American Society for Microbiology

South Central Branch Meeting

October 19-21, 2017

University of Arkansas for Medical Sciences

Department of Microbiology and Immunology

Little Rock, Arkansas

AMERICAN SOCIETY FOR MICROBIOLOGY

UAMS

University of Arkansas for Medical Sciences

ASM Branches

President's Welcome

Dear Colleagues,

It is my pleasure to welcome you to Little Rock, Arkansas for the 2017 Annual Meeting of the South Central Branch of the American Society for Microbiology. The University of Arkansas for Medical Sciences has graciously supported hosting this year's meeting. I would like to thank the organizers of this year's meeting, Drs. Daniel Voth and Jon Blevins from the Department of Microbiology and Immunology, for their efforts in developing this year's program. I am very grateful for the time and effort that all of the organizers and student volunteers have provided in preparing for and hosting this conference.

I am very excited about this year's meeting! We have a fantastic keynote speaker, Dr. Harry L.T. Mobley, Frederick G. Novy Professor and Chair in the Department of Microbiology and Immunology at the University of Michigan Medical School. Dr. Mobley will present an American Society for Microbiology Distinguished Lecturer presentation at the invitation of the South Central Branch of ASM. We also have multiple faculty and student speaker sessions as well as a poster session planned which includes the following topic areas: Pathogenic Microbiology, Immunology and Virology, and Applied and Environmental Microbiology. In addition, we have a special panel for students and postdoctoral fellows in which experts in multiple scientific fields will discuss their career paths in science and education.

Thank you also to the sponsors who have made this meeting possible. The acknowledgement page of the program has a list of all of those that have helped with this conference.

Last, but not least, I would like to thank each of the attendees for continuing to support your ASM branch! THANK YOU ALL!

Best wishes,

Karl J. Indest, *ASM SCB President*

Table of Contents

2017-18 Officers
South Central Branch ASM

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Meeting Agenda

Thursday, October 19, 2017

6:00-8:00 p.m.	Registration and Opening Reception Marriott Lobby	

Friday, October 20, 2017

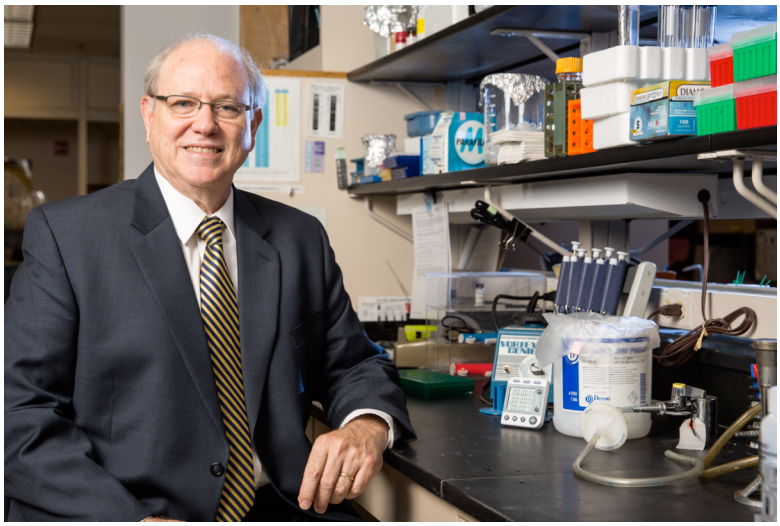
7:00-8:00	Registration, Light Breakfast, and Coffee	
8:00-10:00	**Immunology and Host Response** **Invited Speakers** Conway Lecture Hall	**Applied and Environmental Microbiology** **Selected Oral Presentations** Harris Brake Lecture Hall
10:00-10:15	Coffee Break	
10:15-12:15	**Bacterial Pathogenesis** **Invited Speakers** Conway Lecture Hall	**Immunology and Virology** **Selected Oral Presentations** Harris Brake Lecture Hall
12:15-1:30	Lunch On Own	
1:30-3:30	**Applied and Environmental Microbiology** **Invited Speakers** Conway Lecture Hall	**Pathogenic Microbiology** **Selected Oral Presentations** Harris Brake Lecture Hall
3:30-4:00	Coffee Break Poster Setup	
4:00-5:00	**Keynote Lecture** Conway Lecture Hall	
5:00-7:00	**Poster Session & Refreshments** Salon C Balcony	
7:00-9:00	**Banquet Dinner** Salon C	

Saturday, October 21, 2017

7:00-8:00	Light Breakfast and Coffee	
8:00-9:00	Scientific Career Panel	
9:00-11:00	**Virology** **Invited Speakers** Conway Lecture Hall	**Food Microbiology** **Invited Speakers** Harris Brake Lecture Hall
11:15-12:15	**Charles C. Randall Lecture** Conway Lecture Hall	
12:15-1:30	**Business Meeting and Announcement of Awards** Conway Lecture Hall	
1:30	Meeting Adjourned	

Keynote Speaker

Dr. Harry L. T. Mobley
Frederick G. Novy Distinguished University Professor and Chair
Department of Microbiology and Immunology
University of Michigan Medical School



We are pleased to have Dr. Harry L. T. Mobley as our American Society for Microbiology Distinguished Lecturer at this year's meeting.

Redefining Virulence: Measuring Bacterial Gene Expression during Human Infection

Our traditional definition of bacterial virulence has been based on *in vitro* measurements of adherence, iron acquisition, toxin activity, protein secretion, and motility. Now we must consider what metabolic pathways are in play, what transport systems must be active, and, most importantly, which genes are actually being expressed during human infection. Novel techniques including RNA-Seq and Tn-Seq allow us to identify the most highly expressed genes and which genes are essential during actual infections. This leads to a better understanding of how bacterial pathogens outfox our immune defenses.

BIOGRAPHICAL SKETCH

Dr. Mobley received his B.S. degree in Biology from Emory University in 1975 and Ph.D. in Microbiology and Immunology from University of Louisville in 1981. He conducted postdoctoral training in Biological Chemistry and Bacterial Genetics at the University of Maryland School of Medicine. He served on the faculty there from 1984 until 2004 and led the graduate program. In 2004, Mobley moved to the University of Michigan to chair the Department of Microbiology and Immunology and was installed as the Frederick G. Novy Collegiate Professor. Dr. Mobley, a fellow in AAAS and the American Academy of Microbiology, chaired the Pathogenesis and Host Response Mechanisms group of ASM. He serves on the editorial review board of *Infection and Immunity* and on NIH study sections. His research interests focus on the molecular mechanisms of bacterial pathogenesis. His lab studies virulence mechanisms of uropathogenic *Escherichia coli* and *Proteus mirabilis* and formerly studied *Helicobacter pylori* that causes peptic ulcer disease. Dr. Mobley has published 240 peer-reviewed articles, 38 book chapters and 4 books. His work has been cited in the literature nearly 15,000 times. He has trained 29 Ph.D. students and 34 postdoctoral fellows, and has delivered 201 invited lectures in 20 countries.

Please visit Dr. Mobley's website for more info about his work: http://www.umich.edu/~hltmlab/

Charles C. Randall Lectureship

Dr. Karl W. Boehme
Assistant Professor
Department of Microbiology and Immunology
University of Arkansas for Medical Sciences

The Little Engine That Could: How Sigma 1s Controls Reovirus Spread

Dr. Karl Boehme received his doctoral degree from the University of Wisconsin-Madison in 2005. His postdoctoral training was with Dr. Terence Dermody at the Vanderbilt University Medical Center. He joined the Department of Microbiology and Immunology at the University of Arkansas for Medical Sciences in 2012. Dr. Boehme's research interest is in understanding mechanisms of reovirus pathogenesis, specifically defining how reoviruses establish bloodstream infections.

The lectureship is named in honor of Dr. Charles C. Randall, Professor Emeritus, University of Mississippi Medical Center, Jackson, Mississippi. Dr. Randall conducted pioneering research in the area of viral structure, biochemistry, and molecular biology. As the former Chairman of the Department of Microbiology at the University of Mississippi Medical Center and former President of the South Central Branch, Dr. Randall made enormous contributions to the growth and development of the microbiological sciences within the South Central Branch.

The South Central Branch, American Society for Microbiology, established the Charles C. Randall Lectureship, which is to be awarded annually to an "outstanding young faculty member" who will present a lecture on his or her research at the annual branch meeting. The awardee, selected by a panel of senior faculty members appointed by the Branch President, must hold the rank of Assistant Professor (or equivalent) in a scientific institution (public or private) within the geographic boundary of the South Central Branch of the American Society for Microbiology, and must be in the early stages of a research career with less than five years of experience since completing training, but be independent of a mentor.

