae,* and *ehxA.* STEC prevalence in houseflies in cattle feedlots was relatively low; however, due to very large fly populations, this represents many houseflies carrying STEC. Houseflies may play a role as a vector of non-O157 STEC because of their synanthropic nature and unrestricted movement.

Discovery of Genetic Correlates Encoded by *Chlamydia* that are Important for Mammalian Infection

Kelly Harrison[1] (Doctoral)[*], Robert J. Suchland[2], Brian Brunelle[3], Scott LaBrie[1], P. Scott Hefty[1]

[1]Department of Molecular Biosciences, The University of Kansas, Lawrence, Kansas

[2]Department of Medicine, University of Washington-Seattle, Seattle, Washington

[3]National Animal Disease Center, ARS, USDA, Ames, Iowa

Chlamydial infections are the most commonly reported bacterial STI and the leading cause of preventable blindness worldwide. Notwithstanding this impact on public health, chlamydial pathogenesis remains poorly understood. One feature of *Chlamydia* biology is the variable host specificity and infectability despite highly similar genomic content between strains. An essential property of infection differential between murine (*C. muridarum*) and human (*C. trachomatis*) species is the ability to ascend to the upper genital tract using a mouse infection model: *C. trachomatis* is unable to ascend, whereas *C. muridarum* readily ascends leading to pathology. To investigate possible genetic factors associated with these phenotypes, we employed the ability of *Chlamydia* to undergo lateral genetic exchange between species. Genetic hybrids of *C. muridarum* and *C. trachomatis* were isolated and evaluated for their ability to ascend. Genomes of hybrids were then compared to parental strains to identify regions of genetic variance. Between two hybrids, recombination in *C. trachomatis* occurred near the same regions, losing hypothetical genes and gaining genes unique to *C. muridarum*. Upon analysis of murine infection, however, no detectable ascension or pathology was observed. Further investigations utilizing genetic hybrids may identify correlates of host- and disease-specificity and elucidate critical steps in chlamydial pathogenesis.

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.

Phenotypic analysis of Transposon mutant strains of Chlamydia trachomatis

Scott Labrie (Doctoral Student)*, Kelly Harrison, Greg Peterson, Jason Wickstrum, P. Scott Hefty**,**

Department of Molecular Biosciences, University of Kansas, Lawrence, KS, USA

Chlamydia trachomatis is a gram-negative, obligate intracellular pathogen and, worldwide, is the most commonly reported bacterial STI. *Chlamydia* possesses a unique bi-phasic developmental cycle involving an initial infection of the host cell by a metabolically inert Elementary Body (EB). Upon entering the host cell, the EBs form an inclusion and convert to a metabolically active Reticulate Body (RB). RBs divide and begin reversion back to EBs 18-24 hours post-infection. Eventually, the bacteria exit the cell through lysis or extrusion, allowing EBs to infect neighboring cells. Genetic factors involved in this developmental cycle remain elusive due to the lack of genetic techniques capable of a more comprehensive analysis. This study utilizes a novel transposon mutagenesis system, which has not been previously developed for *Chlamydia*. A library of 13 transposon mutants was created and used to infect host cell monolayers for phenotypic analysis. Morphology and development was assessed by confocal microscopy. Transposon mutants yielded no apparent abnormalities with the exception of Tn::CT696— a hypothetical, cytosolic product with no similarity to a previously defined protein. Analysis showed a reduced ability for the mutant to develop inclusions, lack of nuclear trafficking, and abnormal aggregations of RBs. These observations will offer insight into uncovering the unknowns of *Chlamydia,* and aid in development of methods to combat its associated diseases

Function of Sigma 54 in *Chlamydia trachomatis*

Megan McKinney (Doctoral Student)*, Stephanie Schremmer, Greg Peterson, P. Scott Hefty**,**

Department of Molecular Biosciences, University of Kansas, Lawrence, KS, USA

Chlamydia trachomatis is an obligate intracellular pathogen and is the most common bacterial STI worldwide. *Chlamydia* infections can cause pneumonia, pelvic inflammatory disease, and blindness with over 1,000,000 annual cases reported in the USA alone. In spite of this, basic biology of *Chlamydia* remains poorly understood, including transcriptional regulation of many genes. Sigma (σ) factors have been shown to be essential in initiation of transcription due to their interactions with RNA polymerase. In *Chlamydia*, σ54 has been the least studied, and its effects on gene expression are currently unknown. σ54 is regulated by a two-component system involving the sensor kinase, CtcB and the hexamer-forming kinase, CtcC, containing an ATPase domain. This allows the σ54 and RNA polymerase-holoenzyme to initiate transcription of specific genes. Since it is unknown which genes σ54 is responsible for transcribing, an inducible expression vector containing the ATPase domain was transformed into *Chlamydia*. Expression of candidate genes (based on promoter sequences) was subsequently measured by Droplet Digital PCR. Preliminary studies demonstrated an upregulation of CT683 (tetratricopeptide repeat protein), however, studies to confirm this observation are currently ongoing. Future studies will further define the role of σ54 in transcriptional initiation, offering new insights into the pathogenicity of *Chlamydia.*

Functional importance of the N-terminal region of Photoactive yellow protein

Farzaneh Moghadam, Danny Maples, Masato Kumauchi and Wouter D. Hoff

Department of Microbiology and Molecular Genetics, Oklahoma State University, Stillwater, OK, USA.

