

**Meeting of the Missouri Valley Branches of the American Society of Microbiology**

**March 18-19, 2022**

**Oklahoma State University**

**Stillwater, OK**

**Sponsors:**

**Department of Microbiology and Molecular Genetics, Oklahoma State University**

**Thermo Fisher Scientific**

**Welcome to Stillwater!**

On behalf of the Oklahoma State University and the Department of Microbiology and Molecular Genetics, we are excited to host you all this year and welcome you to Stillwater for the 2022 Meeting of the Missouri Valley Branch of the American Society of Microbiology. This meeting is an excellent platform for undergraduate and graduate students to present their research, network with other students and faculty, and interact with our ASM Distinguished Lecturer and Branch Speakers. This year our meeting is hybrid (in person with a live stream of all presentations) and we have 65 abstracts submitted by undergraduate and graduate students from Oklahoma, Kansas, and Nebraska. We hope you all enjoy this meeting and that it will lead to new friendships, collaborations, and new opportunities. Once again, welcome to Stillwater!

Erika Lutter

President ASM Missouri Valley Branch

Associate Professor

Department of Microbiology and Molecular Genetics

Oklahoma State University



**PARKING GUIDE**

**Address:** N Washington St & W Hall of Fame Ave, Stillwater, OK 74078

Map below shows location of Wes Watkins Center (WWC, designated with a star). WWC is located on the corner of Hall of Fame and Washington St.



HALL OF FAME

Parking is available in lots 99, 9 and 9A. Parking is free after 5PM and on weekends.

**SCHEDULE OVERVIEW**

**Friday, March 18th**

The meeting will be held in the Wes Watkins Center on the Oklahoma State University Campus in Stillwater OK.

**ADDRESS**: N Washington St & W Hall of Fame Ave, Stillwater, OK 74078 [(Google Map)](https://www.google.com/maps/place/Wes+Watkins+Center+-+Oklahoma+State+University/@36.127109,-97.070572,17z/data=!3m1!4b1!4m5!3m4!1s0x87b10a3c52a402a1:0x1377b60cec48bef3!8m2!3d36.1271!4d-97.0683987).  
You can park anywhere next to the Wes Watkins center starting at 5PM.  Parking is free after 5 and on weekends.

Note there is now a mixer from 5-6PM (First drink ticket is provided and additional drinks are available at the cash bar).  The mixer, dinner and Key Note presentation will occur in the International Exhibit Hall. Bar will be open all evening.

|  |  |  |
| --- | --- | --- |
| **Friday, March 18, 2022** | At Oklahoma State University | Virtual |
| 5:00 - 6:00 PM | Mixer | ---------- |
| 6:00 – 7:00 PM | Dinner | Virtual Mixer (starting at 6:30) |
| 7:00 – 7:15 PM | Welcome and Introductions  In person and virtual will be connected via Zoom | |
| 7:15 – 8:15 PM | ASM Distinguished Lecturer via Zoom  Dr. Kim Orth, UT Southwestern  Zoom link: [https://unmc.zoom.us/j/91387779183?pwd=Y2RKakJ4Nk94WnV0S0tTWkp0TVBBUT09](https://nam04.safelinks.protection.outlook.com/?url=https%3A%2F%2Funmc.zoom.us%2Fj%2F91387779183%3Fpwd%3DY2RKakJ4Nk94WnV0S0tTWkp0TVBBUT09&data=04%7C01%7Cerika.lutter%40okstate.edu%7C812fc669479e4be32e4408da011d0a12%7C2a69c91de8494e34a230cdf8b27e1964%7C0%7C0%7C637823521487560117%7CUnknown%7CTWFpbGZsb3d8eyJWIjoiMC4wLjAwMDAiLCJQIjoiV2luMzIiLCJBTiI6Ik1haWwiLCJXVCI6Mn0%3D%7C3000&sdata=Jhbicm%2BbPIHHgL67yifwXsOC6ZhhDmpXzrtApV%2B%2Fqho%3D&reserved=0) Meeting ID: 913 8777 9183 Passcode: 222362 | |
|  |
| 8:15 – 8:45 PM | Students to visit with Dr. Kim Orth  Via Zoom (in person students will go into an adjacent meeting room) | |
| 8:45-9PM | Closing Announcements  In person and virtual connected via Zoom | |

**Saturday March 19th**

The meeting will be held in the Wes Watkins Center on the Oklahoma State University Campus in Stillwater OK.

**ADDRESS**: N Washington St & W Hall of Fame Ave, Stillwater, OK 74078 [(Google Map)](https://www.google.com/maps/place/Wes+Watkins+Center+-+Oklahoma+State+University/@36.127109,-97.070572,17z/data=!3m1!4b1!4m5!3m4!1s0x87b10a3c52a402a1:0x1377b60cec48bef3!8m2!3d36.1271!4d-97.0683987).  
You can park anywhere next to the Wes Watkins center.  Parking is free on weekends.

Note the Poster boards we have can accommodate a poster up to 48” x 36”.

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| --- | --- | --- |
| **Saturday, March 19, 2022** | At Oklahoma State University | Virtual |
| 8:00 - 8:30AM | Coffee and breakfast | Virtual coffee (staring at 8:15) |
| 8:30 – 10:45AM | Oral presentations (in person and virtual connected via Zoom), 4 rooms presenting simultaneously | |
| 10:45 – 11:00AM | Break | |
| 11:00 – 12:00PM | Poster Session, Flash talks in one room from 11-11:30 | |
| 11:45 -12:00PM | Branch Meeting (in person and Zoom) | |
| 12:00-1:00PM | Lunch | |
| 1:00-1:30 PM | Dr. Rey Carabeo | |
| 1:30-2:00 PM | Dr. I-Hsiu (George) Huang | |
| 2:00-2:30 PM | Dr. Stacey Gilk | |
| 2:30-2:45 PM | Break | |
| 2:45-3:15 PM | Dr. Ratnakar Deole | |
| 3:15-3:45 PM | Dr. Austin S. Nuxoll | |
| 3:45-4:15 PM | Awards and closing remarks | |

**ASM Distinguished Lecturer**

# **Dr. Kim Orth, Ph.D.**

Professor, Department of Molecular Biology

University of Texas Southwestern Medical Center



Biographical Sketch

Kim Orth, Ph.D., a professor at the University of Texas Southwestern Medical Center, studies how virulence factors expressed by bacterial pathogens cause disease. Studies using microbial genetics, biochemistry, cell biology and bioinformatics on effectors from Yersinia and Vibrio bacteria have uncovered many mechanisms that bacteria use to subvert host signaling pathways, including the discovery of novel protein modifications. Her team's work has uncovered unexpected strategies that bacteria use to survive and spread. Additionally, her work has also helped reveal normal signaling pathways.  
She has received a variety of prestigious awards, including Burroughs Wellcome Fund Investigators in the Pathogenesis of Infectious Diseases, ASBMB Merck Award, election to the American Academy of Microbiology, and recent election to the National Academy of Science. Orth is a Howard Hughes Medical Institute Investigator.

Zoom Link:

[https://unmc.zoom.us/j/91387779183?pwd=Y2RKakJ4Nk94WnV0S0tTWkp0TVBBUT09](https://nam04.safelinks.protection.outlook.com/?url=https%3A%2F%2Funmc.zoom.us%2Fj%2F91387779183%3Fpwd%3DY2RKakJ4Nk94WnV0S0tTWkp0TVBBUT09&data=04%7C01%7Cerika.lutter%40okstate.edu%7C812fc669479e4be32e4408da011d0a12%7C2a69c91de8494e34a230cdf8b27e1964%7C0%7C0%7C637823521487560117%7CUnknown%7CTWFpbGZsb3d8eyJWIjoiMC4wLjAwMDAiLCJQIjoiV2luMzIiLCJBTiI6Ik1haWwiLCJXVCI6Mn0%3D%7C3000&sdata=Jhbicm%2BbPIHHgL67yifwXsOC6ZhhDmpXzrtApV%2B%2Fqho%3D&reserved=0)  
Meeting ID: 913 8777 9183  
Passcode: 222362

**Dr. Rey Carabeo, Ph.D.**

Professor

University of Nebraska Medical Center



Biographical Sketch

I am a Professor in Department of Pathology and Microbiology at the University of Nebraska Medical Center. I received my B.S. in Microbiology and Molecular Genetics from UCLA, and my Ph.D. in Oncology from the McArdle Laboratory for Cancer Research at the University of Wisconsin-Madison.

My research group investigates the cellular and molecular bases of *Chlamydia*-host cell interaction. We are interested in several topics related to *Chlamydia* pathogenesis. They include the mechanism of invasion of non-phagocytic epithelial cells by *Chlamydia*, and elucidated the initial signaling framework that connected the chlamydial type III effector TarP to the host remodeling machinery. We are currently characterizing the mechanosensitive landscape of invasion, and how contractile forces regulate TarP function. We are also investigating how this pathogen inhibits host cell turnover from the epithelium, which for an obligate intracellular pathogen that loses infectivity during its normal biphasic developmental cycle, is crucial to its survival and replication. We have identified two type III effectors in *Chlamydia* that are responsible for distinct, but complementary mechanisms of enhancing host cell adhesion to the extracellular matrix. My research group is also characterizing the transcriptional response of *Chlamydia* to nutritional stress. The current focus is iron starvation. Our efforts led to the identification of the chlamydial iron-dependent repressor YtgR, and its unusual role in regulating the *trp* operon as a tryptophan-sensing *trans*-acting attenuator of *trp* transcription. The newest project in my laboratory is on epithelial cell-fibroblast communication, and how this is altered by *Chlamydia* infection to activate fibroblasts to become pro-fibrotic myofibroblasts. The overarching hypothesis is that fibrotic sequelae, *e.g*. tubal factor infertility that develop in asymptomatically infected women occur via a cellular mechanism that is independent of the host inflammatory response.

The variety of research topics that we are pursuing illustrates the many interesting, and still unanswered questions on *Chlamydia* biology and pathogenesis. With the relatively recent advent of molecular genetic techniques, we anticipate exciting times ahead.

**Dr. I-Hsiu (George) Huang, Ph.D.**

Assistant Professor

Department of Biochemistry and Microbiology

Oklahoma State University Center for Health Sciences

A person wearing glasses and a tie

Description automatically generated with medium confidence

Biographical Sketch

My lab is focused on understanding the pathogenic mechanism of anaerobic human pathogens *Clostridioides difficile* and *Fusobacterium nucleatum*. In the gut pathogen *C. difficile*, we have been interested in all aspects of *C. difficile* pathogenesis including epidimiological sudies, the isolation and characterization of environmental isolates, and characterizion of novel virulence factors. In recent years our interests in *C. difficile* have shifted to developing novel preventive and theraputic measures. We are currently developing a mucosal vaccine against *C. difficile* using recombinant antigens encapsulated with biodegradable nanoparticles. We are also interested in developing non-antibiotic based therapies to treat *C. difficile* infections. In *F. nucleatum* we seek to characterize its potential role in promoting tumor development in colorectal and oral cancer by developing in vitro and in vivo models to identify bacterial factors involved in tumor proliferation, chemoresistance, and metastasis.

**Dr. Stacey Gilk, Ph.D.**

Associate Professor

Department of Pathology and Microbiology

University of Nebraska Medical Center

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Biographical Sketch

The Gilk Lab is interested in how intracellular pathogens manipulate host cell lipids and lipid metabolism in order to establish the intracellular niche. We study the obligate intracellular bacterium *Coxiella burnetii*, a highly infectious pathogen which is considered a potential bioterrorism weapon. *Coxiella* replicates insides a specialized lysosome-like vacuole known as the *Coxiella* containing vacuole (CCV). We recently discovered that *Coxiella* is very sensitive to CCV cholesterol levels, with elevated cholesterol leading to increased CCV acidification and bacterial degradation. Because cholesterol readily traffics to the CCV and is found in the CCV membrane, we hypothesize that *Coxiella* regulates CCV cholesterol levels to maintain the optimal CCV environment. *Coxiella* expresses two sterol-modifying enzymes, CBU1158 and CBU1206, which may act in lowering CCV cholesterol. Using a mutant in CBU1206, we found that CBU1206 facilitates bacterial growth by reducing CCV cholesterol and 25-hydroxycholesterol (25-HC). Further, we found that dysregulated 25-HC leads to increased CCV proteolytic activity and bacterial death. These studies suggest that cholesterol metabolism is an integral component of *Coxiella*pathogenesis, and therefore may be a viable drug target.

**Dr. Ratnakar Deole, PhD**

Assistant Professor

Department of Biochemistry and Microbiology

Oklahoma State University-Center for Health Sciences



Biographical Sketch

Ecosystems with high salt concentration (hypersaline), are hostile to life as salt tends to be hygroscopic. Yet, microorganisms referred to as halophiles belonging to the three domains of life (archaea, bacteria and eukarya) have acquired the capacity to grow in the presence of high salt concentration. Deole research lab is involved in molecular research of high salinity adaptations by archaea and bacteria which enable them to grow in such environments. Interestingly, salts have been used in the food industry for flavoring and in preservation. However, salt can also be the source of living microorganisms that may affect human health especially the gut microbiome. In Deole lab we isolate and identify halophilic archaea and bacteria present in commercially available edible salts and study their potential impact on the microbiome.

**Dr. Austin S. Nuxoll, PhD**

Associate Professor

Department of Biology, BHS 201D

University of Nebraska – Kearney

Kearney, NE 68849

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Biographical Sketch

Dr. Nuxoll joined the UNK Biology Department in 2016 where he is currently an Associate Professor. He received his undergraduate education at the University of Nebraska – Kearney and PhD from the University of Nebraska Medical Center in Pathology and Microbiology. Following his graduate work, he received postdoctoral training at Northeastern University, his focus was mechanisms of antibiotic tolerance. Since arriving at UNK, he has continued working on antibiotic tolerance in *Staphylococcus aureus*with an emphasis on how tolerant cells contribute to survival within a host.