Invited Speaker Sessions

Immunology and Host Response

October 20, 8:00-10:00 a.m., Conway Lecture Hall

Chairs: Dr. Jason Stumhofer and Dr. Lin-Xi Li, *University of Arkansas for Medical Sciences*

8:00 a.m. **Memory CD4 T Cell Responses in *Chlamydia* Female Reproductive Tract Infection**
Dr. Lin-Xi Li, *Assistant Professor, University of Arkansas for Medical Sciences*

8:30 a.m. **Candida and the Long Strange Road to Vascular Permeability**
Dr. Doug Johnston, *Assistant Professor, Louisiana State University School of Medicine - New Orleans*

9:00 a.m. **Host Protection and Antigen-specific CD4 T Cell Immunity is Dictated by Anatomical Location During Acute and Chronic *Salmonella* Infection**
Dr. James McLachlan, *Assistant Professor, Tulane University*

9:30 a.m. **Naïve CD4+ T Cell Activation in the Nasal Associated Lymphoid Tissue**
Dr. John Bates, *Assistant Professor, University of Mississippi Medical Center*

Bacterial Pathogenesis

October 20, 10:15 a.m.-12:15 p.m., Conway Lecture Hall

Chairs: Clay Litteken and Katelynn Doiron, *University of Arkansas for Medical Sciences*

10:15 a.m. **Regulation of Virulence by *msaABCR* in *Staphylococcus aureus***
Dr. Mohamed Elasri, *Professor, The University of Southern Mississippi*

10:45 a.m. **Evaluating Early Host/Pathogen Interactions During Pneumonic Plague**
Roger Pechous, *Assistant Professor, University of Arkansas for Medical Sciences*

11:15 a.m. **Monocytes, Macrophages and Trojan Horses: How Pathogenic Rickettsial Species Can Disseminate in Fatal Cases of Spotted Fever Rickettsioses**
Juan Martinez, *Professor, Louisiana State University*

11:45 a.m. **Pneumococcal Resistance to Neutrophil-mediated Killing: A Trait for Invasiveness?**
Justin Thornton, *Associate Professor, Mississippi State University*

Applied and Environmental Microbiology

October 20, 1:30-3:30 p.m., Conway Lecture Hall

Chairs: Dr. David Ussery, *University of Arkansas for Medical Sciences* **and Dr. Arijit Mukherjee**, *University of Central Arkansas*

1:30 p.m. **Indigenous Microorganisms in Inoculated Deep Litter Systems**
Dr. Heather Jordan**,** *Assistant Professor, Mississippi State University*

2:00 p.m. **Secondary Metabolites of Therapeutic Value from a Marine *Cyanobacterium* Culture**
Dr. Erik Hom, *Assistant Professor*, *University of Mississippi*

2:30 p.m. **Decoding the Microbes of the Northern Gulf of Mexico "Dead Zone"**
Dr. J. Cameron Thrash, *Assistant Professor, Louisiana State University*

3:00 p.m. **The Symbiotic Potential of *Burkholderia* Bacteria in the Social Amoeba *Dictyostelium discoideum***
Dr. Tamara Haselkorn, *Assistant Professor, University of Central Arkansas*

Food Microbiology

October 21, 9:00-11:00 a.m., Harris Brake Lecture Hall

Chairs: Dr. En Huang, *University of Arkansas for Medical Sciences* **and Dr. Franck Carbonero**, *University of Arkansas*

9:00 a.m. **The Contribution of Plasmids to Antimicrobial Resistance and Virulence in *Salmonella enterica* Isolated from Foods, Food Animals or Human Patients**
Dr. Steven Foley**,** *Research Microbiologist, National Center for Toxicological Research*

9:30 a.m. **The Impact of Oxygen Availability on the Stress Response of *Listeria monocytogenes***
Dr. Janet Donaldson, *Professor*, *The University of Southern Mississippi*

10:00 a.m. **Prevalence of Antibiotic Resistance *Clostridium difficile* in Lettuce**
Dr. Marlene Janes, *Professor, Louisiana State University*

10:30 a.m. **Determination of the Microbiota Dynamics in Fermented Foods and Beverages**
Dr. Franck Carbonero, *Assistant Professor, University of Arkansas*

Virology

October 21, 9:00-11:00 a.m., Conway Lecture Hall

Chairs: Dr. J. Craig Forrest and Dr. Karl Boehme, *University of Arkansas for Medical Sciences*

9:00 a.m. **Tumor Suppressor p53 Controls Chronic Infection and Host Genomic Integrity in a Murine Model of Gammaherpesvirus Pathogenesis**
Dr. J. Craig Forrest**,** *Associate Professor, University of Arkansas for Medical Sciences*

9:30 a.m. **Regulation of Stromal Gene Expression by Human Papillomavirus Type 16**
Dr. Jason Bodily, *Assistant Professor*, *Louisiana State University*

10:00 a.m. **Dynamin Mediated Endocytic Pathways are Critical for Cytomegalovirus Maturation**
Dr. Ritesh Tandon, *Assistant Professor, University of Mississippi Medical Center*

10:30 a.m. **Ubiquitination of Rotavirus NSP1 in Assays with Tandem-Repeat Ubiquitin-Binding Entities (TUBEs)**
Dr. Michelle Arnold, *Assistant Professor, Louisiana State University Health Sciences Center - Shreveport*

Selected Oral Presentation Sessions

Applied and Environmental Microbiology, Oral Presentations

October 20, 8:00-10:00 a.m., Harris Brake Lecture Hall

Chairs: Dr. Mark Hart, *National Center for Toxicological Research* **and Dr. Josh Kennedy**, *Arkansas Children's Research Institute*

8:00 a.m. **Effects and Mode of Action of Plant Aldehydes And Aldehyde-Based Pro-Antimicrobial Polymer Networks (Pans) on the Antibiotic-Resistant Pathogen *Pseudomonas aeruginosa***
Yetunde Adewunmi, *The University of Southern Mississippi*

8:15 a.m. **Determination of the Effects of Different Maillard Reaction Products on the Murine Gut Microbiota**
Nesreen ALJahdali, *University of Arkansas*

8:30 a.m. **A Water Chemistry Analysis, Bacteriological Survey, and Observation of Acquired Antibiotic resistance in Bayou Lafourche, LA**
Kyle Bird, *Nicholls State University*

8:45 a.m. **Scanning Electron Microscopy of *Salmonella* Biofilms on Various Food-Contact Surfaces in Catfish Mucus**
Nitin Dhowlaghar, *Mississippi State University*

9:00 a.m. **Effect of Tetracycline on Bacteria in Carbon and Nitrogen Removal from a Sewage Treatment Plant**
Richard Grabert, *Nicholls State University*

9:15 a.m. **The Use of Reactive Oxygen Sensitive Green Fluorescent Protein to Determine Reactive Oxygen Species Production in *Candida albicans***
Claire Jones, *Louisiana Tech University*

9:30 a.m. **Multiple Protein-DNA Complexes Activate Meiotic Recombination Hotspots Via Chromatin Remodeling**
Tresor Mukiza, *University of Arkansas for Medical Sciences*

9:45 a.m. **Microbiome of the Formosan Termite, *Coptotermes formosanus* Shiraki**
Seth Van Dexter, *Nicholls State University*

Immunology and Virology, Oral Presentations

October 20, 10:15 a.m.-12:15 p.m., Harris Brake Lecture Hall

Chairs: Dr. Jia Liu, *University of Arkansas for Medical Sciences* **and Dr. Laxmi Yeruva**, *Arkansas Children's Research Institute*

10:15 a.m. **CXCL5 Downregulates IL-17A to Enhance Susceptibility to MRSA Induced Pneumonia**
Laxmi Ghimire, *Louisiana State University Health Sciences Center - New Orleans*

10:30 a.m. **Defining Tissue-Specific Requirements for Viral Replication in Chronic Gammaherpesvirus Infection**
Arundhati Gupta, *University of Arkansas for Medical Sciences*

10:45 a.m. **A Recombinant Varicella Vaccine Expressing Simian Immunodeficiency Virus (SIV) Antigens Stimulates Humoral and Cellular Immune Responses Against SIV Following a Prime-Boost Immunization Strategy**
Micheal Hohl, *University of Mississippi*

11:00 a.m. **Rotavirus NSP1-Mediated Inhibition of IRF3 and NF-κB Nuclear Translocation**
Samantha Murphy, *Louisiana State University Health Sciences Center - Shreveport*

11:15 a.m. **Human Papillomavirus E7 Oncoprotein As A Regulator Of Transcriptional Complexes**
Sadie Rice, *Louisiana State University Health Sciences Center - Shreveport*

11:30 a.m. **Differential Delivery of Genomic dsRNA Dictates Reovirus Serotype-Specific IRF3 Activation**
Johnasha Stuart, *University of Arkansas for Medical Sciences*

11:45 a.m. **Regulation of Interferon kappa by Transforming Growth Factor Beta in HPV infection**
Brittany Woodby, *Louisiana State University Health Sciences Center - Shreveport*

12:00 p.m. **The Human Cytomegalovirus Tropism Modulator UL148 Induces Endoplasmic Reticulum Quality Control Compartments during Infection**
Hongbo Zhang, *Louisiana State University Health Sciences Center - Shreveport*

Pathogenic Microbiology, Oral Presentations

October 20, 1:30-3:30 p.m., Harris Brake Lecture Hall

Chairs: Dr. Roger Pechous and Dr. Tiffany Weinkopff, *University of Arkansas for Medical Sciences*

1:30 p.m. **The Stress-Response Protein DDR48 is Required for Adequate Cellular Stress Response in the Pathogenic, Dimorphic, Fungus *Histoplasma capsulatum***
Logan Blancett, *The University of Southern Mississippi*