PYP is a small water soluble protein from the purple sulfur bacterium *Halorhopsira halophile*. This protein functions in bacterial behavior as the photoreceptor of a repellent response of bacteria towards blue light. The absorption of a blue light photon by its p-coumaric acid (pCA) chromophore results in a fast trans-sis isomerization that causes a structural change. This protein was the first member of the PAS superfamily to have its structure determined. Deletion of the N-terminal 25-resisdue in PYP slows down the decay of the pB signaling state in its photo-cycle. Here we explore the importance of the N-terminal region of PYP. We noticed that the presence of an N-terminal 14-residue cleavable Histidine affinity tag, which was constructed in order to facilitate the purification of Δ25 PYP, affects the rate of the pB state. Because our unpublished results suggested that the net negative charge of this peptide is functionally important, we constructed a variant of Δ25 PYP containing an affinity tag with neutral charge to explore the role for electrostatic interactions between N-terminal region and the PAS core. Our results indicate that attachment of an N-terminal His-tag with negative charge to Δ25 PYP accelerates pB decay.

Transcription of T4ASS ORFs in Spotted Fever Group *Rickettsia*

Chris Richards (Doctoral)* and Edward Shaw.

Microbiology & Molecular Genetics, Oklahoma State University, Stillwater, Oklahoma.

The Spotted Fever Group Rickettsia (SFGR) are a pathogenically diverse group of the *Rickettsia* genus ranging from *Rickettsia rickettsii* to avirulent species. SFGR species possess a Type 4A Secretion System (T4ASS), a critical virulence determinant for many pathogens, secreting effector proteins into host cells. The *Rickettsiae* T4ASS possesses eighteen open-reading-frames (ORFs), fifteen of which are in close proximity to each other in two separate loci, designated Region I and Region II. Gene arrangement of these ORFs suggests co-expression and/or transcriptional regulation may exist. Reverse-Transcriptase PCR (RT-PCR) was performed on total RNA harvested from *R. parkeri*, *R. montanensis*, and *R. amblyommii* infected mammalian cell cultures grown at both 34°C and 37°C. To date, we have detected T4ASS transcripts across all three *Rickettsia* species at both 34°C and 37°C. Polycistronic mRNA was detected using primer pairs spanning T4ASS ORFs. RT-PCR identified many transcriptional linkages within both Region I and Region II. These data indicate that SFGR T4ASS ORFs are expressed and co-transcribed during infection of host cells, suggesting these ORFs may be transcriptionally regulated. Further characterization of the expression of this critical virulence determinant will aid in our understanding of these unusual obligate intracytoplasmic pathogens.

Structure and Function Analysis of the Redox-Sensing Transcription Factor Methanogen Specific Vinyl-4-Reductase (MsvR).

Kristen Shelton (Doctoral)*, Vy Trinh, Catherine Isom, Melissa Chanderban, Kylie Gilcrest, Jack Lee,

Elizabeth A. Karr.

University of Oklahoma, Norman, OK.

MsvR is a novel two-domain transcription regulator unique to methanogens with redox-sensitive DNA binding behavior. Methanogens are the only known source of biogenic methane, a potent greenhouse gas. Methanogens are strict anaerobes that have evolved mechanisms to combat oxidative stress. Previous studies of *Methanothermobacter thermautotrophicus* MsvR showed that it regulates transcription of its own gene as well as a divergent operon implicated in oxidative stress response. However, MsvR proteins fall into two groups with varying cysteine signatures in the V4R domain and thus investigation of multiple family members is necessary. In this work, various constructs of the *Methanosarcina acetivorans* MsvR were created to express recombinant full length and truncated proteins containing a single domain. These constructs were used to determine the oligomeric state and DNA binding activity for each domain. Site directed mutagenesis of cysteine residues demonstrated their importance in redox-sensitive DNA binding *in vitro*. Additionally, truncated proteins containing the V4R domain-only were still able to dimerize and DNA binding was no longer detected, as was expected. These data support the hypotheses that several cysteine residues are important for redox-sensitive DNA binding, that both domains are needed for DNA binding and that each domain contains a dimerization interface.