**Oral Presentation Schedule**

**General Microbiology**

**Moderator:** Dr. Matthew Cabeen

**Room:**

**Zoom link:**

**Time Student Title**

|  |  |  |
| --- | --- | --- |
| 8:30AM-8:45AM | Saher Fatima | Regulation and Function of an Atypical MAPK in *Dictyostelium* |
| 8:45AM-9:00AM | Ramee G. Aranda **(virtual)** | Defining Functional Regions of Atypical MAPK Erk2 in *Dictyostelium discoideum* |
| 9:00AM-9:15AM | Christopher Hamm | *Pseudomonas aeruginosa* Pyocins Can Extend Antibiotic Effectiveness |
| 9:15AM-9:30AM | Nicholas A. Wood  **(virtual)** | ClpX Recognition of SsrA-Tagged Substrates Underlies Morphological Commitment to Secondary Differentiation in *Chlamydia trachomatis* |
| 9:30AM-9:45AM | Ravi K. Manjhi | Effect of Media Type on the Growth of Methanotrophic Enrichment Cultures |
| 9:45AM-10:00AM | Sean Carr | Enhancing Production of Isoprene from Engineered Methanogens |
| 10:00AM-10:15AM | Suman Maharjan | Viable Round Mutants of *Escherichia coli* with Point Mutations in *mreB* |

**Environmental Microbiology and General Microbiology**

**Moderator:** Dr. Babu Fathepure

**Room:**

**Zoom link:**

**Time Student Title**

|  |  |  |
| --- | --- | --- |
| 8:30AM-8:45AM | Carrie J. Pratt | Culturing Novel Anaerobic Gut Fungi from Reptilian Hosts |
| 8:45AM-9:00AM | Christopher T. Garner | The Impact of Coculturing on Methane Oxidizing Bacteria |
| 9:00AM-9:15AM | Damilare D. Ajagbe | Survival in Toxic and Multi-Stress Environment: Exploring the Bioremediation Potential of *Modicisalibacter* sp. strain Wilcox for Produced Water |
| 9:15AM-9:30AM | Imam T. Alam | Zodletone Spring Enrichment Aerobically Degrades Methane Under Hypersaline Condition |
| 9:30AM-9:45AM | Myriam M. Achour **(virtual)** | Calcium Regulates Quorum Sensing in *Pseudomonas aeruginosa* |
| 9:45AM-10:00AM | Sarah Chandler | Bacterial Disease Presence in Hall County, Nebraska Tick Populations |
| 10:00AM-10:15AM | Mary J. Erdmann | A Novel Lipase Plays a Role in PstN-Mediated Biofilm Suppression in *Pseudomonas aeruginosa* |
| 10:15AM-10:30AM | John Kincaid/  Babur Mirza | Distribution of Potential Bacterial Pathogens in Bacterially Impaired Watersheds |

**Medical Microbiology Session 1**

**Moderator:** Dr. Avishek Mitra

**Room:**

**Zoom link:**

**Time Student Title**

|  |  |  |
| --- | --- | --- |
| 8:30AM-8:45AM | Aaron A. Jensen **(virtual)** | Characterization of the ClpC AAA+ ATPase of *Chlamydia trachomatis* |
| 8:45AM-9:00AM | Benjamin N. Nelson | Interactions of *Cryptococcus neoformans* with Human Airway Phagocytes |
| 9:00AM-9:15AM | Pramila Lamichhane | A Single-cycle Live Respiratory Syncytial Virus Vaccine Expressing Prefusion F Protein |
| 9:15AM-9:30AM | Natalie A. Sturd **(virtual)** | Characterizing Flightless 1 Localization to the Inclusion Membrane During *Chlamydia trachomatis* Infection |
| 9:30AM-9:45AM | Tarosha B. Salpadoru | Calcium Enhances Resistance of *Pseudomonas aeruginosa* to the Last-resort Antibiotic, Polymyxin B |
| 9:45AM-10:00AM | Miruthula Tamil Selvan | SARS CoV-2 (delta variant) infection kinetics and immunopathogenesis in domestic cats |
| 10:00AM-10:15AM | Joshua S Mytych | *Bacillus anthracis* Peptidoglycan Prevents Efferocytosis by Human M2-like MФ, Reduces Cell Surface Expression of Efferocytic Receptors, but is Not Reversed by ADAM17 Inhibition |
| 10:15AM-10:30AM | Jacob Burch-Konda | Kinetic RNA Seq Analysis Reveals the Role of Calcium Sensor, EfhP, in Regulating Iron Uptake and Biofilm Formation in *Pseudomonas aeruginosa* as a Response to Ca2+. |
| 10:30AM-10:45AM | Mamie Kannon | Blocking Essential Nutrient Acquisition in *Pseudomonas aeruginosa* |
| 10:45AM-11:00AM | Terhüja M | Development of a Respiratory Syncytial Virus based Virus-like Particle Vaccine |

**Medical Microbiology Session 2**

**Moderator:** Dr. Lauren Zenewicz

**Room:**

**Zoom link:**

**Time Student Title**

|  |  |  |
| --- | --- | --- |
| 8:30AM-8:45AM | Prakash Sah | *Clostridioides difficile* Toxin B (TcdB) Activates Group 3 Innate Lymphocytes (ILC3s) |
| 8:45AM-9:00AM | Archana Yadav | A “Reverse Evolution” Approach to Identify Strategies in *Coxiella burnetii* Intracellular Survival |
| 9:00AM-9:15AM | Ray E. Widner | Analysis of VAMP3 Trafficking to the *Chlamydia trachomatis* Inclusion |
| 9:15AM-9:30AM | Emma Weis  **(virtual)** | *Staphylococcus aureus* Persisters Exhibit Increased Survival to Components of the Innate Immune System |
| 9:30AM-9:45AM | Daniel Reed | Contribution of Cell Ultrastructure to the Antibacterial Properties of a Novel Hydrophobic Melanin-Inspired Compound |
| 9:45AM-10:00AM | Samantha Mercer | Jumping Species: Spilling the Beans On The Next Epidemic |
| 10:00AM-10:15AM | Abigail R. Swoboda **(virtual)** | Characterization of the Tail-Specific Protease, Ct441, in *Chlamydia trachomatis* Growth and Development |
| 10:15AM-10:30AM | Hollis C. Holcomb | *Chlamydia trachomatis* and the Characterization of its Inclusion Membrane Protein CT226 |
| 10:30AM-10:45AM | Deepali Luthra | Upregulation of Calcium Stimulated Host Adherence of *Pseudomonas aeruginosa* During Infection |

**Flash Talks**

**Moderator:** Dr. Elizabeth Rucks

**Room:**

**Zoom link:** [https://unmc.zoom.us/j/94764183467?pwd=SUJaY1lsSlljOExwdmZ2c0R2ZE1Pdz09](https://nam04.safelinks.protection.outlook.com/?url=https%3A%2F%2Funmc.zoom.us%2Fj%2F94764183467%3Fpwd%3DSUJaY1lsSlljOExwdmZ2c0R2ZE1Pdz09&data=04%7C01%7Cerika.lutter%40okstate.edu%7C812fc669479e4be32e4408da011d0a12%7C2a69c91de8494e34a230cdf8b27e1964%7C0%7C0%7C637823521487560117%7CUnknown%7CTWFpbGZsb3d8eyJWIjoiMC4wLjAwMDAiLCJQIjoiV2luMzIiLCJBTiI6Ik1haWwiLCJXVCI6Mn0%3D%7C3000&sdata=Fb%2FctrKScLyVboGikWzb%2BPnyY4ikzChFOFNkbEENquo%3D&reserved=0)  
Meeting ID: 947 6418 3467  
Passcode: 046154

**Time Student Title**

|  |  |  |
| --- | --- | --- |
| 11:05AM-11:10AM | Armond J. Isaak | Determination of Lysine Acetylation Susceptibility of DksA and Its Effects on Global Metabolism in *Borrelia Burgdorferi* |
| 11:10AM-11:15AM | Clayton T. Matthews | The Interactions of PhoU1 and PhoU2 Homologs in *Staphylococcus aureus*. |
| 11:15AM-11:20AM | Sydney Marouk | Halophiles isolated from edible salt using a cultromics approach |
| 11:20AM-11:25AM | Rohit Mital | Differential Impact of Diet and Microbiome on Metabolite Molecular Families |

**Survival in Toxic and Multi-Stress Environment: Exploring the Bioremediation Potential of *Modicisalibacter* sp. strain Wilcox for Produced Water.**

Damilare D. Ajagbe (Doctoral)\*, Babu Z. Fathepure, and Mark Krzmarzick. Oklahoma State University, Stillwater, Oklahoma.

Saline and hypersaline wastewater such as produced water (PW) which contains hydrocarbons, benzoates, phenols, biphenyls, and heavy metals are prominent by-products in the industry. PW is generated during oil and natural gas production operations, with over 50 million barrels generated daily in the United States alone. While the possible bioremediation of PW for reuse has gained significant interest recently, practical application has not been realized because not much is known about halophiles that degrade hydrocarbons and tolerate heavy metals commonly found in PW. Genome analysis of our previously isolated halophile, *Modicisalibacter* sp. strain Wilcox, predicted a repository of protein-encoding genes for hydrocarbon metabolism and heavy-metal resistance. Hence, the study seeks to examine Wilcox’s bioremediation capacity for PW. We set up microcosms to study the strain’s ability to degrade toxic aromatics (Benzene, Toluene, Ethylbenzene & Xylene – BTEX) in the presence of 2.5M NaCl and heavy metals at different concentrations, singly and in combination. The data show that Wilcox can tolerate different heavy metals, including Arsenic, Cadmium, Lead, Chromium, Zinc, Nickel, Copper etc. at concentrations ranging from 0.25 mM to 120 mM, and completely degrade 20-35µmol of BTEX in 2-5 weeks, which makes Wilcox a potential candidate for the cleanup of PW.

**Category II: Environmental Microbiology Oral Presentation**

**Zodletone Spring Enrichment Aerobically Degrades Methane Under Hypersaline Condition**

Imam T. Alam (Doctoral)\* and Babu Fathepure

Oklahoma State University, Stillwater, Oklahoma

Oil and gas production wells are one of the significant sources of methane, an important greenhouse gas. The major goal of this study was to investigate the ability of the microbial communities in high salinity environments like oil and gas wells to degrade methane. In this study, we investigated methane oxidation potential of the microbial community in the Zodletone spring, OK. Microcosms were prepared containing 5 grams of Zodletone sediment and 45 ml of mineral salts medium at different salinity ranging from 0.5 to 4 M NaCl. Bottles were closed with rubber septa and aluminum crimps and spiked with 1 ml of methane (41.15 μmol/bottle). Headspace methane was monitored periodically using a gas chromatograph. Results showed that methane degradation occurred in the bottle containing 0 – 2.5 M NaCl and no degradation was seen at 3 and 4M NaCl. Maximum degradation of methane occurred in the presence of 2 or 2.5 M NaCl at a rate of 14.2 µmol/bottle/day. We also tested the enrichment’s ability to degrade ethane, benzene, toluene, ethylbenzene, and xylenes. Interestingly, none of these compounds were degraded despite some of these hydrocarbons are present in the Zodleton spring suggesting highly enriched culture for methane oxidation. This is important because such organisms can be used for the removal methane in oil and gas wells that are saline and a significant source atmospheric methane.

**Category: II Environmental Microbiology Graduate Student Oral**

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

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

In eukaryotic cell signaling, mitogen-activated protein kinases (MAPKs) mediate cellular processes like cell growth, differentiation, and movement. In *Dictyostelium discoideum*, a social amoeba, there are only two of these MAPKs, Erk1 and Erk2. These MAPKs are involved in a developmental lifecycle in which starved cells aggregate to form fruiting bodies. Erk1 is a typical MAPK responsible for developmental kinetics and aggregate size while Ek2 is an atypical MAPK required for chemotaxis and multicellular development and the translocation of a transcription factor GtaC. Not much is known about atypical MAPKs like Erk2. It is known that Erk2 is activated within 30 seconds after stimulation by a chemoattractant and that its function is required for a burst of Erk1 activation in a secondary response to chemoattractants. Atypical MAPKs have a conserved C-terminal motif (CTM) not found in other MAPKs. To test the specificity and function of certain regions of MAPKs, a series of MAPK chimeras (composed of Erk1 and Erk2 sequences) have been created and expressed in strains lacking one or the other MAPK. Recent results suggest the CTM motif is necessary for most Erk2 functions but the CTM motif does not confer Erk2 function to the Erk1 MAPK.

**Category: I General Microbiology Oral Presentation (Virtual)**

**Calcium Regulates Quorum Sensing in *Pseudomonas aeruginosa***.

Myriam M. Achour (Undergraduate)\*, Aya Kubo, and Marianna A. Patrauchan. Oklahoma State University Life Sciences East, Stillwater, Oklahoma.

*Pseudomonas aeruginosa* causes lethal infections in patients with cystic fibrosis. During CF, the abnormally high concentration of calcium ions is found in the lung and nasal fluids. Previously, our group showed that elevated calcium enhances the production of virulence factors, some of which are controlled by quorum sensing (QS). The three QS molecules in *P. aeruginosa* are responsible for cell-to-cell communication and are synthesized by the synthases: LasI, RhlI, and PqsA*.* We aimed to characterize the role of calcium in regulating the expression of the *lasI, rhlI,* and *pqsA* genes. We applied promoter activity assays based on the *lux* construct producing luminescence upon expression. The results showed that calcium alters the promoter activities of all three genes. To identify the specific components of the calcium signaling system that are responsible for this regulation, we used deletion mutants lacking calcium channel (CalC), calcium sensor (EfhP), or calcium regulator (CarR). So far, we showed that in *ΔcalC* mutant, the *lasI* promoter activity lost its calcium sensitivity, whereas the *rhlI* and *pqsA* promoter activities were significantly reduced in comparison to the wild type. This knowledge is of high importance as it provides the regulatory link between two potent signaling systems in *P. aeruginosa.*

**Category: I General Microbiology Oral Presentation (Virtual)**

**Determination of Lysine Acetylation Susceptibility of DksA and Its Effects on Global Metabolism in *Borrelia Burgdorferi***

Isaak, Armond J. (undergraduate)\*, Boyle, William K., Sorensen, Hannah S., Zalud, Amanda K., and Bourret, Travis J.

Creighton University School of Medicine Department of Medical Microbiology & Immunology, Omaha, NE

For its infectious cycle, the Lyme disease spirochete *Borrelia burgdorferi* must overcome a variety of environmental stresses. The DnaK suppressor protein (DksA) is a global gene regulator in *B. burgdorferi* that helps regulate the expression of genes required for infectivity, however, the molecular mechanisms driving DksA-dependent changes in gene expression are unclear. Lysine acetylation is a reversible post-translational modification (PTM) that contributes to the regulation of virulence gene expression in a variety of bacteria pathogens*. In silico* analysis of *B. burgdorferi* DksA suggests that five lysines (L118, L119, L121, L122, and L124) located in the C-terminus of DksA are likely targets for acetylation. In *B. burgdorferi*, lysine acetylation is carried out nonenzymatically through endogenously produced acetyl-phosphate (Ac-PO4). In this study, I observed that recombinant DksA is subject lysine acetylation following incubation with Ac-PO4 *in vitro*. Additionally, our previous work suggested DksA may contribute to lysine acetylation through its regulation of *ackA*, a gene that encodes an enzyme that produces Ac-PO4. Currently, I am comparing the profiles of acetylated proteins in wild-type and *dksA*-deficient *B. burgdorferi* strains to the contribution of DksA to lysine acetylation.

**Category V: Flash talk (virtual only)**

**Evaluating how Resistance to Branched Polyethylenamine (BPEI) Affects the Gram-negative Pathogen *Pseudomonas aeruginosa*** William Best (Doctoral)\*, Maya Ferrell, and Dr. Charles Rice. Department of Chemistry and Biochemistry, The University of Oklahoma, Norman, Oklahoma.