1:45 p.m. **The Role of Phosphodiesterase PdeD in Regulating Intracellular Concentrations of Cyclic di-GMP under Anoxic Conditions in *Listeria monocytogenes***
Sally White, *The University of Southern Mississippi*

2:00 p.m. ***msaABCR* Operon Regulates Antibiotic Resistance Maintaining Cell Wall Integrity in *Staphylococcus aureus***
Bibek GC, *The University of Southern Mississippi*

2:15 p.m. **Bacterial Lipases Differentially Affect the Pore-Formation Activity of Cholesterol-Dependent Cytolysins**
Patrick Gellings, *Louisiana State University Health Sciences Center - Shreveport*

2:30 p.m. **Characterization of Type VI Secretion System in *Edwardsiella ictaluri***
Safak Kalindamar, *Mississippi State University*

2:45 p.m. ***msaABCR* Operon is Involved in the Oxidative Response of *Staphylococcus aureus***
Shanti Pandey, *The University of Southern Mississippi*

3:00 p.m. **Relative Role of *Staphylococcus aureus* SarA and SaeRS Regulators in the Pathogenesis of Osteomyelitis**
Aura Ramirez, *University of Arkansas for Medical Sciences*

3:15 p.m. **Role of Mycolactone in Suppression of *Staphylococcus aureus* Virulence Genes**
Laxmi Dhungel, *Mississippi State University*

Scientific Career Panel

October 21, 8:00-9:00 a.m., Harris Brake Lecture Hall
Chair: Dr. Laura MacDonald, Assistant Professor, *Hendrix College*

Panelists:

Dr. Mark Hart, Research Microbiologist, *National Center for Toxicological Research*

Dr. Tiffany Weinkopff, Assistant Professor, *University of Arkansas for Medical Sciences*

Dr. Richard Morrison, Executive Associate Dean of Research, *University of Arkansas for Medical Sciences*

Dr. Josh Kennedy, Assistant Professor/Clinician Scientist, *Arkansas Children's Research Institute*

Daniel Meeker, MD/PhD Student, *University of Arkansas for Medical Sciences*

The purpose of this panel is to expose students and postdoctoral fellows to multiple scientific career options. The six panelists have expertise in undergraduate education and research, government research, basic research at a medical school, postdoctoral research, and clinical research. Attendees are encouraged to discuss any aspect of these areas with the panelists to obtain information about future career paths and inform their future decisions.

Conference Abstracts

Immunology and Host Response - Invited Speakers

I.1. Memory CD4 T Cell Responses in *Chlamydia* Female Reproductive Tract Infection

Sarah E. House, Jessica I. Gann, Lin-Xi Li, *University of Arkansas for Medical Sciences*

Background: Numerous attempts have been made over the past few decades to develop a vaccine strategy for *Chlamydia*. However, among all immunization strategies tested in mouse models of *Chlamydia* infection, none generates protective immunity any better than a naturally resolved prior *Chlamydia* infection in the female reproductive tract (FRT). In this study, we aimed to understand how immunological memory shaped by a natural episode of *Chlamydia* primary infection confers such potent protective immunity against reinfections.

Methods: Using *Chlamydia*-specific MHC Class II tetramers, we measured antigen-specific CD4 T cell responses to *Chlamydia* FRT reinfection. Moreover, we conducted mouse parabiosis to determine whether *Chlamydia*-specific CD4 T cells in the FRT are non-circulating tissue-resident memory T cells. In addition, we used bone marrow chimeric mice and *in vivo* cell depletion strategies to examine whether a specific antigen presenting cell population is required for *Chlamydia*-specific memory CD4 T cell activation.

Results: We showed that *Chlamydia* induced massive clonal expansion of antigen-specific CD4 T cells in the FRT during the first 7 days of reinfection. In contrast, a robust recall response was not observed in local draining lymph nodes or spleen over the entire course of secondary infection. Surprisingly, although mouse parabiosis revealed that *Chlamydia*-specific memory CD4 T cells in the FRT were exclusively non-circulating tissue-resident memory cells, these CD4 T cells only partially expressed the common tissue-resident memory cell marker, CD69. In addition, our preliminary data showed that neither $CD11c^+$ cells or $CD19^+$ B cells was required for antigen-specific CD4 T cell expansion in the FRT during *Chlamydia* secondary infection.

Conclusion: We conclude that *Chlamydia* primary infection induces the formation of non-circulating tissue-resident memory CD4 T cells in the FRT. A rapid and robust local CD4 T cell recall response correlates with efficient pathogen clearance during *Chlamydia* FRT reinfection. Future studies will dissect the activation, effector function, and maintenance of *Chlamydia*-specific memory CD4 T cells during *Chlamydia* reinfections.

I.2. *Candida* and the Long Strange Road to Vascular Permeability

Douglas A Johnston, *Louisiana State University Health Sciences Center - New Orleans*

Background: *Candida spp* are the most common fungi implicated in blood stream infections (BSIs), contributing to significant morbidity and mortality (30-day mortality rate of 54%). *Candida albicans* is the most predominate isolate; however, non-*albicans Candida* (NAC) species are collectively responsible for more infections worldwide- and many are inherently or increasingly drug-resistant. The aim of this project is to elucidate the earliest stages of the vascular response to *Candida* infections in an attempt to identify potential mechanisms for increasing endothelial resistance to fungal dissemination.

Methods: We used clinical isolates of *C. albicans* and several NAC species in co-culture experiments with human macro- and microvascular endothelial cell monolayers, including an established cell line (HMEC-1) and primary cells from umbilical veins (HUVEC), neonatal dermis (MVEC-neo) and adult cardiac tissue (MVEC-cardiac). Cell culture supernatants were subjected to ELISAs for quantification of protein secretion. Total RNA was purified from cell lysates and used for synthesis of cDNA and subjected to quantitative reverse transcription polymerase chain (qRT-PCR) analyses. Additionally, endothelial cells were co-cultured with *Candida* on glass coverslips and stained for protein expression by fluorescent immunocytochemistry (ICC). Lastly, HMEC-1 cells were transfected with CRISPR-Cas9 Double Nickase plasmids in an attempt to knockout the genes encoding CXCR2 and c-Src.

Results: *C. albicans* induces rapid endothelial activation and dysfunction, including the dissolution of endothelial intercellular adherens junctions (AJs) and the subsequent loss of barrier function. *Candida* induced significant increases in inflammatory cytokines and chemokines, including the CXCR2 ligands CXCL1, CXCL2, and CXCL8. The upstream activation of CXCR2 resulted in a VEGFR2-dependent increase in barrier disassembly. AJ breakdown was consistently rescued by inhibitors of c-Src, CXCR2, and VEGFR2. The major changes in endothelial gene expression and morphology were similar among the different cell types, although *C. albicans* induced the greatest responses in all assays. Interestingly, the emerging "superbug" *C. auris* had minimal effect in many of our assays.

Conclusion: The dissolution of AJs by *Candida* requires a complex series of non-canonical receptor-activation events. We find that the secretion of CXCR2 ligands and subsequent autocrine and paracrine receptor binding, leads to the VEGF-independent activation of VEGFR2 and dephosphorylation of AJ components. Interestingly, endothelial cells from different tissues behaved similarly, but not identically, and different species of *Candida* also had varying effects on this process. These results highlight the importance of choosing the correct model system for in vitro endothelial analyses. Additionally, we hypothesize that *C. auris* likely utilizes a unique infection strategy for infection of endothelial cells, as compared to *C. albicans*.

I.3. Host Protection and Antigen-specific CD4 T cell Immunity is Dictated by Anatomical Location During Acute and Chronic *Salmonella* Infection

Jonathan R Kurtz and James B. McLachlan, *Tulane University School of Medicine*

Background: Certain pathogens, such as *Salmonella*, are able to evade the immune system and persist within the host. In some cases, these persistent infections are asymptomatic for long periods, and represent a significant public health hazard as potential chronic carriers. Many factors, both host and bacterial, may contribute to the ability of a pathogen to establish a persistent infection within the host. The factors that dictate this persistence during *Salmonella* infection are not well understood.
Methods: We used a mouse model of typhoid fever in *Salmonella*-resistant mice to assess immune function in different organs at early and late times after infection. We constructed an epitope tagged strain of virulent *Salmonella* Typhimurium SL1344. Antigen-specific T cells were identified using peptide-MHC class II tetramers. The combination of these tools allowed us to assess *Salmonella*-specific T cell phenotypes and functions using multi-color flow cytometry. We also evaluated organ-specific macrophage phenotypes and functions.
Results: The environment in the hepatobiliary system allows for the persistence of *Salmonella* Typhimurium through liver-resident immunoregulatory CD4 T cells, alternatively activated macrophages, and impaired bactericidal activity.
Conclusion: This work establishes a potential role for certain non-lymphoid organs in chronic bacterial infections and may provide further evidence for the hepatobiliary system as the site of chronic *Salmonella* infection.