Novel synthetic analogs of avian β-defensin-12 with enhanced antimicrobial and immunomodulatory activities.

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

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

Avian β-defensins (AvBD) are a group of small antimicrobial peptides that play multiple important roles in immunity defense against microbial infection. Based on the structure-function study of AvBD-6 and AvBD-12, seven new AvBD-12 analogs were designed and synthesized by residue composition and distribution to increase the antimicrobial activity of wild-type and their active efficiency in high salt condition, and to further test the role of disulfide bonds in chemotactic activity. The results indicated that their antibacterial activity against *E. coli*, *S.* Typhimurium, *S. aureus* and *P. aeruginosa* showed a dose-dependent manner at concentration of 1 to 128 μg/ml, positively correlated with the net positive charge. Treated *S.* Typhimurium with peptides resulted in visible membrane damage observed by SEM, which indicated their main action sites was bacterial membrane. The analog AvBD-12-A3 with net positive charge +9 significantly improved antimicrobial activity in high salt concentration. The analogs with two or less intracellular disulfide bonds by replacing cysteines with isosteric α-aminobutyric acids showed significantly reduced or abolished the chemotactic activity to CCR2 transfected CHO-K1 cells and immature dendritic cells. The hybrid analog of wild-type AvBD-12 and AvBD-6 maintained chemotaxis activity and increased the antimicrobial activity compared to AvBD-12. All analogs and two wild-types at concentration of 256 μg/ml were examined without cytotoxicity to these cells. Our data highlight modification of natural defensins based on their charge and disulfide bonds is an effective strategy for designing novel antimicrobial agents.

Post-doctoral Fellow Poster Presentation Abstracts

Staphylococcus aureus Metabolic Adaptations During the Transition to a Vancomycin-intermediate Susceptibility Phenotype.

Stewart G. Gardner* and Greg A. Somerville. University of Nebraska-Lincoln, Lincoln, Nebraska.

The emergence of methicillin resistant *Staphylococcus aureus* has increased the therapeutic usage of vancomycin, which has increased the isolation of vancomycin-intermediate *S. aureus* (VISA) and vancomycin-resistant *S. aureus* (VRSA) strains. While the isolation of VRSA strains is rare, VISA strains are becoming more common. The most common mechanism by which *S. aureus* acquires intermediate resistance (minimum inhibitory concentration 4-8 µg/ml) is by adapting its physiology and metabolism to permit growth in the presence of vancomycin, a process known as adaptive resistance. The adaptations that increase resistance to vancomycin include, increased cell wall thickness, regulatory mutations, and metabolic changes. The extent of the metabolic changes remains unknown. To determine the metabolic changes associated with the VISA phenotype, the physiological and metabolic differences in an isogenic strain pair isolated from a patient before (Q2275) and after (Q2283) vancomycin treatment failure were examined. Q2283 had a slightly delated entry into the stationary phase, which is reflected in its ability to catabolize acetate. Further characterization will include NMR metabolomics and enzymatic analysis. The longer-term goal of this work is to develop lead compounds that target metabolic pathways necessary for the vancomycin-intermediate susceptibility phenotype, which used in combination with vancomycin may reduce treatment failures.

The Fatty Acid Kinase of *Staphylococcus aureus* Controls Virulence

Christina N. Krute* and Jeffrey L. Bose

Department of Microbiology, Molecular Genetics and Immunology, University of Kansas Medical Center, Kansas City, KS, USA

Virulence factor regulation in *Staphylococcus aureus* is under complex transcriptional and post-transcriptional control. We identified mutants in an operon encoding two hypothetical proteins, VfrA and VfrB, which had a dramatic decrease in α-hemolysin production. Further analysis revealed that the second protein, VfrB, was the primary contributor to conditionally-controlled α-hemolysin, protease production, and expression of secreted virulence factors. This regulation may be mediated by VfrB activation of SaeRS, as α-hemolysin production in a *vfrB* mutant is restored in *S. aureus* Newman, which encodes a constitutively active SaeRS system. Additionally, expression of *saeRS*-controlled genes is down-regulated in *vfrB* mutants. In conjunction with a role in virulence, VfrB was identified as a fatty acid kinase essential for uptake of exogenous fatty acids. When proposed active site residues of VfrB are mutated, the hemolytic activity of the enzyme is abolished, indicating a role for these residues in kinase activity. Furthermore, it was discovered that VfrB works with two previously hypothetical proteins, FakB1 and FakB2, which are fatty acid-binding proteins with differing specificity. *In vivo* studies using a murine model of dermonecrosis revealed the *vfrB* mutant is hyper-virulent. Together, these studies reveal the connection between fatty acid metabolism and virulence factor regulation in *S. aureus*.