*Pseudomonas aeruginosa* is a Gram-negative pathogen that is notorious for infecting the lungs of Cystic Fibrosis (CF) patients and the wounds of burn victims, where it produces persistent biofilms that are difficult to treat. Further contributing to this issue are the emergence of drug-resistant strains that eliminate treatment options for patients, leading to more negative clinical outcomes. It is therefore increasingly important to find new ways to combat and treat these types of infections. One strategy to combat drug-resistant infections is through the use of a potentiator molecule which restores susceptibility to an antibiotic in a previously resistant organism. One such potentiator is Branched Polyethylenamine (BPEI), which potentiates at low concentrations but is fatal to the cell at high concentrations. The bactericidal nature of BPEI leaves open the potential for resistance to develop in pathogens which are exposed to it, as has occurred with antibiotics and other antimicrobial agents. Therefore, it is critical to understand what impact resistance to BPEI has on an organism, as it could impact the effectiveness of BPEI as a therapeutic agent. Preliminary studies have found that, though Pseudomonas aeruginosa is capable of developing resistance to BPEI, doing so has deleterious consequences for the organism.

**Category IV**: **Poster Presentation**

**Calcium Leak Channel, CalC, Plays Role in Calcium Regulation of Virulence and Antibiotic Resistance in *Pseudomonas aeruginosa***

ReyganBraga (Masters)\*, Aya Kubo, Michelle King, Sergio Mares, Tarosha Salpadoru, and Marianna Patrauchan. Department of Microbiology and Molecular Genetics, Oklahoma State University, Stillwater, USA

Cystic fibrosis (CF) is a genetic disease resulting in increased levels of Ca in the nasal and pulmonary fluids. Previously, our group showed the increased production of virulence factors by *P. aeruginosa* when grown at clinically relevant levels of Ca. Such virulence enhancement may contribute to *P. aeruginosa* being the predominate cause of death in CF patients. We showed that the earlier identified calcium leak channel, CalC, plays a key role in the influx of Ca into the cytoplasm leading to the transient increase of cytoplasmic Ca. The genome-wide RNA-Seq analysis of wild type PAO1 and Δ*calC* mutant grown at different levels of Ca revealed that CalC is involved in the regulation of 73% of Ca-regulated genes, which in turn represent ~25% of the genome. So far, by using RT-qPCR, we have validated that Ca-induction of phospholipase plcN requires CalC. We also showed that Ca-dependent increase in tobramycin resistance requires CalC, which likely depends on the Ca-induced expression of *mexAB* RND efflux transporter. By using *Galleria melonella* as an animal model, we showed the Ca-regulated enhancement in virulence requires CalC. This study establishes the regulatory role of intracellular Ca transients that require CalC in *P. aeruginosa* virulence and antibiotic resistance.

**IV. Poster Presentation (in person only)**

**Kinetic RNA Seq Analysis Reveals the Role of Calcium Sensor, EfhP, in Regulating Iron Uptake and Biofilm Formation in *Pseudomonas aeruginosa* as a Response to Ca2+.** Jacob Burch-Konda (Master’s)\*, Biraj Kayastha, and Marianna Patrauchan.Department of Microbiology and Molecular Genetics, Oklahoma State University, Stillwater, Oklahoma.

The human pathogen *Pseudomonas aeruginosa* poses a major risk for severe infections in immunocompromised individuals, particularly those suffering from cystic fibrosis (CF). Increased levels of calcium (Ca2+), as in pulmonary fluids of CF patients, have previously been shown to increase production of secreted virulence factors in *P. aeruginosa*. A Ca2+-binding EF-hand protein, EfhP (PA4107), was demonstrated to be critical for the Ca2+-regulated virulence in *P. aeruginosa* strain PAO1. Here we describe the results obtained from RNA sequencing of an *efhP* deletion mutant and PAO1 after rapid (10 min, 60 min), and adaptive (12 h) exposure to elevated Ca2+. Consistent with previous results, RNA seq data suggests that deletion of *efhP* significantly hinders both rapid and adaptive Ca2+ induction of several *P. aeruginosa* virulence factors, including the iron sequestering siderophores pyoverdine and pyochelin. Pull-down assays using EfhP expressed in *P. aeruginosa* periplasm identified the pyoverdine recycling pump PvdR, anti-σ factor SbrR, and tyrosine phosphatase TpbA as potential interacting partners of EfhP. These proteins may mediate the role of EfhP in the Ca2+-regulated iron uptake and biofilm formation. Overall, the results suggest a regulatory interconnectedness between Ca2+, iron, and biofilm formation signaling systems integrating multiple host signals and controlling *P. aeruginosa* virulence.

**Category: I General Microbiology Graduate Student Oral presentation**

**Detecting Protein-Protein Interactions with a Modified BACTH/FRET Assay**

Lydia Burger (Undergraduate)\*1, Christina Bourne1

1 University of Oklahoma, Norman, Oklahoma

The objective of this project is to modify an existing bacterial two-hybrid assay (BACTH) using principles of the fluorescent resonance energy transfer (FRET) assay for use with proteins of different sizes. BACTH is commonly used to detect protein-protein interactions using two fragments of adenylate cyclase that reveal interactions in the form of high beta-galactosidase activity when combined. However, one drawback to the BACTH system is that it requires proteins of similar sizes for each fragment of adenylate cyclase to interact. This prohibits the detection of interactions between large and small proteins using BACTH. FRET is more forgiving of size differences between proteins as the fluorescent proteins are able to transfer energy in a distance-dependent manner. Fluorescent proteins were fused to BACTH constructs for FRET to create a modified assay for use with proteins of different sizes. We established positive and negative controls of the resulting modified BACTH/FRET hybrid assay as a foundation for future research in the detection of protein-protein interactions.

**Category IV: Poster Presentation**

**Enhancing Production of Isoprene from Engineered Methanogens**

Sean Carr (Doctoral)\* and Nicole R. Buan. School of Biological Sciences, University of Nebraska – Lincoln, Lincoln, Nebraska

Methanogens are obligately anaerobic archaea noteworthy for producing methane from C1 compounds and acetate. The energetic limitations of these low-energy substrates require methanogens to utilize a highly efficient central metabolism which greatly favors respiratory byproducts over biomass. This metabolic strategy creates high substrate:product conversion ratios which is industrially relevant for the production of biomethane, but may also allow for the production of value-added commodities. Particularly of interest are terpene compounds, as methanogen membranes are composed of isoprenoid lipids resulting in a higher flux through isoprenoid biosynthetic pathways compared to Eukarya and Bacteria. To assess the metabolic plasticity of methanogens, our laboratory has engineered *Methanosarcina acetivorans* to produce the hemiterpene isoprene. We that isoprene producing strains would result in a decreased growth phenotype corresponding to a depletion of metabolic precursors needed for isoprenoid membrane production. We found that the engineered methanogens responded well to the modification, directing up to 4% of total towards isoprene production and increasing overall biomass despite the additional metabolic burden. Using flux balance analysis, RNA sequencing, and scale bioreactor growth we investigated how the engineered strains respond to isoprene production and how production can be enhanced.

**Category: I General Microbiology Oral Presentation**

**Bacterial Disease Presence in Hall County, Nebraska Tick Populations**

Sarah Chandler (Undergraduate)\*, Sam Mercer, Julie Shaffer

Biology Department, University of Nebraska at Kearney, Nebraska 68849

The *Amblyomma americanum* and *Dermacentor variabilis* tick species are established along the Platte River in Nebraska. Of concern are the pathogens they vector. *A. americanum*, the Lone Star tick,can host such bacteria as *Ehrlichia chaffeensis*, *Francisella tularensis*, *Anaplasma phagocytophilum*, *Rickettsia amblyommatis*, and the yet unknown causative agent of Southern Tick Associated Rash Illness. *D. variabilis*, the American Dog tick, typically hosts *Rickettsia rickettsii* and *Francisella tularensis,* but recent research done has also shown evidence that bacterial pathogens may be jumping between tick species. To further study the presence of these bacterial diseases and their transfer between species, tick specimens were collected in Hall County, NE. Previous research on *A. americanum* and *D. variabilis* has been focused on Buffalo and Dawson Counties, while Hall County lies just east of Buffalo County and extends the range of the study. During the summer 2021, 431 ticks were collected, identified, and DNA extracted. Within these specimens, two invasive tick species, the Gulf coast tick and Black legged tick were collected, underlining the concern of new tick-borne disease in Central Nebraska. Future research will focus on the analysis of positive bacterial DNA present in the samples via multi-plex PCR and DNA sequencing.

**Category: I General Microbiology Undergraduate Student Oral Presentation (In Person)**

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

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

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

**Category IV: Poster presentation**

**Effects of Organoantimony Compounds on Fungal Pathogens *Cryptococcus neoformans* and *Candida albicans***

*Kaitlyn Cotton (Undergraduate)\*1, Benjamin N. Nelson1, Nikolay Gerasimchuk2 , and Karen L. Wozniak1*. Department of Microbiology and Molecular Genetics, Oklahoma State University, Stillwater, Oklahoma1, Chemistry Department, Missouri State University, Springfield, MO2

*Cryptococcus neoformans* is an opportunistic pathogen that causes pulmonary cryptococcosis and cryptococcal meningitis in immune-compromised individuals. *Candida albicans*, also opportunistic, can cause pulmonary candidiasis, genitourinary tract infections, candidemia, and oral candidiasis. Fungal infections are responsible for approximately 1.7 million annual deaths. With few antifungal drugs, high toxicity, and increased resistance to antifungals, the importance of finding new antifungal therapies is crucial. We hypothesized that novel organoantimony compounds would effectively restrict fungal growth. We tested approximately 20 compounds against *C. neoformans* and *C. albicans* in minimum inhibitory concentration (MIC) assays. Compounds A, B, E, I, F, and G were effective against *C. neoformans* with MIC concentrations of 10.94 μg/ml, 19.79 μg/ml, 18.75 μg/ml, 12.5 μg/ml, 20.83 μg/ml, and 2.60 μg/ml, respectively. Compounds E and G were effective against *C. albicans* at 15.625 μg/ml and 25 μg/ml, respectively. Compounds I and G were fungicidal against *C. neoformans* at concentrations 50 μg/ml and 25 μg/ml, respectively, and compound G was fungicidal against *C. albicans* at 50 μg/ml. Cytotoxicity assays showed that antifungal compounds A, B, E, I, F, and G, and were non-toxic. RNA sequencing studies have identified several *C. neoformans* genes involved with the compounds’ inhibitory effects.

**Category IV: Poster Presentation**

**Generation of Antibodies to the Chlamydial Protein, CT226**

Clay Deal\*(Undergraduate), Christian Holcomb, Biraj Kayastha and Erika Lutter

Oklahoma State University, Stillwater, Oklahoma.

*Chlamydia trachomatis* is an important human pathogen and the leading cause of bacterial sexually transmitted infections in the United States. *C. trachomatis* is an obligate intracellular pathogen that resides in a parasitophorous vacuole, called an inclusion, that is decorated by chlamydial proteins called inclusion membrane proteins. These inclusion membrane proteins are considered to be the nexus for *Chlamydia­*-host interactions by directly interacting with host proteins. One of these inclusion membrane proteins, CT226, has multiple host interacting partners that have been identified in other studies. One of the limiting factors in characterizing this protein has been the lack of antibodies to CT226. In this project we have generated CT226 antibodies and show antibody specificity from different bleeds against purified CT226 protein.

**Category IV: Poster Presentation**

**Structure-Function Studies using Ancestral Sequence Reconstruction of Photoactive Yellow Protein as a Model System**. Rosalie L. Dohmen (Doctoral)\*, Gunnar Hoogerwerf, and Wouter D. Hoff. Oklahoma State University, Stillwater, Oklahoma.

The photoactive yellow protein (PYP) is a small, water soluble protein, first identified in *Halorhodospira halophila*. PYP is a bacterial photoreceptor that has emerged as a powerful model system for protein biophysics. It exhibits a light-triggered photocycle that proceeds in three basic steps: first the chromophore is photo-isomerized. Next, proton transfer occurs, triggering large structural changes of the protein. Lastly, thermal re-isomerization and resetting of the initial protonation states occurs, which cause the protein to re-fold and to recover the initial ground state. In total, 11 PYPs have been studied experimentally to determine the speed of the recovery of the initial state of PYP. Some PYPs have a recovery rate of 0.5 seconds while others of 60 minutes. We aim to determine which residues cause this change in photocycle recovery rate by using an ancestral protein triangulation approach. We identified 998 PYP homologs for which we could obtain a high-quality multiple sequence alignment. This alignment allowed us to predict the amino acid sequences of all 996 nodes in the phylogenetic tree of these PYPs. An ancestor of interest was picked and we designed a synthetic *pyp* gene encoding it. Biochemical and spectroscopic studies of its functional rate are under way.

**Category: IV Poster Presentation (in person)**

**Bacterial Photobiology: Photoactive Yellow Protein as a Model System for Light Regulation of Microbial Physiology and Protein Function.** Rosalie Dohmen, Catalina Bradley, Breden Heise, Gunnar Hoogerwerf, Saylor Hampton, Clarice Huffman, Scout Powell, Sarah Teeman, Aihua Xie, Wouter D. Hoff\*. Oklahoma State University, Stillwater, Oklahoma.

Bacterial photoreceptors are of interest for three reasons. (1) Approximately one quarter of all sequenced bacterial genomes encode one or more photoreceptor. This observation implies the existence of a large field of mostly unexplored bacterial photobiology: what processes are under light-regulation, and what signal transduction pathways are involved? (2) In general, proteins are complex nanoscale devices that contain thousands of moving parts, often operating in the time window picoseconds to milliseconds. The light-triggered nature of photoreceptors offers a unique experimental opportunity into the molecular mechanisms underlying protein function. (3) Photoreceptor domains can be engineered into fusion proteins to place a range of functions, such as DNA binding or enzymatic activity under light control (often referred to as optogenetics). Here we summarize our work on the photobiology and protein science of photoactive yellow protein, a unique family of bacterial photoreceptor proteins. We (1) find bioinformatics evidence that PYP can trigger a range of responses through a variety of signal transduction chains, (2) identify a novel type of conserved interactions in the protein superfamily encompassing PYP, and (3) are developing vibrational spectroscopic approaches to probe active site structure and dynamics.

**Category IV: Poster Presentation**

**Characterization of the Role of Outer Membrane Porins in *Escherichia coli* Colonization of the Mammalian Intestine**

Sudhir Doranga (Doctoral) \*, Dr. Tyrrell Conway

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

Outer membrane of *Escherichia coli* contains channel-forming proteins which allow exchange of nutrients and waste products with the environment. Previous studies showed that the GI tract of streptomycin treated mice fed with wild type *E. coli* MG1655 selects for *envZ* missense mutants which have altered outer membrane protein profiles than the wild type (WT). This suggests a possible role for outer membrane porins (OMPs) in colonization of the mouse intestine. We offer the hypothesis that OMPs play a key role in colonization of mouse intestine by *E. coli*. We showed that in competitive colonization experiments, *ompA* and *ompC* knockout mutants are outperformed by the WT during 15 days of competition and an *ompC* mutant failed to saturate all the niches in the intestine. Interestingly, the *ompF* mutant colonized better than the WT in competitive colonization experiment and appears to outcompete the WT in the same niche. Further experiments to understand the competitive advantage in colonization suggest that *ompF* mutant overexpresses OmpC. However, both overexpression of OmpC and deletion of OmpF are required to confer the colonization advantage. These experiments provide a better understanding of how outer membrane porins affect the ability of *E. coli* to compete in the mammalian intestine.