I.4. Naïve CD4+ T Cell Activation in the Nasal Associated Lymphoid Tissue

John T. Bates, *University of Mississippi Medical Center*

Background: The nasal associated lymphoid tissues (NALT) in mice are the equivalent of the human tonsils and are generally considered to be an immune inductive site. The NALT's small size and location make them a difficult compartment to study. Consequently, less is known about antigen sampling and immune activation in the NALT than in other compartments.
Methods: NALT were harvested from C57BL/6 mice and analyzed by scanning electron microscopy and fluorescent microscopy. Naïve CD4+ T cells with a transgenic T cell receptor specific for chicken ovalbumin were transferred into recipient mice one to two days prior to intranasal immunization with ovalbumin-flagellin fusion protein. Activation of antigen-specific CD4+ T cells was observed based on clustering of OVA-specific CD4+ T cells in the T cell zone of the NALT one day following immunization. Cell division by CFSE-labeled OVA-specific T cells was measured by flow cytometry three days following immunization.
Results: The NALT is covered by ciliated epithelium, though non-ciliated cells were also observed in some samples. Transepithelial sampling of fluorescently labeled antigen was observed. One day following intranasal immunization with ovalbumin-flagellin fusion protein, OVA-specific CD4+ T cells form clusters in the T cell zones of the NALT. Three days following immunization, CFSE-labeled OVA-specific CD4+ T cells in the NALT had undergone extensive cell division. The level of division by the OVA-specific cell population in the NALT was similar to that in the cervical lymph nodes, and populations in both compartments significantly increased in cell number.
Conclusion: The NALT is an efficient immune induction site for naïve CD4+ T cells, and the kinetics of CD4+ T cell activation in the NALT are similar to what has been observed in other compartments.

Bacterial Pathogenesis - Invited Speakers

I.5. Regulation of Virulence by *msaABCR* in *Staphylococcus aureus*

Mohamed O. Elasri, *The University of Southern Mississippi*

Staphylococcus aureus has a complex regulatory network for controlling the production of virulence factors. Our laboratory focuses on the *msaABCR* operon which regulates virulence, biofilm development, and antibiotic resistance. We have also determined the organization of the *msaABCR* operon and found that it includes three functional noncoding RNAs (*msaA, msaC*, and *msaR*) that are essential for its function. We have shown that MsaB is a new transcription factor that controls the production of capsule in response to nutrient availability. We have also shown that MsaB interacts with other regulators (CodY) to carry out its function. When nutrients (branched chain amino acids) are abundant, CodY binds to the promoter region of the cap operon and represses its transcription. However, when nutrient concentrations decrease, MsaB, rather than CodY, binds to the cap promoter. Binding of MsaB to the cap promoter activates transcription of the cap operon. We hypothesize that MsaB uses a similar mechanism to control the expression of other target genes. Furthermore, we are examining the role of the non-coding RNAs in the functions of the *msaABCR* operon. This operon is a potential therapeutic target to treat *S. aureus* infections.

I.6. Evaluating Early Host/Pathogen Interactions During Pneumonic Plague

Samantha Huckuntod, Srijon Banerjee, Roger D. Pechous, *University of Arkansas for Medical Sciences*

Background: Primary pneumonic plague results from the inhalation of respiratory droplets containing *Yersinia pestis* and is nearly 100% fatal in the absence of timely delivered antibiotics. Pneumonic plague progresses in two distinct phases as defined by host immune responses and disease pathology. The initial "pre-inflammatory phase" involves rapid replication of bacteria in the lungs in the absence of any observable innate immune activation, followed by the abrupt and irreversible switch to a "pro-inflammatory phase" characterized by extensive neutrophil influx into the airways and a massive pro-inflammatory cytokine storm. Our lab is working to define the early host/pathogen interactions occurring in the lung that drive the biphasic progression of primary pneumonic plague. Key to establishing the early pro-inflammatory disease phase is the type 3 secretion (T3S) of *Yersinia* outer protein (Yops) effectors into target host cells in the lung. It was recently shown that *Y. pestis* specifically targets alveolar macrophages for T3S early after infection.
Methods: We seek to identify the mechanism of *Y. pestis* target cell selection, and to evaluate the immediate effects of Yop translocation *in vivo*. To this end, we have initiated a CRISPR/Cas9-mediated screen to identify host molecules required for Yop translocation into macrophages. We have also devised an approach that couples a fluorescence reporter with flow cytometry to isolate Yop-injected cells from mice for direct comparison to cells of the same type that have not been targeted for Yop translocation.
Results/Conclusions: We are currently screening a human CRISPR RNA library to identify host genes contributing to Yop translocation. In addition, we are using FACS isolation of Yop-injected cells to evaluate the effects of Yop translocation on target cells *in vivo* during infection. Characterizing the earliest host/pathogen interactions occurring in the lung during infection is essential to understanding of the mechanisms of pneumonic plague pathogenesis, and how the failure of the innate immune system to control infection leads to severe and lethal pneumonia.

I.7. Monocytes, Macrophages and Trojan Horses: How Pathogenic Rickettsial Species Can Disseminate in Fatal Cases of Spotted Fever Rickettsioses

P. Curto[1], S. Riley[2], I. Simoes[1,2], Juan J. Martinez[2], [1]*University of Coimbra, Portugal,* [2]*Louisiana State University*

Spotted fever group (SFG) Rickettsia are recognized as important agents of emerging human tick-borne diseases worldwide such as Mediterranean spotted fever (*R. conorii*) and Rocky Mountain spotted fever (*R. rickettsii*). Recent studies in several animal models have provided evidence of non-endothelial parasitism by different *Rickettsia* species suggesting that the interaction of rickettsiae with cells other than the endothelium may play an important role in the pathogenesis of rickettsial diseases. These studies raise the hypothesis that the role of macrophages in rickettsial pathogenesis may have been underappreciated over the last years. We evaluated the ability of several pathogenic SFG *Rickettsia* species (*R. conorii*, *R. akari*, and *R. africae*) and several related species that are not recognized as causing disease in mammals (*R. montanensis*, *R. amblyommatis* and *R. bellii*) to proliferate in THP-1 macrophage-like cells and within non-phagocytic cell lines, Vero and Eahy.926. Growth dynamics in each of these cell lines were initially evaluated by quantitative PCR (q-PCR). In addition, the ability of individual species to associate with and invade into these mammalian cells lines was evaluated by standard immunofluorescence microscopy-based assays. Our results clearly demonstrated that pathogenic rickettsial species were able to survive and proliferate in both phagocytic, endothelial and epithelial cells *in vitro*. In contrast, species that are not recognized as human pathogens including, *R. montanensi*s, *R. amblyommatis* and *R. bellii* were able to grow in non-phagocytic cells, but were drastically compromised in the ability to proliferate within THP-1 cells. Interestingly, our results demonstrated that the few *R. montanensis* that could invade the THP-1-derived macrophages were rapidly destroyed, and in appeared to localize with LAMP-2 and cathepsin-D, two markers of lysosomal compartments. In contrast, *R. conorii* and other pathogenic species were intact and free in the cytoplasm in both phagocytic and non-phagocytic cells. These findings suggest that a major phenotypic difference between a non-pathogenic and a pathogenic rickettsial species may lie in their respective ability to proliferate in macrophage-like cells and may provide an explanation as to why certain SFG rickettsial species are not associated with disease.

I.8. Pneumococcal Resistance to Neutrophil-Mediated Killing: A Trait for Invasiveness?

James H. Jackson III, Mary A. Carr, Jessica Surma, Leah B. Drumheller, Keun S. Seo, Jason W. Rosch, and Justin A. Thornton, *Mississippi State University*

Background: *Streptococcus pneumoniae* (pneumococcus) is the leading cause of community acquired pneumonia and is also responsible for other invasive infections including bacteremia and meningitis. However, the pneumococcus usually asymptomatically colonizes the nasopharynx or leads to less serious infections such as otitis media and sinusitis. It is not well established exactly what factors are important during the transition from harmless commensal to invasive pathogen. In the absence of capsule type-specific antibody, clearance of pneumococcus is dependent upon the phagocytic and anti-microbial activities of innate immune cells. Neutrophils are the primary responder to pneumococcal infection and are critical for controlling bacterial replication and clearance. It was previously identified that, unlike macrophages which primarily kill pneumococcus via reactive oxygen species, neutrophils use granule components including the proteases elastase and cathepsin G. Given the importance of neutrophils for bacterial clearance, we hypothesized that *S. pneumoniae* has evolved mechanisms to protect against granule-mediated killing.

Methods: We exposed a panel of more than twenty invasive pneumococcal isolates to supernatant from degranulated neutrophils and compared survival after one hour of incubation at 37°C. Additionally, we assessed whether capsule type was related to resistance to degranulate-mediated killing. To determine if the protective factor was a secreted product, we performed degranulate killing assays of sensitive strains in the presence of supernatant from resistant strains. We also compared chromosomal sequences of resistant versus sensitive strains.
Results: Invasive pneumococcal isolates were identified as being either extremely resistant to degranulate-mediated killing or extremely susceptible. Very few strains had intermediate resistance. Resistance does not appear to be capsule type-specific since strains of the same capsule type displayed both resistant and susceptible phenotypes. Additionally, supernatant from resistant strains was unable to rescue sensitive strains from killing.
Conclusions: A large proportion of invasive pneumococcal isolates are highly resistant to neutrophil granule-mediated killing. It appears that the protection is not dependent upon a secreted factor, but may rather be a cell-associated characteristic of the resistant strains. This could be a critical factor in why some strains proceed to cause invasive disease rather than simply colonizing. Targeting this factor either pharmacologically or with a vaccine could improve immune clearance of this important pathogen.