**Category: IV General Microbiology Graduate Poster presentation**

**A Novel Lipase Plays a Role in PstN-Mediated Biofilm Suppression in *Pseudomonas aeruginosa*.** Mary J. Erdmann (Undergraduate)\*, Somalisa Pan, and Dr. Matthew Cabeen

Oklahoma State University, Department of Microbiology and Molecular Genetics

Stillwater, Oklahoma

*Pseudomonas aeruginosa* strain PA14 is an opportunistic pathogen that causes nosocomial infections and whose antibiotic resistance poses an ongoing medical threat. One mechanism of drug therapy resistance is the production of biofilm - a self-secreted substance made of polysaccharides, DNA, and proteins that binds cells together, shielding bacteria from their environment. Unraveling the mechanisms behind *P. aeruginosa* biofilm regulation would provide better an understanding for how this species manages virulence. In PA14, PtsN, part of a phosphorelay called the Nitro-PTS, was identified as a negative regulator of biofilm formation when unphosphorylated. The Nitro-PTS is known to regulate various cellular functions; identifying potential biofilm-suppressive targets of unphosphorylated PstN would contribute to understanding how unphosphorylated PstN decreases biofilm. Using transposon mutagenesis, an unnamed gene (*04030*) was observed to have a biofilm-suppressive function in an unphosphorylated PtsN background. Predicted protein structures suggested that 04030 has a folding pattern like lipase enzymes. Furthermore, our studies suggest that 04030 has a catalytic serine that is important for 04030 function and appears in a signature catalytic motif typical of lipase enzymes. When unphosphorylated, PstN is potentially interacting with a downstream gene encoding a putative lipase, 04030, to achieve biofilm suppression in PA14.

**Category: I General Microbiology Oral Presentation**

**Regulation and Function of an Atypical MAPK in *Dictyostelium***

Saher Fatima (Doctoral)\* and Jeffrey A. Hadwiger. Department of Microbiology and Molecular Genetics, Oklahoma State University, Stillwater, Oklahoma

MAPKs are common regulatory proteins in eukaryotes that play vital roles in various signaling pathways. Not much is known about the function and regulation of one subgroup of MAPKs (atypical MAPKs). The soil-dwelling amoeba *Dictyostelium discoideum* encodes only two MAPKs, an atypical MAPK Erk2 (homologous to mammalian MAPK15) and typical MAPK Erk1. *Dictyostelium discoideum* is an excellent model for understanding chemotactic movement and development. Previous studies have demonstrated that *Dictyostelium* Erk2 is important for chemotaxis to cAMP and folate. Erk2 is activated in response to chemoattractant stimulation and required for the burst of Erk1 activation in a secondary response. However, the mechanism behind the activation of atypical MAPKs is not understood. To see if Erk2 is responsible for its own activation or whether another protein kinase phosphorylates Erk2, a kinase-dead Erk2 mutant (Erk2KD) was created and expressed in erk2- cells. Immunoblot analysis indicates phosphorylation at the activation motif of Erk2KD after chemoattractant stimulation illustrating the absence of autophosphorylation. Chimeric MAPKs comprising parts of both MAPKs are being created and tested to better understand the function and regulation in *erk2-* and *erk1-* cells. Our study of MAPKs in *Dictyostelium* is likely to provide insight into MAPK signaling pathways in other eukaryotes.

**Category:** **I** **General Microbiology Graduate Student Oral Presentation**

**In-Silico Screening of Peptide Mimetics for Binding to a TA System ParE Toxin**  
Felipe Ferreira (Undergraduate Student) \*, Kevin Snead, Christina Bourne.

University of Oklahoma, Norman, Oklahoma

Toxin-antitoxin (TA) systems are widespread in bacterial genomes. These proteins have potential for new antibiotic approaches due to their overlap in activity with known antibiotics. This study focuses on the type-II TA system ParDE1 from *Pseudomonas aeruginosa* with an objective of identifying potential competitors for the critical interaction between the toxin ParE1 and the antitoxin ParD1. The approach uses in-silico screening of peptide mimetics for interaction with the toxin ParE1 surface that interacts with ParD1, and to then compare the strength of the predicted interactions. Computational tools (AutoDock Vina and UCSF Chimera) were utilized for docking and visualization of the predicted interactions. After screening, some major characteristics of scaffolds were identified, and in particular, an indole-like moiety seems critical for the interaction, as the predicted energetics were significantly greater in compounds containing this. Additionally, these ring structures closely align with a tryptophan binding pocket found in the wild-type interaction. When successful, the identification of the competing compounds could liberate toxin activity to control bacterial cell growth.

**CATEGORY: IV POSTER PRESENTATION**

**The Impact of Coculturing on Methane Oxidizing Bacteria.**

Christopher T. Garner (Doctoral)\*, Jared Gregston, Lee R. Krumholz. University of Oklahoma, Norman, OK.

Methane is a greenhouse gas that contributes to global warming and understanding how methane producing ecosystems function is of great interest. The primary biological methane sink is oxidation to carbon dioxide by methane oxidizing bacteria (MOB) which are a critical component of how methane producing ecosystems function and are a primary focus of understanding these systems. There is much to understand about how MOB interact with non-methane oxidizing heterotrophic bacteria (NMOHB). We conducted experiments comparing methane oxidation rates (MOR) of three monoculture MOB to cocultures of the same MOB with various NMOHB. We have shown that the MOB *Methylosinus trichosporium* OB3b has a MOR of 0.01 ± 0.004 h-1 in monoculture but the MOR increased significantly to 0.02 ± 0.003 h-1 when in coculture with a species of *Pseudomonas* (p-val = 0.04). However, when *Methylosinus trichosporium* OB3b was in coculture with species of *Cupriavidus* or *Flavobacterium* there was no increases or decrease in the MOR compared to monoculture. Similar trends are present across other species of MOB as well. These results indicate that different species of MOB and NMOHB interact in different ways, implying that the relationships between microbial members in methane oxidizing systems are complex and diverse.

**Category II: Environmental Microbiology Oral Presentation (In-Person)**

**Title: Effect of Vancomycin on *Elizabethkingia* *anophelis***

Sushim Kumar Gupta (PhD)a#, William L. Johnsona, Suman Maharjanb, Randy M. Morgensteinb, and John E. Gustafsona\*

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The Gram-negative *Elizabethkingia* represent opportunistic pathogens that cause severe disease which is complicated by a multiple antimicrobial resistance mechanism. Although a Gram-positive drug, vancomycin has been used extensively to treat *Elizabethkingia* infections, so we examined the effects of vancomycin on *Elizabethkingia anophelis*. *E. anophelis* was relatively vancomycin susceptible and challenge with this drug led to morphological changes indicating cell lysis. Vancomycin growth challenge however demonstrated that vancomycin-resistance readily emerged, vancomycin-selected mutants appeared at high mutation frequencies, and vancomycin-selected mutants demonstrated reduced susceptibility to antimicrobials other than vancomycin. Genome analysis revealed that vancomycin-selected mutants possessed a mutation in a transcriptional regulator gene (*vsr1* = vancomycin-susceptibility regulator) located in a bicistronic operon (*vsr1* - *ORF551*)that was upregulated in vancomycin-selected mutants. Phage shock systems affect inner membrane permeability and ORF551 has 5 transmembrane spanning regions and a phage shock protein conserved domain. We propose that *E. anopheles* is inherently vancomycin-resistant, the *vsr1* mutation supports a multiple antimicrobial reduced susceptibility mechanism, and *vsr1* acts as a negative regulator of *vsr1*-*ORF551*. This is the first report that a PadR homologue (Vsr1) affects antimicrobial susceptibility in a Gram-negative organism. Additional work is required to determine if ORF551 overexpression alters membrane permeability in *E. anopheles*.

**Key words:**

*Elizabethkingia anophelis*, vancomycin, whole genome sequencing, PadR, and vancomycin-susceptibility regulator 1 (Vsr1).

**Category: IV Poster Presentation**

***Pseudomonas aeruginosa* Pyocins Can Extend Antibiotic Effectiveness**

*Christopher Hamm* (Doctoral candidate) *\*, Matthew T. Cabeen.* Department of Microbiology and Molecular Genetics, Oklahoma State University, Stillwater, OK.

*Pseudomonas aeruginosa* is a notorious gram-negative human pathogen that often resists antibiotic treatment. Pyocins, phage tail-like complexes that *P. aeruginosa* uses to kill and compete with other *P. aeruginosa* strains, represent a possible alternative or adjunct to antibiotics. Pyocins are typically released in response to DNA damage via RecA, and like phages they are released by producer cell lysis. We recently found that strains lacking the tyrosine recombinase XerC strongly express pyocins via a novel RecA-independent but DNA damage-inducible pathway. We sought to test whether Δ*xerC* cells, which are hypersensitive to ciprofloxacin, would extend the effect of this antibiotic to other pyocin-sensitive strains. We found that a sublethal concentration of ciprofloxacin did not substantially block the growth of strain 13S, a pyocin-sensitive strain. However, when 13S was co-cultured with Δ*xerC* cells, sublethal ciprofloxacin treatment effectively inhibited the growth of the co-culture, with preferential killing of the 13S cells. Moreover, the inhibition depended on pyocin production. Our data represent a proof of principle that XerC-deficient strains can increase the effectiveness of antibiotics against *P. aeruginosa*, opening the door to future therapeutic strategies.

**I. General Microbiology Oral Presentation (in person)**

**Effect of Low Molecular Weight Branched Polyethylenimine (BPEI) on Planktonic Carbapenem-Resistant *Enterobacteriaceae* and their Biofilms.** Neda Heydarian (PhD Candidate) \*, Cassandra L. Wouters, Andrew J. Neel, Charles V. Rice PhD.

Department of Chemistry and Biochemistry, University of Oklahoma, Norman, Oklahoma.

**Abstract**

Carbapenem-Resistant *Enterobacteriaceae* (CRE) are emerging pathogens causing a variety of severe infections. CRE evade antibiotic treatments because these bacteria produce enzymes that degrade a wide range of antibiotics including carbapenems and β-lactams. Due to formation of biofilms, CRE infections are difficult to treat. The persistence of the biofilms leads to non-healing wounds. There is a need for new therapeutic adjuvants to overcome CRE antimicrobial resistance, disrupt its biofilms, and improve healing of infectious wounds. Recent studies in our lab show that 600-Da branched polyethyleneimine (BPEI) and its derivative PEG350-BPEI can overcome antimicrobial resistance and eradicate biofilms in methicillin-resistant *S. aureus*, methicillin-resistant *S. epidermidis*, *P. aeruginosa*, and *E. coli*. In this study, the ability of 600-Da BPEI and PEG350-BPEI to eradicate carbapenem-resistant *Enterobacteriaceae* bacteria and their biofilms is demonstrated. We show that both BPEI and PEG-BPEI have anti-biofilm efficacy against CRE family. Furthermore, our results illustrate that BPEI can disrupt the normal cell cycle of planktonic CRE bacteria. These data validate the multi-functional properties of 600 Da BPEI and PEG350-BPEI to reduce biofilm formation and mitigate virulence in carbapenem-resistant *Enterobacteriaceae*.

**Preferred Presentation Format:** Poster

***Chlamydia trachomatis* and the Characterization of its Inclusion Membrane Protein CT226**

Hollis C. Holcomb (PhD Student)1, Prakash Sah1, Madison Tryzbiak2, Jennifer H. Shaw3, Erika I. Lutter1. *1Department of Microbiology and Molecular Genetics, Oklahoma State University, Stillwater, OK, USA, 2Department of Veterinary Pathobiology, Oklahoma State University, Stillwater, OK, USA, 3Department of Biomedical Sciences, Philadelphia College of Osteopathic Medicine, South Georgia, GA, USA*

*Chlamydia trachomatis* remains a significant human pathogen. *Chlamydia*’s biphasic lifecycle has its replicative stage inside of a parasitophorous vacuole termed an “inclusion”. *Chlamydia* must secrete inclusion membrane proteins (Incs) into the membrane of the inclusion to receive nutrients and communicate with the host cell. These proteins are unique to *Chlamydia*. This study focuses on the Inc CT226. Several of the potential interacting partners with this protein include LRRFIP1, LRRFIP2, FLII, and TMOD3, proteins that bind to and modify the actin cytoskeleton of the host cell and are negative regulators of the inflammasome. We hypothesize that the Inc CT226 and its interacting partners are important for chlamydial infection.An L2ΔCT226 mutant was developed in our lab, and it shows a lack of recruitment of FLII and altered recruitment of LRRFIP1 and TMOD3 *in vitro*. A murine cervicovaginal infection model was performed to quantify infectious forming units shed by our two strains and found that they were higher in the L2ΔCT226 mutant than the L2 wild-type. By deleting this protein, it has altered the infectivity of the pathogen and provides context for how the deletion of the interaction between CT226 and the host protein interactions change how the pathogen infects.

**Category: III Medical Microbiology/Immunology Graduate Student Oral presentation (in person)**

**CarP, a Putative Phytase, Regulates the *Pseudomonas aeruginosa* Metabolome in Response to Elevated Ca2+.** Mackenzie Hull (Doctoral)\*, Michelle King, Marianna Patrauchan. Oklahoma State University, Stillwater, Oklahoma.

*Pseudomonas aeruginosa*, a Gram-negative opportunistic pathogen, is notorious for its ability to adapt to different environments. One example is the lungs of patients with Cystic Fibrosis (CF), which is associated with a dysregulation in ion homeostasis, including Ca2+. Previously we have shown that *P. aeruginosa* responds to elevated Ca2+ via a two-component system, CarSR, leading to an alteration in gene transcription and attenuation in virulence. One of the known regulatory targets of CarSR, *carP*, encodes a predicted 5-bladed β-propellor with a phytase-like domain. To identify the function of CarP, a global metabolomics analysis was performed. For this, wild type PAO1 and ∆*carP* were grown to mid-log and stationary phases at 0 or 10 mM Ca2+ followed by GC-MS. We found that 88 of the 187 detected metabolites were dysregulated by Ca2+ in mid-log cells with 73% of them requiring CarP. The stationary phase cells had 127 metabolites that were Ca2+-dependent with 48% being CarP-dependent. These metabolites represented metabolic pathways associated with biosynthesis of amino acids, nucleotides, fatty acids, and carbohydrates. These findings suggest that CarP plays a major role in Ca2+ regulation of *P. aeruginosa* metabolism, which is likely due to its enzymatic activity or interactions with other proteins.

**IV. Poster Presentation (in person only)**

# **Characterization of the ClpC AAA+ ATPase of *Chlamydia trachomatis***

Aaron A. Jensen (Doctoral)**a**\*, Nicholas A. Wood**a**, Derek J. Fisher**b**, Scot P. Ouellette**a**

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The obligate intracellular Gram-negative pathogen *Chlamydia trachomatis* is the leading cause of preventable infectious blindness and bacterial sexually transmitted infections worldwide. *Chlamydia* has a biphasic developmental cycle consisting of the infectious but non-dividing elementary body and the non-infectious but replicating reticulate body. Given its two distinct forms, protein degradation is critical for proteome alteration during differentiation between one form and the other. One way bacteria degrade proteins is through the caseinolytic protease (Clp) system, which uses an unfoldase (e.g. ClpX or ClpC) that hydrolyzes ATP to unfold target proteins into the associated proteolytic barrel—comprised of ClpP. We hypothesize that ClpC, typically found in Gram-positive bacteria and mycobacteria, recognizes specific substrates to effect chlamydial differentiation. Bioinformatics studies indicate *C. trachomatis* ClpC has two ATP binding and hydrolysis Walker A/B motifs. *In vitro* assays confirmed the expected ATPase and protease activity of the protein. *In vivo* assays demonstrated that overexpression of wild-type and ATPase-null mutants leads to a significant decrease in growth and number of infectious progeny as assessed by immunofluorescence assay and inclusion forming units, respectively. Drastic impacts on development and stability when altering ClpC levels show its importance for *Chlamydia* growth and differentiation.

**Category: III Medical Microbiology/Immunology Graduate Student Oral presentation (virtual)**

**Structure Function Studies of The Ndhd4 Protein of the CO2 Uptake Mechanism in Synechococcus Elongatus (PCC 7942)**

Clark K. Jett1(Masters)\*, Anton P. Avramov1, Minquan Zhang1, Ross Walker1, Robert L. Burnap1

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Carbon dioxide (CO2) uptake and fixation by cyanobacteria, algae and plants is one of the unique and distinctive features of photosynthetic organisms. The CO2 fixation allows these organisms to create sugars, create biomass and effectively store nutrition and energy in the form of complex organic molecules. To overcome the poor Km value of Rubisco, cyanobacteria employ a unique carbon concentrating mechanism (CCM). The cyanobacterial CCM utilizes a modified NADH dehydrogenase oxidoreductase (Ndh-13,4) to hydrate CO2 to more practical bicarbonate (HCO3-).

The purpose of this study is to investigate the NdhD4 protein, which is part of the NDH-14 complex, and is positioned in the thylakoid membrane. Upon the hydration of CO2 into HCO3- via CupB, a proton is released on the cytosol side of the thylakoid membrane. It is hypothesized that the NdhD4 protein functions to remove these protons by pumping them across to the luminal side of the membrane away from the anhydrase active site and avoiding the back reaction. This hypothesized ‘product removal mechanism’ allows the reaction in CupB to maintain energetic favorability in the direction of HCO3- production.

**Category: V Poster Presentation Masters/Graduate Student**

**Blocking Essential Nutrient Acquisition in *Pseudomonas aeruginosa.***

Mamie Kannon\* (Masters) and Avishek Mitra.

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

*Pseudomonas aeruginosa* (*Pa*) is a serious lung pathogen which has been declared by the World Health Organization as a “Priority 1: Critical Pathogen” needing immediate new strategies for chemotherapy. Since iron is an essential micronutrient required by *Pa* to colonize the human host, numerous inhibitors have been developed to block *Pa* siderophore-iron acqsuition, which is essential for capturing host ferric iron. But*Pa* also utilizes backup systems to acquire ferrous and heme iron from the host, making the siderophore inhibitors ineffective in treating *in vivo Pa* infections. *We hypothesize that all iron acquisition systems of Pa must be blocked simultaneously in vivo to make inhibition of iron acquisition a viable antipseudomonal chemotherapy*. Our research goals are to identify molecules to block the backup iron acquisition systems of *Pa*. After screening 1600 molecules, we identified 7 and 4 molecules that specifically inhibit the growth of *Pa* in the presence of heme or ferrous iron, respectively. In future experiments, we will determine efficacy of these novel *Pa* heme and ferrous iron inhibitors against drug-resistant-*Pa* and in biofilm assays. Our long-term goal is to produce a cocktail of molecules containing inhibitors that can simultaneously block the major iron acquisition pathways of *Pa*.

**Category: III Medical Microbiology/Immunology Graduate Student Oral presentation**

**Mechanistic Basis for Stress Sensing by the *Bacillus subtilis* Stressosome**

Rabindra Khadka (PhD)\*, Brannon Maravich (Undergraduate)\*, and Matthew Cabeen. Oklahoma State University, Stillwater, Oklahoma

*Bacillus subtilis* uses cytoplasmic multiprotein complexes called stressosomes for sensing a variety of environmental stressors. The stressosome complex consists of two types of core proteins: the RsbRs (RsbRA, RsbRB, RsbRC, RsbRD, and YtvA) and RsbS. It is not known exactly how proteins in the stressosome sense stress to initiate and activate the σB stress response. A longstanding model assumed that σB activation by environmental stress depends on the RsbR proteins, which are organized by RsbS, which serves a scaffolding function. Here, we present preliminary genetic evidence that contradicts this model, suggesting that RsbS or RsbR proteins might individually be sufficient to control environmental stress signaling. Ongoing studies are examining whether RsbS alone can independently respond to environmental stress. We also have evidence that the presence of any single RsbR protein is sufficient to modulate the σB stress response. Using RsbRA as a model, we highlight the impact of individual amino acid residues in its putative N-terminal stress-sensing domain on stress response patterns via alanine scanning. We expect that our studies will provide significant insight into how the stressosome proteins are flexible in exerting control over stress sensing.

**Category IV: Poster Presentation**

**Distribution of Potential Bacterial Pathogens in Bacterially Impaired Watersheds**

John C. Kincaid1, Marc R. Owen2, Robert T. Pavlowsky2 and Babur S. Mirza1\*

1 Biology Department, Missouri State University, Springfield, Missouri

2 Ozark Environmental and Water Resources Institute, Missouri State University, Springfield, Missouri

Bacterial impairment of freshwater systems is a commonly studied global problem. However, studies on the relative distribution of bacterial pathogens in different impaired aquatic systems have been limited. Frequently, impaired freshwater systems are classified by the presence of fecal indicator bacteria (FIB) and the identification of sources of fecal contamination through microbial source tracking. We assessed the relative abundance of DNA sequences related to potential human bacterial pathogens along with human fecal indicator bacteria in three impaired watersheds. These watersheds consistently showed a high abundance of FIB for the past several years. Using Illumina paired-end DNA sequencing of 16S rRNA gene amplicons, we observed variation in the relative distribution of DNA sequences related to *Legionellaceae*, *Enterobacteriaceae* and *Bacteroidaceae* families across different sites. We identified potential hotspots sites in these impaired water systems, which showed a relatively high abundance of pathogen-related DNA sequences. This study demonstrates the significance of Next-Gen DNA sequencing for the initial screening of waterborne pathogens and the identification of high-risk sites for preferential remediation efforts in impaired water systems. Secondly, the frequent temporal monitoring of specifically identified pathogens that are in high abundance in a watershed can help in the accurate prediction and prevention of disease outbreaks.

**Category: II** **Environmental Microbiology** (Oral Presentation)

**A Single-cycle Live Respiratory Syncytial Virus Vaccine Expressing Prefusion F Protein**

Pramila Lamichhane1\*(Doctoral), Megan E. Schmidt2, Megolhubino Terhuja1, Steven M. Varga2, Tim Snider1, Christina A. Rostad3, Antonius G.P. Oomens1

1College of Veterinary Medicine, Oklahoma State University, Stillwater OK

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Respiratory syncytial virus (RSV) is responsible for close to 200,000 deaths per year in children worldwide and a vaccine is not available. To develop an efficacious yet safe live vaccine, we generated RSV-preF, a single-cycle live RSV expressing a membrane-anchored prefusion-stabilized form of F (preF) (based on DS-Cav1 mutations, McLellan et al, 2013) in place of native F protein. Because preF is no longer functional and the native F gene is absent, RSV-preF is amplified in baculovirus GP64 complementing cells and has single-cycle characteristics in normal cell types and vaccine recipients, as it cannot spread beyond the site of application. Intranasal prime-boost vaccination of mice with RSV-preF induced a high level of anti-preF antibodies and virus specific anti-viral T cells. RSV-preF induced antibodies neutralized wildtype RSV in vitro and protected mice from wildtype RSV challenge-induced lung pathology and prevented viral multiplication in lungs. A virus solely expressing a membrane-anchored prefusion variant of F is unique in combining a stringent single-cycle safety feature with broad efficacy including anti-preF humoral and cellular immunity, and has potential to overcome previous challenges in the balance of vaccine efficacy and safety.

**Category: III Medical Microbiology/Immunology Graduate Student Oral presentation**

**Identification of Critical Virulence Factors of *Fusobacterium nucleatum* in Promoting Oral Squamous Cell Carcinoma.** Serene B. Y. Lim (doctoral)\*, I-Hsiu Huang.

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

*Fusobacterium nucleatum*, a Gram-negative oral commensal in human, has recently been shown to contribute to the initiation and progression of colorectal cancer (CRC) and oral squamous cell carcinoma (OSCC). However, the mechanism of *F. nucleatum*-induced oral cancer has not been well established. In this study, we aim to identify the key regulators of *F. nucleatum* which play important roles in direct and indirect interactions with OSCC to promote the aggressiveness and invasiveness of the disease. Interactions will be analyzed through cell proliferation and migration assays with the treatment of bacterial cells or supernatant. Our preliminary data reproduced some of the initial critical observation published previously. By treating HCT 116, a CRC cell line, with bacterial supernatant, cell proliferation and migration were enhanced by *F. nucleatum* wild type strains. The same approaches with the addition of cell invasion and adhesion assays will be applied on the SCC-15, a OSCC cell line. Our preliminary data on clinical strains have shown that the *F. nucleatum* isolates which were extracted from OSCC patients to be more invasive than the non-OSCC group. Further investigation will be demonstrated to expand the knowledge towards the development of novel OSCC prevention and treatment strategies.

**Category: IV Poster Presentation (in person only)**

**Upregulation of Calcium Stimulated Host Adherence of *Pseudomonas aeruginosa* During Infection**

Deepali Luthra (Doctoral)\*, Aya Kubo, Ty Lutze, Marianna Patrauchan, Erika Lutter.

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

*Pseudomonas aeruginosa*is the key bacterial agent that forms biofilms on airway mucosal epithelia in the lungs of cystic fibrosis (CF) patients. Calcium (Ca2+), a cellular signaling molecule that accumulates in the pulmonary fluids of CF patients is shown to act as a trigger for expression of virulence factors in *P. aeruginosa*, but little is known about how itregulates host-pathogen interactions. Our project focuses on understanding how Ca2+ affects the adherence of *P. aeruginosa* during infection. Adherence studies were performed in low and high Ca2+ using A549 and CuFi5 (homozygous for the ∆508 mutation in CF) lung epithelial cells infected with *P. aeruginosa* PAO1 and pulmonary isolate FRD1. A significant increase in adherence of all bacterial strains was observed in high Ca2+when compared to low Ca2+ conditions using both cell lines, along with the calcium deposition by FRD1 on the surface of CuFi5 cells, as observed by scanning electron microscopy (SEM). By quantitative RT-PCR (qRT-PCR), we determined that the transcription of adhesins (*fliC, pilA* and *lecA*) is elevated in response to Ca2+ in *P. aeruginosa* isolates during adherence. Additionally, increased concentration of Ca2+ enhances the flagellation frequency of *P. aeruginosa.*

**Category: III Medical Microbiology/ Immunology- Graduate Student Oral presentation (In person)**

**Development of a Respiratory Syncytial Virus based Virus-like Particle Vaccine.**

TerhüjaM(Doctoral)\*, Siddappa M, Lamichhane PL, and Oomens AG.Department of Veterinary Pathobiology, College of Veterinary Medicine,Oklahoma State University, Stillwater, Oklahoma.

Respiratory Syncytial Virus (RSV) is a major cause of bronchiolitis and pneumonia in infants, young children, and the elderly people worldwide. Currently development of an anti-RSV vaccine is a WHO priority. Among the many vaccine approaches, virus-like particles (VLPs) have gained increased vaccine potential as they lack the viral genetic material. VLPs also accurately mimic the natural virus thereby inducing relevant immune responses. We hypothesize that a novel RSV-based VLP vaccine containing multiple viral epitopes can induce a safe and effective immune response. The most important RSV antigens are fusion protein F and attachment protein G. Using 293 cells, VLPs displaying either prefusion stabilized form of F or that of the central conserved region of G(GCR)were generated and vaccine potential of these VLPs was tested in a mouse model. BALB/c mice (n=5/group) were vaccinated intranasally in a prime-boost regimen. Antibody levels and specificities were determined three weeks after the boost. Initial testing shows mice infected intranasally with VLPs had higher RSV specific antibodies than their controls. Insights gained from these findings suggest successful induction of antibodies after intranasal vaccination and the potential of this method to elicit a local mucosal immune response in the respiratory tract.

**Category: III Medical Microbiology/ Immunology Graduate Student Oral presentation**

**Effect of Media Type on the Growth of Methanotrophic Enrichment Cultures.**

Ravi K. Manjhi (Masters)\*, Chuang Li, and Lee R. Krumholz. The University of Oklahoma, Norman, Oklahoma.

Methanotrophic bacteria play a crucial role in regulating methane (an important greenhouse gas) release into the environment by oxidizing it. Using 16S rRNA gene analysis, we previously observed a very high relative abundance (~15%) of novel methanotrophs in Norman landfill soil. The aim of the study was to determine the effect of media type on the growth of methanotrophic enrichment cultures and try to cultivate the novel methanotrophs from Norman Landfill and OU Duck Pond soil samples. Soil samples were inoculated from both environments into 11 different media types at different dilutions. Methane oxidation was monitored, and cells were harvested from the highest two dilutions, which exhibited methane oxidation. It was observed that methanotrophs grew more slowly in Ammonium Mineral Salts (AMS) media compared to the other media types. No methane oxidation was observed in the landfill enrichment cultures with 20% O2 but was observed at lower O2 concentrations. Methanotrophic growth was also faster at higher pH in duck pond enrichment cultures. It appears that AMS media inhibits growth of some methanotrophs from these two systems.

**Category: II Environmental Microbiology Graduate Student Oral Presentation (in person)**

**Viable Round Mutants of *Escherichia coli* with Point Mutations in *mreB***

Suman Maharjan **(**Doctoral)**\*,** Ryan Sloan, Jada Lusk, Rose Bevienguevarr, and Randy Morgenstein. Oklahoma State University, Stillwater, Oklahoma.