Applied and Environmental Microbiology - Invited Speakers

I.9. Indigenous Microorganisms in Inoculated Deep Litter Systems

C.N. Baugher[1], A.M. Bryant[1], J.K. Tomberlin[2], M.E. Benbow[3], T.L. Crippen[4], J. Cammack[2], M.W. DuPonte[5], A. Farris[2], J.L. Pechal[3], Heather R. Jordan[1], *[1]Mississippi State University, [2]Texas A&M University, [3]Michigan State University, [4]United States Department of Agriculture, [5]University of Hawaii*

As the human population continues to grow, so are the numbers of confined animal feeding operations (CAFOs), including piggeries. Conventional methods for waste management within piggeries involve housing with grated floors, allowing waste to flow to holding areas below the facilities, where it is then transferred for storage until it can be applied as fertilizer, or processed at a treatment facility. These methods lead to incredibly foul odors, environmental and pathogen pollution, animal stress, and an increase in workforce labor requirements. To combat these issues, Inoculated Deep Litter Systems (IDLS) have been implemented in small-scale swine operations as a sustainable farming method. IDLS provide natural waste management that reduces moisture, eliminates foul odors, decreases pollution and pest flies, and leads to an increase in production through reduced animal stress and illness. IDLS is a five-layer system of green waste inoculated with indigenous microorganisms (IMO), for low maintenance, federally approved waste management. IMO mixtures must be cultivated, but specific microbial species have not been identified. Furthermore, it is not known whether core microbial taxa are shared between multiple IDLS farms. To identify the responsible microbes, we used the isolation and sequencing of nucleic acids from feces, soil, IMO mixed cultures, and feed samples collected from three piggeries that incorporate IMOs into their bedding system and compared them to three piggeries that do not. Results showed that the Family Pseudomonadaceae dominated the IMO mixed cultures, as well as other IDLS components. Further, we identified differences between dominant family taxa in IMO+ and Non-IMO fecal and soils samples with potential for further system optimization.

I.10. Secondary Metabolites of Therapeutic Value from a Marine *Cyanobacterium* Culture

Erik Hom, *University of Mississippi*

My lab is interested in understanding how microbes interact in a persistent fashion with the goal of designing microbial communities that perform specified functions. I will briefly highlight the logic of our approach and then describe an ongoing effort to elucidate the activity and nature of a coral reef-associated cyanobacterium culture that produces secondary metabolites of therapeutic value.

I.11. Decoding the Microbes of the Northern Gulf of Mexico "Dead Zone"

J. Cameron Thrash, *Louisiana State University*

Coastal marine systems that experience losses of dissolved oxygen (hypoxia- DO < 2 mg/L) due to eutrophication are growing in number and severity worldwide. One of the world's largest of these so called "dead zones" occurs in the northern Gulf of Mexico as a result of fertilizer runoff from the Mississippi River and strong seasonal stratification of the water column. Dissolved oxygen is consumed by aerobic microorganisms respiring plentiful organic carbon supplied by phytoplankton responding to fertilizer-based nutrients. However, in spite of being largely a microbially-generated phenomenon, we know little about the specific microorganisms inhabiting the dead zone or their functional capacity. To determine microbial roles during hypoxia, we reconstructed functional potential through metagenomic assembly and quantified gene expression through community transcriptomics. We recovered over 70 high quality genomes spanning numerous groups of varying abundances, facilitating detailed reconstruction of their metabolic contributions during hypoxia. Our results indicate that the majority of taxa rely on aerobic respiration even in the lowest dissolved oxygen samples, although multiple terminal electron accepting processes

likely occur simultaneously. Furthermore, many poorly understood uncultivated groups; such as SAR406, SAR202, and some Candidate Phylum members; appeared highly adapted for the degradation of complex organic matter like algal cell wall components. This work provides the most comprehensive metabolic assessment of microorganisms in the northern Gulf of Mexico dead zone thus far, and provides important hypotheses about the roles of uncultivated taxa in regions of hypoxia.

I.12. The Symbiotic Potential of *Burkholderia* Bacteria in the Social Amoeba *Dictyostelium discoideum*

Tamara Haselkorn, *University of Central Arkansas*

The ability to interrogate host-microbe interactions is what makes the amoeba, *Dictyostelium discoideum* an ideal model for studying the dynamics of symbiosis. This soil-dwelling amoeba has diverse interactions with bacteria: bacteria are prey, pathogens, and symbionts. Certain species of *Burkholderia* are able to survive amoebal digestion and maintain a persistent relationship, conferring to its amoeba host the ability to carry other food bacteria within its spores, with the potential benefit of seeding its own food crop. To characterize the prevalence and diversity of this association in nature we screened over 700 isolates of *D. discoideum* and found a 25% infection prevalence. Using a multilocus phylogenetic analysis, we discovered at least two introductions of the *Burkholderia* symbiont into *D. discoideum*. There are at least 10 distinct symbiont strains that fall into two distinct clades, one of which shows evidence of rapid sequence evolution. To assess the symbiotic potential of these bacteria, we infected each of these symbiont strains, as well as non-symbiont *Burkholderia* strains into novel *D. discoideum* hosts. For the different *Burkholderia* strains, we found variation in their ability to colonize, persist, and enter into spores, suggesting that *Burkholderia*'s relationship with *D. discoideum* exists on a symbiotic continuum that will be useful for exploring the transition to the symbiotic lifestyle and mechanisms of this interaction. Only *D. discoideum Burkholderia* symbionts (from both clades) were able to fully express all symbiont traits, and we have sequenced the genomes of 12 symbiont and closely related non-symbiont strains to look for patterns of genome evolution consistent with the transition to the intracellular lifestyle.

Food Microbiology - Invited Speakers

I.13. The Contribution of Plasmids to Antimicrobial Resistance and Virulence in *Salmonella enterica* Isolated from Foods, Food Animals or Human Patients

Steven L. Foley[A], Bijay Khajanchi[A], Jing Han[A], Yasser M. Sanad[A,B], Pravin Kaldhone[A,C] and Carl E. Cerniglia[A], *[A]National Center for Toxicological Research, [B]University of Arkansas at Pine Bluff, [C]University of Arkansas*

Background: *Salmonella enterica* are important foodborne pathogens, they are estimated to cause more than 1 million infections annually in the United States, leading to nearly 20,000 hospitalizations and 400 deaths. While most infections manifest as self-limiting gastroenteritis, a small percentage of cases involve more invasive infections that warrant antimicrobial therapy. Therefore, antimicrobial resistance is an important concern and many of resistance determinants and potential virulence factors are located on plasmids that may be transferred among bacteria. The food safety research program in the NCTR/FDA Division of Microbiology has focused efforts to better understand the role of plasmids in virulence and antimicrobial resistance. This presentation includes some of our latest research findings on the topic.

Methods: Antimicrobial susceptibility data from 1,275 *Salmonella* isolates from foods, food animals and human patients, representing multiple serotypes were evaluated. Over 20 of these isolates were subjected to whole genome and/or plasmid sequencing to identify the presence of resistance, virulence and plasmid-transfer associated genes. Representative strains containing putative plasmid-associated virulence factors were evaluated for their abilities to invade and persist in intestinal epithelial and/or macrophage cell lines. Strains were further evaluated for their ability to conjugatively transfer antimicrobial resistance and virulence genes.

Results: Several different plasmid types, including incompatibility group (Inc) A/C, FIB, HI1, HI2, I1, and IncX4 plasmids, were detected among the sequenced strains. Many of the plasmids were conjugative and carried multiple antimicrobial resistance genes and genes potentially associated with virulence, including those for bacteriocins, iron acquisition and VirB/D4 type 4 secretion systems (T4SS). Plasmids containing the VirB/D4 T4SS and to a smaller extent the iron acquisition systems increased the abilities of strains to invade and/or persist in host cells.

Conclusions: Genes encoded on transmissible plasmids are important for antimicrobial resistance and in many cases virulence, thus understanding factors that impact plasmid transfer may provide ways to limit the emergence of increasingly resistance and virulent *Salmonella*.

I.14. The Impact of Oxygen Availability on the Stress Response of *Listeria monocytogenes*

Janet R. Donaldson, *The University of Southern Mississippi*

Background: *Listeria monocytogenes* is a Gram positive, foodborne pathogen and is the cause of nearly 28% of all food related deaths. Pathogenesis of *L. monocytogenes* is dependent upon its ability to survive stressors encountered within the gastrointestinal tract. Here, we analyzed the impact that oxygen availability has on the resistance of *L. monocytogenes* to stressors.
Methods: Survival of *L. monocytogenes* strains F2365, EGDe, 10403S, and HCC23 to bile and acid stress was analyzed under either aerobic or anaerobic conditions. Real-time PCR and mass spectrometry were used to analyze the expression of putative oxygen sensors under aerobic and anaerobic conditions. A gerbil animal model was used to test the impact of anaerobiosis on virulence.
Results: Survival of *L. monocytogenes* to bile increased under anaerobic conditions, but only among virulent strains. This also correlated with an increase in the expression of the bile salt hydrolase and putative oxygen sensors. Exposure to anaerobic conditions prior to infection increased the morbidity associated with infections with *L. monocytogenes* and increased survival within the cecum.
Conclusion: This study shows that virulent strains of *L. monocytogenes* become more resistant to stressors under anaerobic conditions. This impact may be due to variations in the regulation of oxygen sensing proteins. Due to the role of putative oxygen sensors in regulating intracellular concentrations of c-di-GMP, further work is needed to determine whether c-di-GMP is involved in regulating virulence factors.