The actin-homolog *mreB* is a crucial component of the rod-machinery that adds new peptidoglycan material to the cell wall for lateral elongation during growth. It is necessary for maintaining the rod shape of *Escherichia coli* and many other rod-shaped bacteria. MreB is functionally active as polymeric nanofilaments localized beneath the inner membrane along the long axis of the cell. As expected, cells lacking MreB or in the presence of depolymerizing agent A22 suffer loss of rod shape that ultimately leads to cell death. Previous studies looking at random mutations in MreB observed varying degrees of alteration in cell shape and size implying the effect of each amino acid residue for rod-shape dynamics is independent. Here, we have taken a systematic approach by creating an alanine-scanning library to better understand the role of each residues in MreB. We have analyzed over 250-point mutants and observed a varying degree of cell shape modulation. Surprisingly, fourteen mutants have a round shape with a similar shape-phenotype to A22 treated cells. The fact they are viable despite this shape defect suggests MreB has vital functions related to growth in addition to shape maintenance. In support of this, cell shape suppressors analysis of mutants able to restore rod shape only revealed either reversions or mutations in MreB suggesting that MreB is necessary for rod shape.

**Category: I General Microbiology Oral Presentation**

**Halophiles isolated from edible salt using a cultromics approach.**

Sydney Marouk (Graduate Certificate) \* and Dr. Ratnakar Deole

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

Named for their affinity for salt, halophiles survive and grow in some of the harshest and unhabituated environments due to their unique ability to compensate for the physical stressors’ hypertonicity imposes on cells. Consequently, unlike the non-extremophilic microorganisms, halophiles are not effectively compromised when salt is being used to decrease bacterial growth.  Salt's use as a preservative in foods had long been employed despite evolving mechanisms for preservation.  By decreasing the amount of unbound water for microorganisms and their chemical reactions, salt works to reduce the growth of pathogens and microorganisms which could lead to spoilage or illness.  However, Halophiles can resist the preservative properties of the salt and continue to grow.  It is not yet determined the roles halophiles can play in health, especially mouth and gut microbiomes.  In this study, an investigation was done to determine the microbial presence in edible salts using a cultromics approach.  Four commercially used, edible table salts were purchased at a grocery store, dissolved in solutions, and plated onto salt enriched Difco marine agar and broth medium. Edible coarse sea salt showed growth of a halophile. This halophilic organism was purified using plating techniques and further identified using biochemical tests and 16S ribosomal RNA sequencing.

**Category: V Flash talk (virtual only)**

**The Interactions of PhoU1 and PhoU2 Homologs in *Staphylococcus aureus***.

Clayton T. Matthews (Masters)\* and Stewart G. Gardner. Emporia State University, Emporia, Kansas.

The pathogenesis of *S. aureus* is closely related to virulence factors and persister formation. PhoU homologs are critical in defining the regulation of persister cell formation and phosphate metabolism in invasive bacteria. Specifically, two PhoU protein homologs in *S. aureus*, PhoU1 and PhoU2, are instrumental in regulating the PstSCAB phosphate transport complex and key to persister formation. How these homologs interact within *S. aureus* phosphate regulation remains unclear. This comprehensive research focused on the two proteins interaction using a Bacterial Adenylate Cyclase Two-Hybrid system. Furthermore, the level of interactions between proteins was determined using quantitative Beta-galactosidase and qualitative colorimetric assays. Protein structures were predicted using Phyre2 and Cluspro protein modeling software. These observations indicate PhoU1 self-dimerizes. In addition, PhoU2 and PhoR also self-dimerize. The analyses revealed there is some, although weak, interaction between PhoU1 and PhoU2. PhoU1 interacts with PstB; however, there is no interaction with PhoU2 and PitA. Research findings also indicate the structure of PhoU1 and PhoU2 dimers and possible PhoU1-PhoU2 heterodimer protein structure models. This study provides a coherent understanding of PhoU1 and PhoU2 interaction with other proteins and points to potential models of how PhoU1 and PhoU2 are involved in phosphate signaling and virulence gene regulation.

**Category: V. Flash Talk**

**Inhibition of *Clostridioides difficile* by a Probiotic Derived Recombinant Bacteriocin.** Joseph H. McCreary (Masters)\*1, Hsiang-Ning Chang2, Yuan-Pin Hung3, Jenn-Wei Chen2, and I-Hsiu Huang1.

1Oklahoma State University Center for Health Sciences, Tulsa, Oklahoma

2National Cheng Kung University, Tainan, Taiwan

3National Cheng Kung University Hospital, Tainan, Taiwan

*Clostridioides difficile* is a gram-positive bacterial pathogen and the leading cause of antibiotic-associated diarrhea worldwide. Disruption of the gut flora by antibiotic use is the major reason for *C. difficile* induced pseudomembranous colitis. Traditional treatment of CDIs relies on further use of antibiotics which often result in multiple relapses and increase risk for antibiotic resistance. In our laboratory, we previously identified CBMB, a bacteriocin secreted by a commercially available probiotic strain of *Clostridium butyricum*, as capable of inhibiting *C. difficile* growth in vitro. Recombinant CBMB (rCBMB) was shown to display a selective spectrum of activity targeting *Clostridium* species. In vitro growth inhibition assays indicated rCBMB exhibited potent bactericidal activity against *C. difficile*. Intra-rectal administration of rCBMB along with vancomycin in a mice model revealed less body weight reduction and bacterial load in feces of infected animals. In this study, in order to further understand the mechanism of action of rCBMB and to improve its stability, we created various truncations of the 7-kDa rCBMB and assessed their antibacterial activity in vitro. The eventual goal of this project is to generate potentially novel alternative treatment for *C. difficile* infections.

**Category IV: Poster Presentation**

**Jumping Species: Spilling the Beans On The Next Epidemic**

Samantha Mercer (Undergraduate)\*, Darby Carlson, Julie Shaffer, Department of Biology, the University of Nebraska at Kearney, NE 68849.

*Dermacentor variabilis* is the primary native tick species in Nebraska and a vector of intracellular pathogens, including several spotted fever group rickettsia and *Francisella tularensis*. Our objective was to survey female *D. variabilis* ticks collected along the Platte River in south-central Nebraska during the 2021 tick season to determine the bacterial pathogen prevalence. Total DNA was extracted from 332 female ticks for PCR analysis and confirmation of pathogen identity using amplicon sequencing. Out of 332 females*,* 41% (139) tested negatives to the pathogens. The presumptive positives consisted of 30% (102) *Rickettsia amblyommatis*, 2% (8) *Ehrlichia chaffeensis*, 6% (23) *Rickettsia montanensis*, 1% (4) *E. chaffeensis* and *Rickettsia amblyommatis*, 1% (2) *F. tularensis,* and *Rickettsia rickettsii* was not detected. This increase in *R. amblyommatis* may be due to increased overlap of *Amblyomma americanum* and native *D. variabilis*. Higher concentrations of *A. americanum* have been collected each year since 2016. This information indicated that there will be an increase in clinical cases of tick spotted fever disease.

**Category 3 Medical Microbiology: Undergraduate Student Oral Presentation**

**Differential Impact of Diet and Microbiome on Metabolite Molecular Families.** Rohit Mital (undergraduate)1\*, Morgan Harris1, Adwaita Parab1, Benedikt Hild2, Mitchelle Katemauswa1, Ji Hoon Oh2, Min Kyung Jung2, Barbara Rehermann2 and Laura-Isobel McCall1. 1 University of Oklahoma, Norman, Oklahoma. 2  Immunology Section, Liver Diseases Branch, NIDDK, National Institutes of Health, DHHS, Bethesda, Maryland.

Lab mice, as experimental subjects, are valuable for the ability to standardize their genome. However, the effects of the controlled animal facility environment and the absence of the complex, wild-derived microbiome that co-evolved with mammals mean that results might not be fully applicable to humans. Here, we investigated how the wild-derived microbiome in combination with diet affected the organ-specific metabolome. Random forest models were applied to organ metabolomics data. Pathway Activity Level Scoring analyses identified specific chemical families which differed between combinations of sampling site, microbiome, and diet. To control for baseline metabolite presence, for each sampling site, the ratios of the significantly changing family members in each diet and microbiome combination to the family members simply present at a given sampling site were compared. Many of the chemical families exhibited similar general trends across diet and microbiome groups. Trends for some families, like amino acids and peptides, were different for each diet and microbiome combination. Many smaller families were exclusively impacted in certain sample locations, like 1-hydroxy-2-unsubstituted benzenoids in gastrointestinal contents. These results are informative regarding the role of the gut microbiome in shaping locally active metabolic pathways, and in helping improve generalizability of mouse models to human research.

**Category: V. Flash talk (virtual only)**

**Isolation and Characterization of Environmental *Clostridioides difficile* Isolates from Wastewater Treatment Plants and Farmed Mollusks in Taiwan.** Gianna Moulis (master’s)\*1, Ya-Ru Lee2, Bo-Yang Tsai2, Chin-Shiang Tsai3, Jenn-Wei Chen2, Pei-Jane Tsai2, and I-Hsiu Huang1. 1Oklahoma State University Center for Health Sciences, Tulsa, Oklahoma. 2National Cheng Kung University, Tainan, Taiwan. 3National Cheng Kung University Hospital, Tainan, Taiwan.

*Clostridioides difficile* (*C. difficile*) is an anaerobic, spore-producing, toxin-producing, gram positive bacterium. *C. difficile* is a large burden on the healthcare system as it is one of the most frequent causes of hospital-acquired antibiotic associated diarrhea and is responsible for approximately 500,000 cases and 29,000 deaths a year in the United States. Previously, our lab in Taiwan isolated *C. difficile* from seafood and multiple wastewater treatment plants. Toxinotyping and PCR ribotyping was performed for all 97 environmental isolates and 24 different ribotypes were represented. Multiple isolates belong to the hypervirulent ribotypes 027, and 078 lineages were found in the samples. We also performed phenotypic characterizations such as colony morphology, swimming and swarming motility, biofilm formation, and sporulation efficiency. Our preliminary results indicated significant variation in all phenotypes tested. This work represents the first time that toxigenic C. *difficile* isolates were isolated from water samples in Taiwan. The presence of C. *difficile* from wastewater treatment plants and farmed seafood suggest a possible reservoir for zoonotic and human transmission. In our study at OSU-CHS, we will analyze the phenotypic characteristics of these isolates.

**Category: IV Poster Presentation**

***Bacillus anthracis* Peptidoglycan Prevents Efferocytosis by Human M2-like MФ, Reduces Cell Surface Expression of Efferocytic Receptors, but is Not Reversed by ADAM17 Inhibition.** Joshua S Mytych1,2 (Doctoral)\*, Zijian Pan1, Charmaine Moya1, Christina Lawrence1, Judith James1, Narcis Popescu1, Mark Coggeshall1,2, Darise Farris1,2; **1**Oklahoma Medical Research Foundation*, Oklahoma City, OK. 2University of Oklahoma Health Science Center, Dept. Microbiology and Immunology, Oklahoma City, OK.*

Peptidoglycan (PGN) makes up a large portion of the bacterial cell wall and is common to both gram-positive sepsis and fulminant anthrax infection. *Bacillus anthracis* PGN plays a key role in promoting the host inflammatory response, as well as contributing to host coagulation defects. Increases in apoptotic lymphocytes are a late-stage feature of anthrax and sepsis, suggesting there is a defect in apoptotic clearance. Clearance of apoptotic cells (efferocytosis) is thought to be mediated by tissue-resident macrophages (MΦ), modeled *in vitro* as human M2-like MΦ. We investigated the effects of PGN on human M2-like MΦ efferocytosis, efferocytic receptor expression and the role of ADAM17 membrane-bound protease. PGN prevented efferocytosis by human M2-like MΦ and reduced cell surface expression of the pro-efferocytic receptors MerTK, Tyro3, Axl, αVβ5, CD36 and Tim-3. Soluble forms of MerTK and Tim-3 were increased in cellular supernatants, suggesting involvement of ADAM17 protease. However, inhibition of ADAM17 failed to restore efferocytosis by PGN-treated MΦ. We conclude that PGN impairs human MΦ efferocytosis and that strategies in addition to ADAM17 protease inhibition are required to mitigate this effect for the treatment of late stage anthrax.

**Category: III Medical Microbiology/Immunology Graduate Student Oral Presentation**

**Bacterial Two Hybrid Analysis of *Chlamydia Trachomatis* Inclusion Membrane Protein, CT226**

Kayli Nail\*(Undergraduate), Christian Holcomb, and Erika Lutter, Oklahoma State University, Stillwater, Oklahoma.

*Chlamydia trachomatis* is an obligate intracellular bacterial pathogen which poses severe health problems throughout the world. It is imperative to understand how *Chlamydia* manipulates the host cell and potentially develop future treatments. The mechanisms by which *C. trachomatis* alters immune response is not well understood, but recent work has identified an interaction between the chlamydial inclusion membrane protein, CT226, and the potential interacting host proteins, Flightless homologue II (FLII), TMOD3 and Leucine Rich-Repeat Flightless-Interacting Proteins 1&2 (LRRFIP1 and LRRFIP2). Currently, it is unknown how CT226 interacts with multiple interacting partners; this will be investigated through the bacterial two hybrid system. My hypothesis is that CT226 will directly interact with one of the 4 potential interacting partners. Current efforts have focused on cloning CT226 into PUT18 and the three host proteins (FLII, TMOD3, LRRFIP1 and LRRFIP2) individually into PKNT25. PUT18 and PKNT25 are the bait and prey plasmids adapted for use in the bacterial two hybrid system. Once each pair of plasmids are properly transformed, they will be screened using traditional Beta-galactosidase assays or cAMP assays to determine the specific interactions.

**Category IV: Poster Presentation (in person only)**

**Interactions of *Cryptococcus neoformans* with Human Airway Phagocytes**

Benjamin N. Nelson (Doctoral)\*1, Cheyenne Daugherty1, Vineet I. Patel2, Jordan P. Metcalf2, and Karen L. Wozniak1

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*Cryptococcus neoformans* is an opportunistic fungal pathogen that causes over 180,000 annual deaths in HIV/AIDS patients. Innate phagocytes such as dendritic cells (DCs) and macrophages are the first cells to interact with the pathogen. Six subsets of airway phagocytes (three macrophage and three DCs) have been characterized in healthy human lungs. However, the specific subsets responsible for the fate of *C. neoformans* are unknown. We hypothesize there are differences with uptake and survival of *C. neoformans* among the subsets. Healthy human bronchoalveolar lavage containing these six phagocytic subsets was incubated with *C. neoformans* for two hours. Cells were examined by flow cytometry to determine association with the fungus and by imaging flow cytometry to visualize intracellular cryptococcal morphology, indicating killing or replication. Single cell RNA sequencing (scRNA-seq) was used to determine relative gene expression following cryptococcal interaction. Results showed that all phagocyte subsets interacted with *C. neoformans*, and different fungal morphologies were observed. scRNA-seq revealed differential gene regulation of autophagy regulators which are necessary for cryptococcal killing. These findings suggest it is possible to elucidate the causative elements that are different between the killing and permissive subsets. Future studies include trajectory analysis to understand phenotypic differences and their anti-fungal role.

**Category: III Medical Microbiology/Immunology Graduate Student Oral Presentation**

**Analysis of Different Strategies of *Serratia marcescens* When Exposed to Sub-MIC Concentrations of Triclosan.** Elizabeth Grace O. Pascual (Osteopathic Medical Student)\*, Sue Katz Amburn, and Franklin R. Champlin. Oklahoma State University Center for Health Sciences, Tulsa, Oklahoma.

*Serratia marcescens* is intrinsically resistant to the biocide triclosan and requires outer membrane permeabilization for triclosan to inhibit enoyl-acyl carrier protein reductase. Recent literature indicates that even without membrane permeabilization, triclosan exposure alters gene expression. Previous RNAseq data has been re-analyzed using PATRIC (Pathosystems Resource Integration Center). The differential expression analysis allowed identification of both highly up- and down-regulated genes, many which were not observed in the original analysis, and FPKM counts (Fragments per Kilobase Million) identified potential housekeeping genes. Primers were designed using IDT PrimerQuest to these targets. Future qPCR experiments will monitor the expression profile of these genes at specific time points. Further experimentation will seek to determine the expression profile of these genes in cells treated not only with triclosan but with permeabilizer. With the addition of permeabilizer, *S. marcescens* ceases growth for a short period of time, in a pattern different from that found with another organism resistant to triclosan, *Pseudomonas aeruginosa*, an observation that leads to the conclusion that *S. marcescens* has a constitutively expressed efflux gene rather than an induced. FPKM counts were used to analyze the expression profile of known efflux genes, allowing identification of one which does appear to be constitutively expressed.

**Category IV:** Poster Presentation

***Clostridioides difficile* Toxin B (TcdB) Activates Group 3 Innate Lymphocytes (ILC3s)**

Rosemary Pope, Alisha Chitrakar, Prakash Sah (Post-doc)\*, Tyler Shadid, Jimmy Ballard, and Lauren Zenewicz.

Department of Microbiology and Immunology, College of Medicine, The University of Oklahoma Health Sciences Center, Oklahoma City, OK, 73104, USA

Group 3 innate lymphocytes (ILC3s) are rare immune cells enriched at mucosal surfaces, especially the gastrointestinal (GI) tract. Despite their rarity, they are a major source of the cytokine IL-22, which protects the GI epithelium during inflammation and infection. Though ILC3s have been demonstrated as important for defense against *Clostridioides difficile* infection, the exact mechanisms through which they sense productive infection and become activated to produce IL-22 remain poorly understood. In this study, we identified a novel mechanism of ILC3 activation after exposure to *C. difficile*. TcdB from *C. difficile* directly induced production of IL-22 in ILC3s, and this induction was dependent on the glucosyltransferase activity of the toxin, which inhibits small GTPases. Pharmacological inhibition of the small GTPase Cdc42 also enhanced IL-22 production in ILC3s, indicating that Cdc42 is a negative regulator of ILC3 activation. Further gene expression analysis revealed that treatment with TcdB modulated the expression of several inflammation-related genes in ILC3s. These findings demonstrate that *C. difficile* toxin-mediated inhibition of Cdc42 leads to the activation of ILC3s, providing evidence for how these cells are recruited into the immune response against the pathobiont.

**Category: III Medical Microbiology/Immunology Post-doc Oral presentation**

**Culturing Novel Anaerobic Gut Fungi from Reptilian Hosts**

Carrie J. Pratt (PhD)\*, Noha H. Youssef, and Mostafa S. Elshahed.

Oklahoma State University, Stillwater, OK.

In the herbivorous gut, a multitude of microorganisms interact to break down plant biomass into simpler compounds for host uptake. Members of the anaerobic gut fungi (AGF, phylum Neocallimastigomycota) are some of the most unstudied members of this community and have been exclusively cultured out of mammalian herbivores. Through culture independent analysis, AGF were identified in the fecal samples of several reptiles. The goal of this research was to culture AGF, particularly novel taxa, from the digestive tracts of reptilian herbivores. We were able to successfully culture an AGF isolate from the fecal sample of a ploughshare tortoise, a critically endangered species endemic to Madagascar. Through amplification and sequencing of the D1/D2 region of the 28S rRNA gene, we were able to determine that the isolate belongs to a novel family of AGF and represents one of the most ancestral taxa within the phylum. Documenting the diversity of AGF in non-mammalian hosts will allow us to trace the evolution of AGF and uncover more knowledge about this relatively unstudied group of microorganisms.

**Category: II Environmental Microbiology Graduate Student Oral Presentation**

**Contribution of Cell Ultrastructure to the Antibacterial Properties of a Novel Hydrophobic Melanin-Inspired Compound.** Daniel Reed1,2(undergraduate)\*, Katherine Nehmzow3, Toby Nelson1, Erika Lutter2, Gabriel Cook1, Franklin R. Champlin3. 1Department of Chemistry, Oklahoma State University, Stillwater, Oklahoma; 2Department of Microbiology and Molecular Genetics, Oklahoma State University, Stillwater, Oklahoma; 3Department of Biochemistry and Microbiology, Oklahoma State University Center for Health Sciences, Tulsa, Oklahoma.

Melanin-inspired compounds (EIPE) possess a core that provides scaffolding suitable for attachment of various functional groups. There is a need for the development of novel compounds to combat refractory bacterial pathogens. The purpose of this study was to determine the antibacterial properties of hydrophobic (EIPE-1) and hydrophilic (EIPE-HCl) EIPE derivatives. A standardized disk agar diffusion bioassay was performed to screen susceptibility and resistance levels of 12 gram-positive and 13 gram-negative bacteria to EIPE-1 and EIPE-HCl. The hydrophobic derivative EIPE-1 exhibited a gram-positive spectrum, while the hydrophilic derivative EIPE-HCl exhibited no antibacterial properties. Turbidimetric growth curves were constructed to investigate the EIPE-1 mechanism of action. Bacteriolysis occurred immediately upon treatment for *Staphylococcus epidermidis* SK01 and later at the five-hour mark for *B. subtilis* ATCC 6633, thereby suggesting a membrane-directed modality. Minimal inhibitory concentration (MIC) and minimal bactericidal concentration (MBC) bioassays were employed to quantitatively determine efficacy of EIPE-1. MIC/MBC values below 2.0 µg/mL were obtained against gram-positive bacteria, while values greater than 128 µg/mL were obtained against gram-negative bacteria. No synergistic sensitization was observed when outer membranes of selected gram-negative organisms were chemically permeabilized. We surmise that intrinsic resistance involves concomitant involvement of outer membrane impermeability and constitutive efflux systems.

**Category: III Medical Microbiology/Immunology Undergraduate Student Oral Presentation**

**Potential for Viral Replication within the Brain of Nora Virus-Infected *Drosophila melanogaster.*** Blase Rokusek (master’s)\*, Britney de Leon, Sunanda Rajput, Nicholas Hobbs, and Kimberly Carlson. Department of Biology, University of Nebraska at Kearney, Kearney, NE

Nora virus (NV), a positive-sense single-stranded picornavirus, was first described less than two decades ago when it was found to infect *Drosophila melanogaster.* Since its discovery, replication of NV appeared to be largely confined to the gut. The purpose of the present study is to determine whether NV is capable of invading the nervous system in *D. melanogaster.* We removed the heads from the bodies of flies collected from chronically infected stocks and extracted RNA. We amplified NV *ORF1* by means of RT-PCR and confirmed the presence of NV in the heads of infected flies by gel electrophoresis. We also extracted RNA at various time points over the course of adult infection from heads and bodies. We are in the process of utilizing qRT-PCR to compare viral loads between heads and bodies throughout the infection. Finally, we are optimizing protocols to dissect the heads of infected flies and section them using a cryostat. We will use immunohistochemistry and confocal microscopy in an attempt to visualize NV within the brains of infected flies. Confirmation of viral replication within the brain would further our understanding of this endemic fruit fly virus that chronically infects laboratory stocks.

**Category IV: Poster Presentation**

**Impact of ParE Toxin Expression on the Antibiotic Susceptibility of *Pseudomonas aeruginosa*.**Shengfeng Ruan (doctoral)\*, Christina R. Bourne. University of Oklahoma, Norman, Oklahoma.

ParDE is a Type-II toxin-antitoxin (TA) system encoded in many pathogenic bacteria such as *Pseudomonas aeruginosa*. In this TA family, the ParE toxin is a DNA gyrase inhibitor that binds to DNA gyrase when not complexed with the cognate ParD antitoxin via protein-protein interactions. The accumulation of DNA breaks caused by inhibition of gyrase likely triggers error-prone polymerases to repair these breaks to prevent cell death. Because of the low fidelity of these repair responses, we hypothesize that low levels or sub-toxic expression of ParE may increase antibiotic resistance due to the arising mutations, whereas higher concentrations of ParE toxin cause too many breaks to be repaired, resulting in cell death. Our study seeks to determine the relative toxicity and resulting mutation rate as a function of ParE expression in their host bacteria. We performed viability assays to determine the highest level of ParE induction allowing for cell survival, thus providing a window for potential mutations, and then used fluctuation assays to determine the mutation rate upon ParE expression. We are also using Etest strips, which we expect will correlate with any observed changes in mutation rates. Overall this study will address a potential intrinsic driver of antibiotic resistance through the reversible inhibition of DNA gyrase by the ParDE TA system.

**Category: IV Poster Presentation**

**Calcium Enhances Resistance of *Pseudomonas aeruginosa* to the Last-resort Antibiotic, Polymyxin B*.*** Tarosha B. Salpadoru1 (Doctoral)\*, Sharmily Khanam1, Anna Khanov1, Kerry Williamson2, Prasadi Gallage1, Spencer Pitre1, Michael J. Franklin2, and Marianna A. Patrauchan1.

1Oklahoma State University, Stillwater, Oklahoma, 2 Montana State University, Bozeman, Montana.

*Pseudomonas aeruginosa* accounts for high morbidity and mortality in Cystic Fibrosis (CF) patients. It is resistant to most available antibiotics,including the last resort polymyxin B (PMB). *P. aeruginosa* resistance to PMB is multifactorial and remains poorly understood. Here, we show that the presence of elevated calcium (Ca) levels, often detected in airways of CF patients, enhances the PMBresistance in *P. aeruginosa*. Several known mechanisms that confer resistance to PMB did not contribute to this increase suggesting the involvement of novel Ca-dependent mechanisms. Through random chemical mutagenesis, we identified three genes contributing to Ca-induced resistance. One of these genes, PA2803 encodes a predicted phosphonatase, and its transcription is regulated by Ca and PhoB-mediated phosphate starvation. PA2803 provides Ca-dependent growth advantage at low-phosphate but shows no *in-vitro* phosphonatase activity congruent with sequence-based predictions. Currently, we are characterizing binding properties of PA2803. Further, we show that during growth at elevated Ca, *P. aeruginosa* acquires several Ca-dependent changes in the membrane properties, such as increased permeability of the outer and inner membranes and alterations in the LPS and proteomic profiles. We aim to investigate these membrane modifications and the newly-discovered proteins to improve our understanding of PMB resistance in *P. aeruginosa.*

**Category: III Medical Microbiology/Immunology Graduate Student Oral presentation**

SARS CoV-2 (delta variant) infection kinetics and immunopathogenesis in domestic cats. Miruthula Tamil Selvan 1 (Ph.D.) \*, Sachithra Gunasekara 1, Shannon Cowan 1, Ping Xiao 2, Darren Hagen2, Jerry W. Ritchey1, Jennifer M. Rudd 1, Craig A. Miller 1

1Department of Veterinary Pathobiology, College of Veterinary Medicine, Oklahoma State University; Stillwater, OK, USA.

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The persistence of Covid-19 has made it critical to understand the pathogenesis of SARS CoV-2 variants in both animals and humans. Current animal models for SARS CoV-2 (transgenic mice, primates, ferrets) have limited lower respiratory lesions, divergence from clinical Covid-19 disease, and requirements for host genetic modifications to permit infection. We, therefore established a feline model to evaluate the infection kinetics and immunopathology of the rapidly circulating delta variant (B.1.617.2) of SARS-CoV-2. In this study, specific-pathogen-free domestic cats (n=24) were inoculated intranasally and intratracheally with SARS CoV-2 (B.1.617.2) to evaluate clinical disease, histopathological lesions, and viral infection kinetics at 4- and 12-days post-infection (dpi). Viral loads and ACE-2 expression were quantified in the trachea, lung and the nasal turbinates were more pronounced at 4 dpi than at 12dpi. RNA sequencing analysis of lung samples from infected cats identified upregulation of multiple gene pathways associated with cytokine receptor interactions, chemokine signaling, and viral protein-cytokine interactions during acute infection with SARS-CoV-2. Collectively, the results of these studies help delineate the role of domestic cats in disease transmission and response to variant emergence, establish a flexible translational model to develop control strategies to prevent the spread of SARS-CoV-2, and identify potential targets for downstream therapeutic development.

**Category : III Medical Microbiology/ Immunology Graduate Student Oral Presentation**

**Altering a TA System Protein-Protein Interface to Manipulate Bacterial Cell Growth**  
Kevin J. Snead (doctoral)\*, Christina R. Bourne. University of Oklahoma, Norman, Oklahoma

To combat the ever growing antibiotic resistance crisis, it is vital that we develop new strategies for controlling bacterial cell growth. One strategy is to target toxin-antitoxin (TA) systems, which are widely dispersed throughout bacteria. TA systems are composed of a non-secreted metabolic regulator protein (toxin) and a cognate immunity protein (antitoxin) which directly binds to the toxin neutralizing its cellular toxicity. This work focuses on the chromosomally encoded type-II TA system ParDE1 from *Pseudomonas aeruginosa* to identify the minimal length of antitoxin and most vital interactions required for formation of the protein-protein interaction. Using both biophysical and bacterial cell-based techniques to measure interactions with antitoxin-derived peptides we determined that that the C-terminal alpha-helix of ParD1 is required for the interaction with ParE1. This helix contains a position that significantly weakens the interaction when mutated, and we have established this arose from altering the disorder-to-order transition of this antitoxin region upon complexation. These results, combined with previously published data, are highly suggestive that targeting this region or mimicking this interaction could be a viable target for future therapeutics. Ultimately the secrets of these interactions provide proof of concept for unlocking a new therapeutic way of controlling bacterial cell growth.

**Category: IV Poster Presentation**

**Characterizing Flightless 1 Localization to the Inclusion Membrane During *Chlamydia trachomatis* Infection.**

Natalie A. Sturd (Doctoral)\* and Elizabeth A. Rucks. University of Nebraska Medical Center, Omaha, Nebraska.