I.15. Prevalence of Antibiotic Resistance *Clostridium difficile* in Lettuce

Marlene Janes, Yi Han, Joan King, *Louisiana State University Agricultural Center*

Background: *Clostridium difficile* (*C. difficile*) is the major cause of antibiotic-associated diarrhea in humans in health care facilities. *C. difficile* infections in individuals with no contact with health care facilities have been identified. Foodborne transmission has been suggested as a possible route for Community-associated *C. difficile* infections. Limited data is available regarding the prevalence of *C. difficile* in food and water. This study determined the prevalence of antibiotic resistance toxigenic *C. difficile* isolated from retail lettuce.
Methods: Lettuce samples grown in California, Arkansas, and Louisiana were purchased from retail stores for seven months (297 lettuce samples). Standard plating methods were used for isolation of *C. difficile* from lettuce. Positive colonies were tested for toxin A and toxin B by real-time polymerase chain reaction (PCR). Antibiotic-resistance was determined using the micro-dilution minimal inhibitory concentration (MIC) test for five common clinical-selected antibiotics (metronidazole, vancomycin, clindamycin, erythromycin, and cefotaxime). Ribotype was determined by PCR.
Results: Toxigenic *C. difficile* was found in 41 of 297 (13.8%) of the lettuce samples. Among the toxigenic isolates, 82.9% (34/41) only produce toxin B, 17.1% (7/41) produced both toxin A and toxin B, and two of the Louisiana *C. difficile* isolates were ribotype 027. The virulence *C. difficile* isolates were antibiotic resistance to metronidazole, vancomycin, and erythromycin.
Conclusion: The present study reports the highest prevalence of toxigenic *C. difficile* in *USA* retail lettuce. The antibiotic resistance to metronidazole, vancomycin, and erythromycin of the isolated *C. difficile* from retail lettuces could lead to public health concerns.

I.16. Determination of the Microbiota Dynamics in Fermented Foods and Beverages

Lindsey Rodhouse, Margaux Vallee, Laura Fassier and Franck Carbonero, *University of Arkansas*

Background: Fermented foods and beverages have been produced for centuries, as microbial fermentation has been found to improve shelf-life and reduce foodborne pathogens contamination risk. More recently, there has been an additional trend associating fermented foods with health benefits, specifically the belief that fermented foods act as probiotics. However, evidence for probiotic effect is sparse except for fermented dairy products. Here, we assessed the microbiota associated with several different fermented food and beverages.
Methods: Fermented vegetables, kombucha and beer/cider samples were obtained from local producers, and repeated sampling along the fermentation process was performed. Microbial DNA was extracted from all samples, and bacterial amplicons were submitted to Illumina MiSeq sequencing resulting in thousands of reads for each sample. Reads were analyzed with the Mothur pipeline, and microbiota profiles compared by multivariate analyses as well as univariate analyses of taxa of interest.
Results: Somewhat surprisingly, Archaea were detected by qPCR in most of the samples. Cabbage, okras and jalapenos fermentation were shown to be mainly performed by *Leuconostoc* initially and *Lactobacillus* in the last stages and final products. However, microbial diversity was relatively high, presumably due to survival of the diverse raw vegetables. Intriguingly, unfermented tea was found to harbor a diverse microbiota, influencing to some extent the fermentation, which mainly involved *Acetobacter* species. While ciders produced with starter cultures were almost exclusively contaminated with the expected *Oenococcus oeni*, 'home-made" ciders from organic apples led to the potentially dangerous development of *Yersinia spp*.. Extensive sampling in craft breweries showed that diverse bacterial microbiota were associated with malts and hops and persisted in the mashing process. Boil kettle and fermenter surfaces were shown to harbor specific bacterial microbiota, which influenced significantly the composition of the products at these stages. Remarkably, the final products harbored raw materials and vessel-associated bacteria in roughly equal amounts.
Conclusion: For all products, a much higher bacterial diversity than hypothesized was observed, suggesting potential roles (detrimental or beneficial) for rare taxa. A very limited stool batch culture experiment revealed that kombucha supernatant exerted remarkable prebiotic properties. These preliminary studies suggest that bacterial compositions of fermented foods are complex, and claims of probiotic properties may only be justified in specific cases. In addition, we show that craft brewing processes allow for maintenance of a diverse

bacterial microbiota, which is likely associated with observed spoilage issues with packaged products. We intend to perform similar analyses to determine the fungal microbiota profiles on these samples.

Virology - Invited Speakers

I.17. Tumor Suppressor p53 Controls Chronic Infection and Host Genomic Integrity in a Murine Model of Gammaherpesvirus Pathogenesis

Jeffrey M. Sifford[1], Shana M. Owens[1], Gang Li[1], Eduardo Salinas[1], Debopam Ghosh[1], Andrew D. Miller[2], Jason Stumhofer[1], and J. Craig Forrest[1], *[1]University of Arkansas for Medical Sciences, [2]Cornell University*

Gammaherpesviruses (GHVs) establish life-long infections and cause cancer in humans and other animals. To facilitate chronic infection, GHV oncoproteins promote cellular proliferation and differentiation. Aberrant cell-cycle progression driven by viral oncogenes should trigger activation of tumor suppressor p53, unless p53 is functionally deactivated during GHV latency establishment. However, interactions of GHVs with the p53 pathway during the establishment and maintenance of latent infection are poorly defined. Here we demonstrate in vivo that p53 is induced specifically in infected cells during latency establishment by murine gammaherpesvirus 68 (MHV68). In the absence of p53, MHV68 latency establishment was significantly increased, especially in germinal center B cells, and correlated with enhanced cellular proliferation and reduced apoptosis. However, enhanced latency was not sustainable, and MHV68 exhibited a defect in long-term latency maintenance in p53-deficient mice that was characterized by genomic instability in B cells. Moreover, infected p53-null mice exhibited accelerated mortality that was dependent on chronic MHV68 infection. These data demonstrate that p53 intrinsically restricts MHV68 latency establishment and reveal a paradigm in which a host restriction factor provides a long-term benefit to a chronic pathogen by limiting infection-associated damage.

I.18. Regulation of Stromal Gene Expression by Human Papillomavirus Type 16

Gaurav Raikhy, Brittany Woodby, Matthew Scott, and Jason Bodily, *Louisiana State University Health Sciences Center – Shreveport*

The impact of the stromal microenvironment in the development of cancers and the ability of tumor cells to regulate the behavior of stromal cells is increasingly recognized. Human papillomavirus infections share many characteristics with cancers, but the impact of infection on cells in the neighboring stroma, and vice versa, is not well understood. We have observed that viral oncogene expression leads to the alterations in the levels of many paracrine-acting factors, including interferons, cytokines, and growth factors. To investigate the impact of viral infection on stromal gene expression, we created organotypic cultures containing human fibroblasts in the stromal compartment and either HPV16-containing keratinocytes or uninfected keratinocytes in the epithelial compartment. Gene expression profiling revealed a significant downregulation of innate immune genes, including interferon response genes, in fibroblasts from cultures containing HPV as compared to controls. We found that knockdown of STAT1 or the type I interferon receptor in the fibroblasts of organotypic cultures resulted in dramatic upregulation of viral late gene expression in the epithelium. We also observed that the ability of HPV to suppress innate immune responses, both in the stroma and in the epithelium, depended in part on the expression of E5. These findings demonstrate that the stromal innate response to HPV infection is important for suppressing the viral life cycle, and they identify a new function for E5 as a regulator of paracrine epithelial-stromal interactions.

I.19. Dynamin Mediated Endocytic Pathways are Critical for Cytomegalovirus Maturation

Mohammad Hasan, Rinkuben Parmar, Leslie Davis, and Ritesh Tandon, *University of Mississippi Medical Center*

Background: Cytomegalovirus secondary envelopment occurs in a virus-induced cytoplasmic assembly compartment (vAC) generated via a drastic reorganization of the membranes of the secretory and endocytic systems. Dynamin is a euokaryotic GTPase that is implicated in membrane remodeling and endocytic membrane fission events. Dynamin is also important in the entry of several viruses in cell types where the primary method of virus entry is clathrin-mediated endocytosis. The role of dynamin in cellular trafficking of viruses beyond virus entry is only partially understood.
Methods: Mouse embryonic fibroblasts (MEF) engineered to excise all three isoforms of dynamin by a tamoxifen inducible knockout strategy were infected with mouse cytomegalovirus (MCMV-K181) at a high multiplicity of infection. Viral gene expression and maturation stages were analyzed.
Results: Equivalent levels of immediate early (IE1; m123) viral protein were detected in triple dynamin knockout (TKO) and parental uninduced (UI) cells at early times post infection indicating intact virus entry; however, virus growth was reduced several folds in TKO cells compared to UI cells. Analysis of later time points in infection revealed similar distribution of viral early (m04) and late (m55; gB) proteins in TKO and UI cells. Ultrastructural analysis revealed intact stages of nuclear virus maturation in both cases with equivalent numbers of nucleocapsids containing packaged viral DNA (C-capsids) indicating successful viral DNA replication, capsid assembly and genome packaging in TKO cells. Most importantly, severe defects in virus envelopment and egress were visualized in TKO cells but not in parental UI cells.