The obligate intracellular pathogen *Chlamydia trachomatis* is the most common bacterial sexually transmitted disease worldwide. Infection can lead to development of chronic conditions, such as pelvic inflammatory disease and tubal infertility in women. Chlamydiae have a biphasic developmental cycle, alternating between the infectious elementary body and the non-infectious, replicative reticulate body. The developmental cycle occurs within a pathogen-specific vacuole, termed the chlamydial inclusion. *Chlamydia* encode inclusion membrane proteins, called Incs, which are inserted into the inclusion membrane and can interact with host proteins. Prior studies in our lab identified host protein Flightless 1 (FLII) and its interacting partner, LRRFIP1 (LRRF1), at the inclusion membrane. FLII is a negative regulator of wound healing and a transcriptional coactivator of nuclear receptors. We’ve demonstrated that FLII localizes to the IM during early-mid development and remains stable up to 48h hours post infection. By co-immunoprecipitation, FLII and LRRF1 bind in complex to the Inc, CT226. This interaction requires LRRF1, which was previously demonstrated to interact with Ct226. Following inducible knockdown of *ct226*, we no longer observe IM localization of FLII nor LRRF1. We plan to further investigate downstream functions of FLI1 and LRRF1 to identify signaling pathways altered during chlamydial infection.

**Category III: Medical Microbiology/Immunology Graduate Student Oral presentation**

**Characterization of the Tail-Specific Protease, Ct441, in *Chlamydia trachomatis* Growth and Development.**

Abigail R. Swoboda (Undergraduate)\*, Nicholas A. Wood, and Scot P. Ouellette

University of Nebraska Medical Center, Omaha, Nebraska

*Chlamydia trachomatis* (Ctr)undergoes a unique biphasic developmental cycle where it differentiates between two functionally and morphologically distinct forms: the elementary body (EB) and the reticulate body (RB). The EB is the smaller, non-dividing, infectious form, and the RB is the larger, dividing, non-infectious form. The molecular mechanisms driving differentiation are poorly understood but require significant remodeling of the bacterial proteome. We hypothesize that periplasmic protein degradation is critical for the reorganization of an RB to an EB during secondary differentiation specifically to halt RB division. Ct441 is an ortholog of periplasmic tail-specific proteases (i.e. Tsp, Prc). In Gram-negative bacteria, Prc is constitutively expressed and aids in cell wall homeostasis through degradation of peptidoglycan (PG) processing proteins. Interestingly, Ctr lacks a classical PG sacculus, using PG only at the division septum. Additionally, Ct441 is expressed as RBs differentiate to EBs, further suggesting a function in this process. We determined the effect of altering Ct441 levels on chlamydial growth and development. Changes in bacterial morphology and EB production suggest that Ct441 levels and/or activity are carefully regulated for proper developmental cycle progression and secondary differentiation. These data indicate that Ct441 plays a crucial role in production of functional EBs in *Chlamydia*.

**Category: General Microbiology Undergraduate Student Oral Presentation (virtual)**

**Archaea in Mammalian Gut Microbiomes.**

Shilpa Dange (Masters)\*, Alejandro Torres, Senait Assefa, Steven Rivera, Thomas Richardson, Brenda Perez, Dolores Vazquez Sanroman, and Gerwald Koehler

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

Archaea are the most enigmatic of the three domains of life. Most archaea are extremophiles found in highly acidic, high-salt, or high-temperature environments. However, members of the archaea also have been found in animal and human intestines, albeit their functional roles in host health or disease are poorly understood. This study aims to mine rodent 16S rRNA gene amplicon or whole-genome shotgun metagenomic sequencing data sets in our laboratory for the presence of archaeal sequence reads. Additionally, we want to investigate whether these sequences are derived from allochthonous or autochthonous microorganisms. Taxonomic profiling workflows in the Qiagen CLC Genomics Workbench and QIIME 2 are being used to elucidate the relative abundances of archaeal and bacterial reads in sequence libraries prepared from DNAs isolated from intestinal digesta and feces. Our results indicate that archaeal sequences, mostly from methanogens and halophiles, can be found in low abundances in rodent gut microbiomes. Future studies will include confirmation of the presence of archaea in the mammalian intestine by quantitative PCR and, if possible, in vitro culture of select isolates. Furthermore, we will investigate archaea-bacteria co-occurrence networks and correlations with host metadata and health status.

**Category: IV Poster Presentation**

**Characterization of *V. cholerae* ParE toxin proteins from ParDE TA systems**

Chih-Han Tu (doctoral)\*; Christina Bourne

University of Oklahoma, Stephenson Life Science Research Center, Norman, OK

DNA gyrase is an essential enzyme in bacteria, where it functions in regulating DNA topology by temporarily rendering double-strand breaks when cells undergo DNA replication or transcription. Compounds capable of stabilizing gyrase-mediated double-strand breaks are valuable antibacterial therapeutics but these also impart higher rates of mutations and thus development of resistance. Therefore, developing new forms of gyrase inhibitors may yield additional antibacterial strategies.

In bacterial TA systems, the ParE family of toxins are characterized to poison DNA gyrase; however, the molecular mechanism of this inhibition has remained elusive. Therefore, the overall goal of this study is to deduce the inhibition mechanism of the two ParE toxins from *Vibrio cholerae* as these have been previously characterized as potently toxic. Our studies have revealed a dose-dependent bactericidal activity to the host *V. cholerae* cells, but never achieve complete sterilization of cultures. On-going work is characterizing the propensity of ParE toxicity to result in altered DNA mutation rates, and to purify the recombinant ParE toxin proteins for structural studies. The outcome of these studies is expected to reveal a new modality for the inhibition of DNA gyrase, thus contributing to the development of new antibacterial strategies.

**Category: IV. Poster Presentation (in person only)**

**Characterization of the Nitro-PTS in *Pseudomonas aeruginosa*.** Simon A.M. Underhill (postdoctoral fellow)\*, Somalisa Pan (doctoral student)\*, Mary Erdmann (Undergraduate student) and Matthew T. Cabeen. Department of Microbiology and Molecular Genetics, Oklahoma State University, Stillwater, OK.

*Pseudomonas aeruginosa* is a bacterium whose biofilm lifestyle causes chronic, deadly infections in patients with cystic fibrosis or immune disorders. This biofilm behavior has previously shown responsiveness to the nitrogen-related PTS. The Nitro-PTS is a phosphorelay chain with largely unknown signaling outputs. It consists of a GAF domain-containing kinase, PtsP,an intermediary receptor/kinase, PtsO, and the terminal receptor, PtsN. Unphosphorylated PtsN was suggested by previous work to suppress biofilm behavior by lowering the concentration of the biofilm signaling molecule c-di-GMP. In this work we use western blotting to understand how phosphate is transferred, demonstrating that PtsN is phosphorylated on a single histidine residue. We also show that while PtsP is strictly required for PtsN phosphorylation, PtsO is not. Further, we demonstrate that the GAF domain of PtsP is required for transfer through the intact chain, whereas deletion of *ptsO* results in insensitivity to GAF presence. Finally, we query the output of unphosphorylated PtsN using RNA sequencing and find that while there is a clear effect on pyoverdine siderophore production, no biofilm or c-di-GMP related genes show obvious transcriptional changes, arguing that PtsN impacts biofilm development at the protein level rather than altering transcription.

**Category: IV. Poster presentation**

***Staphylococcus aureus* Persisters Exhibit Increased Survival to Components of the Innate Immune System**

Emma Weis (Undergraduate)\*, Trenten Theis, Alexis Hobbs, and Austin Nuxoll

University of Nebraska at Kearney, Kearney, Nebraska

*Staphylococcus aureus* is an opportunist pathogen which causes foreign device mediated infections that are recurrent or relapsing in nature. The phenomenon of relapsing infections caused by susceptible bacteria is hypothesized to be due to the presence of persister cells. Persister cells are a subset of dormant-like cells that survive antibiotic treatment. Little is known about the interaction between persister cells and the innate immune system. Based on previous findings of a *fumC* knockout exhibiting increased persister cell formation and increased survival to antimicrobial peptides, we hypothesized that the *fumC* knockout will survive better to other components of innate immunity. Following macrophage infection, the *fumC* knockout had increased survival compared to wild type *S. aureus,* HG003. Reactive nitrogen species (RNS) were measured in macrophages infected with either the *fumC* knockout or with HG003*.* RNS levels were similar between both strains indicating the same macrophage response to both strains. By examining the growth under various conditions, it was revealed that *fumC* had increased growth in subinhibitory concentrations of NaNO2 and paraquat. Furthermore, the *fumC* knockout had increased survival to lethal NaNO2 and paraquat concentrations. These results indicate that a *fumC* knockout survives RNS better leading to enhanced survival within a macrophage.

**III. Medical Microbiology/Immunology Oral Presentation- undergraduate (in person)**

**Analysis of VAMP3 Trafficking to the *Chlamydia trachomatis* Inclusion.**

Ray E. Widner (Doctoral)\*1 and Elizabeth A. Rucks1**.**

1University of Nebraska Medical Center, Omaha, Nebraska.

*Chlamydia trachomatis* is an obligate intracellular bacterial pathogen. *Chlamydia* develop within a eukaryotic host cell-derived vacuole termed the inclusion, where the bacteria alternate between two distinct forms—the infectious elementary body (EB) and the replicative reticulate body (RB). The inclusion is modified with bacterial inclusion membrane proteins, or Incs. Most studies examining Inc-host protein interactions have focused on a single Inc and protein binding partner. Further, it has been widely accepted that eukaryotic proteins recruited to the inclusion have a single Inc binding partner. Prior studies have demonstrated recruitment of eukaryotic SNARE proteins to the inclusion. Specifically, we have shown that one SNARE, VAMP3, interacts with 5 different Incs temporally throughout chlamydial development. We hypothesize that VAMP3 is recruited to the *Chlamydia trachomatis* inclusion through interactions between specific domains of VAMP3 with the different individual Inc proteins. To screen VAMP3 domains important for interaction with Incs, we used bacterial adenylate cyclase two-hybrid (BACTH) analyses. Further, we have created new chlamydial knockdown strains targeting the Incs with which VAMP3 interacts. We will use a Dendra2-VAMP3 construct to observe VAMP3 trafficking in live chlamydial-infected cells with wild-type and mutant bacteria. Combined, these data will determine mechanisms of dynamic host-chlamydial interactions.

**Category: III. Medical Microbiology/Immunology Oral Presentation**

**ClpX Recognition of SsrA-Tagged Substrates Underlies Morphological Commitment to Secondary Differentiation in *Chlamydia trachomatis***

Nicholas A. Wood (Doctoral)\*1, Abigail R. Swoboda1, Amanda M. Blocker2, Derek J. Fisher2, and Scot P. Ouellette1.

1University of Nebraska Medical Center, Omaha, Nebraska.

2Southern Illinois University Carbondale, Carbondale, Illinois.

Despite extensive genome reduction in evolving to obligate intracellular dependence, *Chlamydia* undergoes a complex developmental cycle in which the bacteria differentiate between two functionally, proteomically, and morphologically distinct forms: the infectious, non-replicative elementary body (EB) and the non-infectious, replicative reticulate body (RB). However, the trigger for differentiation remains unidentified. Unlike in other bacteria, the transitions between EBs and RBs are not mediated by division events that re-distribute intracellular proteins. Rather, both primary (EB to RB) and secondary (RB to EB) differentiation likely require protein turnover. One system for targeted protein degradation is the *trans*-translation system for ribosomal rescue that marks polypeptides stalled during translation with an SsrA tag. ClpX recognizes both specific (untagged) substrates and SsrA-tagged substrates, leading to their degradation by the ClpXP protease. As such, we hypothesize that ClpX plays a critical role during chlamydial differentiation through targeted protein degradation. Both overexpression of a ClpX isoform that cannot target the SsrA tag or replacement of the canonical SsrA degron with a 6xHis tag prevent secondary differentiation but not replication. Our data support a model where these accumulated SsrA-tagged substrates effectively shift the balance of ClpX substrate recognition from untagged to tagged ClpX in a probabilistic manner to initiate secondary differentiation.

**Category I: General Microbiology Oral Presentation (Virtual)**

**A “Reverse Evolution” Approach to Identify Strategies in *Coxiella burnetii* Intracellular Survival** Archana Yadava (Ph.D.) \*, Melissa N. Brewer a,b, Mostafa S. Elshaheda, and Edward I. Shaw a,ca Department of Microbiology and Molecular Genetics. Oklahoma State University. Stillwater, OK.

b Biological Sciences. Southeastern Oklahoma State University. Durant, OK.

c Medical Microbiology and Immunology. Philadelphia College of Osteopathic Medicine. Moultrie, GA.

*Coxiella burnetii* (*Cb*) is an obligate intracellular pathogen that infects macrophages, and successfully propagates in the parasitophorous vacuole (PV). To identify genes and proteins crucial to their normal intracellular growth lifestyle, we applied a “Reverse Evolution” approach where *Cb* was grown for 67 passages in a chemically defined ACCM-D media and gene expression patterns and genome integrity from various passages were compared to intracellularly grown cultures. Transcriptomic analysis over 67 subsequent passages of *Cb* identified a marked downregulation of genes encoding type 4B secretion system (T4BSS), general secretory (sec) pathway, several chaperones, LPS, and peptidoglycan biosynthesis and a few previously identified effector proteins. A general marked downregulation of central metabolic pathways balanced by a marked upregulation in transporters gene was observed which reflects the richness of axenic media and diminishing anabolic needs in the media. Finally, genomic analysis showed an extremely low mutation level signifying a relatively stable genome. Our work implies that the adaptation of *Cb* in axenic media is reflected more with changes in gene expression compared to changes in genomic architecture and provide us with genes vital in *Cb* intracellular growth that could be used for further investigation.

**Category: III Medical Microbiology/Immunology Graduate Student Oral presentation**

**Impact of RecA Functions on *Pseudomonas aeruginosa* Biofilm Suppression** *Amal H. Yahya* (doctoral student) *\*, William Colton, and Matthew T. Cabeen.* Department of Microbiology and Molecular Genetics, Oklahoma State University, Stillwater, OK.

Bacterial biofilms are a common cause of chronic and persistent infections. Biofilms are a community of bacterial cells that are held together and encapsulated by a self-produced extracellular matrix. The biofilm matrix provides extra protection to *P. aeruginosa* cells, allowing them to tolerate harsh environmental conditions and resist antibiotics. We observed that disruption of *recA* increased biofilm matrix production in a biofilm-suppressed (Δ*16550*) PA14 strain. Moreover, deletion of *recA* increased biofilm production in the wild type. Because this recombination protein functions both in recombination and in the DNA damage response, we asked which function of RecA is important for impacting biofilm formation. To answer this question, we separated the two functions, constructing a point mutation in RecA (N304D) that disables homologous recombination or using a non-cleavable point mutant of LexA (S125A) to specifically disable the SOS DNA-damage response. Like *recA* deletion, *recAN304D* and *lexAS125A*showed similarly increased biofilm matrix levels in our biofilm-suppressed background. Our results suggest that both functions of RecA are important to achieve biofilm suppression.

**Category: IV Poster Presentation**