Conclusion: Dynamin-mediated endocytic pathways are dispensable for early stages of MCMV infection in fibroblasts but are critical for cytoplasmic maturation.

I.20. Ubiquitination of Rotavirus NSP1 in Assays with Tandem-Repeat Ubiquitin-Binding Entities (TUBEs)

Michelle M. Arnold, *Louisiana State University Health Sciences Center - Shreveport*

Background: The covalent attachment of ubiquitin to proteins regulates many cellular processes, including the innate immune response to viruses. Some viruses counteract host immune defenses by encoding E3 ubiquitin ligases to target proteins within the innate immune pathway for degradation. Rotavirus nonstructural protein 1 (NSP1) inhibits the production of type I interferon (IFN) by inducing the degradation of key signaling proteins including IFN regulatory factors (IRF3, IRF5, IRF7) and the β-transducin repeat-containing protein (β-TrCP), which is essential for NF-κB activation. NSP1 has long been thought to act as an E3 ubiquitin ligase, but recent data suggest that NSP1 may instead hijack host cullin-RING ligase complexes (CRLs) to promote degradation of its target proteins. Our goal is to define the mechanism of NSP1-induced degradation of target proteins.
Methods: Tandem-repeat ubiquitin-binding entities (TUBEs) consist of ubiquitin binding associated domain repeats that recognize polyubiquitin chains and efficiently protect ubiquitinated proteins from degradation. To determine if NSP1 proteins are ubiquitinated, HaloTag-NSP1 from different strains of rotavirus were expressed in 293T cells and lysates were subjected to pull-down analysis using TUBEs. To confirm our results with TUBEs, *in vitro* ubiquitination assays were performed using 293T cells exogenously expressing HaloTag-NSP1 treated with and without the Nedd8 inhibitor MLN4924.
Results: The TUBE pull-downs were immunoblotted to detect ubiquitin and showed a smear, suggesting NSP1 itself is ubiquitinated. To further characterize the polyubiquitin linkages that associate with NSP1, Lys^{48} and Lys^{63} specific TUBEs were used. NSP1 proteins were ubiquitinated through the attachment of both Lys^{48} and Lys^{63}-linked polyubiquitin chains. The association with Lys^{48}-linked ubiquitin chains was expected because that specific linkage is known to be involved in targeting proteins to the proteasome for degradation. The ability of NSP1 to also associate with Lys^{63}-specific ubiquitin linkages suggests NSP1 associates with mixed ubiquitin chains or that it may have additional unknown functions. Results of the in vitro assays with the Nedd8 inhibitor indicated that activated CRLs were not required for NSP1-mediated degradation of target proteins.
Conclusion: These data support the hypothesis that NSP1 functions as an E3 ubiquitin ligase to induce degradation of proteins required for IFN induction. Although we have not excluded a role for CRLs in NSP1-mediated host protein degradation, future experiments will be aimed at examining other possible effects of the NSP1 interaction with CRLs in infected cells. By delineating the mechanisms for rotavirus antagonism of immune responses we will improve the understanding of how the innate immune system functions to protect against viral infections.

Applied and Environmental Microbiology - Selected Abstracts

S.1. Effects and Mode of Action of Plant Aldehydes and Aldehyde-Based Pro-Antimicrobial Polymer Networks (Pans) on the Antibiotic-Resistant Pathogen *Pseudomonas aeruginosa*

Yetunde Adewunmi, Dahlia Amato, Douglas Amato, Olga Mavrodi, Derek Patton, and Dmitri Mavrodi, *The University of Southern Mississippi*

Background: The continuous emergence of resistant pathogens, scarcity of new drugs in the pharmaceutical discovery pipeline, and public demand for antibiotic-free food have led to growing interest in natural, plant-derived extracts as alternatives to conventional antibiotics. Plant essential oils (EOs) contain metabolites that possess a broad range of antimicrobial activity and kill microorganisms without promoting resistance. Despite the demonstrated antimicrobial potential, the use of EOs continues to be hampered by knowledge gaps in their mode of action, their poor solubility, volatility, and oxidative instability. We screened different EO constituents against a panel of Gram-positive and -negative pathogens, including the antibiotic-resistant pathogen *Pseudomonas aeruginosa* PAO1. We further demonstrated that some plant-derived aldehydes (cinnamaldehyde, cyanobenzaldehyde, methoxybenzaldehyde, chlorobenzaldehyde and bromobenzaldehyde) and their combinations effectively inhibited PAO1. Finally, we generated a transposon insertion library of *P. aeruginosa* PAO1 and screened it to identify clones with enhanced susceptibilities to plant aldehydes.
Methods: A standard broth microdilution method was used to determine the antimicrobial activity of EOs constituents and measure their Minimal Inhibitory and Bactericidal Concentrations (MIC and MBC). Synergistic interactions between various combinations of compounds and their Fractional Inhibitory Concentrations (∑FICs) were determined using the checkerboard method. The transposon insertion library of 3,000 mutants was generated by electroporating *P. aeruginosa* PAO1 with EZ::TN<TET-1> transposon. The library was screened for the susceptibility to plant aldehydes using the broth microdilution method at 0.5x MIC.

Results: The combination of methoxybenzaldehyde with cinnamaldehyde, as well as chlorobenzaldehyde with bromobenzaldehyde, had partial synergistic effects against PAO1 (∑FICs of 0.75 and 0.625, respectively). By performing two rounds of screening the mutant library, we identified 34 clones with enhanced susceptibility to plant-derived aldehydes (MICs as low as >0.4 mg/ml). We are currently analyzing these mutants to determine the cellular pathways targeted by EO-derived aldehyde antimicrobials.
Conclusion: Results of this study identified plant-derived aldehydes and their combinations that effectively kill pathogenic bacteria. The mutational analysis of PAO1 will help to elucidate molecular mechanisms underlying the biological activity of these EO constituents. The five aldehydes identified in this study will be incorporated into pro-antimicrobial polymer networks (PANs) that degrade and release antimicrobials upon exposure to changes in pH and humidity. The approach enables the high loading efficiency and sustained release of antimicrobials and offers a viable solution to the challenges associated with the use of EOs as alternative to antibiotics.

S.2. Determination of the Effects of Different Maillard Reaction Products on the Murine Gut Microbiota

Nesreen ALJahdali[1], Pascale Gadonna-Widehem[2], Pauline M Anton[2], and Franck Carbonero[1], *[1]University of Arkansas, [2]Institut Polytechnique UniLaSalle, Beauvais, France*

Background: The Maillard Reaction (MR) is a non-enzymatic chemical reaction which results in the linkage between the amino group of amino acids and the carbonyl group of reduced sugars. This reaction generates Maillard reaction products (MRPs) are not naturally in foods and are responsible for a range of colors, odors, flavors, and palatability. Conflicting reports of MRPs impacts on human health are probably due to the fact that bioconversion of these digestible molecules by the gut microbiota has been marginally taken into account. Here, we assessed the impact of MRPs on the murine gut microbiota through three studies.
Methods: Study 1 focused on the effect of NεCarboxymethyllysine (CML) on the composition of mice gut microbiota and potential association with severity of experimental colitis. Study 2 focused on the impact of model bread crust melanoidins (and model bread crumbs) on healthy and experimentally-induced inflammation mice. Study 3 focused on the impact of consumption of increased concentration of melanoidin-rich malts on short and long-time.
Results: CML induced limited changes in healthy mice, but was found to significantly relieve the bacterial dysbiosis, especially the Proteobacteria bloom, incurred by one (but not the other) chemically-induced colitis. Melanoidin-rich bread crust had a significant impact on the gut microbiota, with significant decrease of Enterobacteriaceae and increase of Ruminococcaceae, *Oscillibacter* and Lachnospiraceae, while surprisingly bread crumbs stimulated *Lactobacillus*. Experimental colitis appeared to buffer the intensity of gut microbiota responses. High amounts of melanoidins rich malt rapidly and persistently led to significantly different gut microbiota profiles. There was a trend for decrease of *Lactobacillus* and *Ruminococcus* and increase of *Akkermansia* and *Bifidobacterium* with higher amounts of dietary melanoidins.
Conclusion: Overall, our findings suggest that CML and melanoidins are not detrimental in terms of their impact on the gut microbiota. CML was shown to be possibly beneficial in case of disturbed microbiota. Moreover, melanoidins appeared to have a prebiotic-like impact, with inhibition of potentially pathogenic Proteobacteria, and stimulation of fermentative Firmicutes for bread crust, or probiotic genera for the malts. Melanoidins structure are extremely variable from one food to another, therefore additional studies will be needed to better assess their potential beneficial properties on gut health.

S.3. A Water Chemistry Analysis, Bacteriological Survey, and Observation of Acquired Antibiotic resistance in Bayou Lafourche, LA

Kyle Bird and Raj Boopathy, *Nicholls State University*

In recent years, concern has grown around the presence of antibiotic resistant bacteria (ARB) and antibiotic resistance genes (ARG) due to their impacts on public health. While many instances of antibiotic resistance are often associated with healthcare facilities, antibiotics in the environment could greatly exacerbate the potential threats of drug-resistant pathogens. Bayou Lafourche of Southeastern Louisiana serves as the raw source of drinking water for 300,000 people in the region. Four sites along the bayou and one site from its' input source on the Mississippi River were monitored for water chemical composition, total and fecal coliform estimates, and presence of ARB/ARG. Water chemical analysis includes pH, dissolved oxygen, organic carbon, nitrite, nitrate, ammonia, sulfate, and phosphate. Total and fecal coliforms were estimated by means of the most probable number method (MPN). Four bacterial isolates (*Escherichia coli, Klebsiella pneumonia, Enterobacter sp,* and *Enterococcus sp*) were tested for resistance to antibiotics (sulfamethoxazole/trimethropin, tetracycline, cefoxitin, meropenem, imipenem, and vancomycin) and resistant bacteria were further examined with PCR to confirm the presence of antibiotic resistance genes (Sul1, tet(A), tet(W), tet(X), IMP, KPC, and OXA-48). Results showed consistent presence of ARB and ARGs in this water body.

S.4. Scanning Electron Microscopy of *Salmonella* Biofilms on Various Food-Contact Surfaces in Catfish Mucus

Nitin Dhowlaghar, Mohit Bansal, Mark W. Schilling, and Ramakrishna Nannapaneni, *Mississippi State University*

Background: USDA-FSIS prioritized *Salmonella* as foodborne pathogen due to its prevalence in imported catfish, farms and in processing environments. Reports indicate that fish mucus promotes growth, colonization and adherence of fish pathogens to the skin surface. The role of catfish mucus in the growth and survival and the persistence of *Salmonella* in processing environments has not been studied.
Methods: The growth and survival of *Salmonella* spp. was determined in low (15 µg/ml) and high (and 375 µg/ml) concentrations of catfish mucus at 22°C or 10°C for 63 days. The biofilm formation of *Salmonella* Blockley 7175 was determined in the presence of 375 µg/ml of catfish mucus on the processing surfaces. Also, biofilm formation was observed using scanning electron microscopy. The surface properties, surface roughness and surface energies on different processing surfaces were determined using contact angle measurement and atomic force microscopy.
Results: Growth and survival of all *Salmonella* strains was greater in the 375 µg/ml of catfish mucus at 10°C or 22°C in comparison to 15 µg/ml of mucus concentration. *Salmonella* strains were non-detectable in the absence of catfish mucus by 21-28 days at 10°C or 22°C. Scanning electron microscopy revelead that biofilm formation by *Salmonella* Blockley was less in 15 µg/ml than 375 µg/ml of catfish mucus extract on stainless steel. In addition, SEM indicated that the visible biofilms were least on buna-n rubber as compared to other three processing surfaces.
Conclusion: *Salmonella* can grow and form biofilms in a very low concentration of catfish mucus extract on different food-contact surfaces. Contact angle and atomic force microscopy confirmed that buna-n rubber was highly hydrophobic with low surface energy and low roughness when compared to other three surfaces. This may have contributed to a lower adherence of *Salmonella* to buna-n rubber as compared to the other surfaces.

S.5. Effect of Tetracycline on Bacteria in Carbon and Nitrogen Removal from a Sewage Treatment Plant

Richard Grabert, Joselyn Braimah, Sarah Rogers, and Raj Boopathy, *Nicholls State University*

Antibiotic and their overuse has become a huge problem over the last couple of decades and their future use is under much scrutiny. Residual antibiotics, antibiotic resistance genes (ARGs), and antibiotic resistant bacteria (ARBs) have been reported in sewage treatment plants before, and ARGs and ARBs have even been found in the local sewage treatment plant in Thibodaux. These antibiotics and ARGs may be influencing the bacteria in sewage treatment that carry out nitrogen assimilation processes to remove nitrogen as well as removal of carbon from the wastewater. Therefore, this study was conducted to study the effect of tetracycline on nitrogen assimilation rates and removal of carbon in sewage plants. Samples of sewage were collected from the aerobic ponds (activated sludge) and from the anaerobic digester sludge of the Thibodaux Sewage Treatment Plant, Thibodaux, LA. Consortia of nitrogen assimilation bacteria were isolated from these samples and their ability to assimilate ammonia and nitrate at different concentrations of tetracycline were measured along with carbon removal. EC-50 values for tetracycline for each consortium was determined for nitrate and ammonia assimilating bacteria. Nitrogen assimilating bacteria were isolated from the consortia. These isolates will be genetically analyzed to determine if they carry the tetracycline-resistant genes such as tet(A), tet(X), and tet(W).

S.6. The Use of Reactive Oxygen Sensitive Green Fluorescent Protein to Determine Reactive Oxygen Species Production in *Candida albicans*

Claire E. Jones and Patrick L. Hindmarsh, *Louisiana Tech University*

Background: *Candida albicans* is an opportunistic fungal pathogen commonly found in the mucosal tissue of the human body. Antifungal drugs, commonly used in treating fungal infections, causes *C. albicans* to produce reactive oxygen species (ROS) as a stress response. We have produced a *C. albicans* optimized, reactive oxygen sensitive GFP (royGFP) in order to measure production of ROS when the cells are presented with antifungals.
Methods: Using site directed mutagenesis, we introduced point mutations at S147 and Q204 in yeast enhanced GFP (yEGFP), where the amino acids are replaced with cysteine. When presented with ROS, these cysteine cause a disulfide bridge which will bend the chromophore and shifts the fluorescent emission wavelength. royGFP will be transfected in *C. albicans* via homologous recombination, and tested using a verity of antifungal drugs.
Results: The two amino acid mutations have been incorporated into yEGFP and fluorescent emission has been observed. The royGFP cassette in the experimental plasmid has been completed to knockout the His1 gene in *C. albicans* and an alternative plasmid to knockout Ura3 gene is currently being completed.
Conclusion: The presentation will cover preliminary results observed from transfecting royGFP in *C. albicans* cells.

S.7. Multiple Protein-DNA Complexes Activate Meiotic Recombination Hotspots via Chromatin Remodeling

Tresor O. Mukiza, Reine U. Protacio, Mari K. Davidson, and Wayne P. Wahls, *University of Arkansas for Medical Sciences*

Background: Meiotic homologous recombination increases genetic variability and ensures the proper segregation of homologs. Errors in meiotic recombination lead to abnormal chromosomal segregation, which causes pathologies such as trisomy 21. In the fission yeast

Schizosaccharomyces pombe, like many organisms, meiotic recombination occurs preferentially at hotspots that regulate its frequency and distribution across the genome. There are currently five identified DNA sequence motifs that activate hotspots in *S. pombe*, but only one, the *M26* motif, has a well-defined mechanism of activation. The *M26* hotspot is bound by a heterodimeric transcription factor, Atf1-Pcr1, leading to chromatin remodeling and high levels of recombination. The hypothesis of this study is that the four other regulatory motifs, *CCAAT, Oligo-C, 4095* and *4156*, employ similar mechanisms.
Methods: We constructed different strains harboring individually the five hotspot DNA sequence motifs, and a control allele that lacks these motifs, at the same genomic locus, the *ade6+* gene. We used genetic crosses to measure rates of recombination in the presence and absence of the motifs and transcription factors that bind to the motifs. In addition, the chromatin landscapes at the hotspot motifs, in mitotic cells and meiotic cells, were analyzed at single-nucleosome resolution with an assay that uses micrococcal nuclease and qPCR.
Results: Each of the five DNA sequence motifs increased recombinant frequencies, relative to the basal recombination control lacking the motifs. The deletion of each gene encoding a binding protein under study reduced hotspot recombination levels in the strain bearing its binding motif. Recombination at the control allele and at each of the other motifs was largely unaffected by the deletion, revealing high specificity for each hotspot. But while these protein-DNA complexes functioned with high specificity (independently and redundantly) to promote recombination, all five motifs triggered dynamic changes in chromatin structure during meiosis. In each case, the motifs caused the displacement of nucleosomes to generate a more open chromatin configuration.
Conclusions: High levels of homologous recombination at each of the hotspot motifs examined require the presence of their cognate transcription factor, and are accompanied by changes in nucleosome organization in the region surrounding the motif. These data suggest that the binding of a transcription factors to their cognate DNA sequence motifs is a general mechanism for activating meiotic recombination hotspots, in each case triggering nucleosome remodeling to promote access for the basal recombination machinery.

S.8. Microbiome of the Formosan Termite, *Coptotermes formosanus* Shiraki

Seth Van Dexter and Raj Boopathy, *Nicholls State University*

The subterranean Formosan termite, *Coptotermes formosanus* Shiraki is an insect pest in Louisiana responsible for $1 billion in damage annually in New Orleans alone. There has lately been interest in the gut microbiome of termites, both as a model system for microbial ecology due to its stability and as a potential target for pest controllers. In this experiment, we collected Formosan termites from dead wood in the field and homogenized their abdomens in a buffer solution. Diluted homogenates were incubated with both general purpose and three single-carbon source agars containing phenol, cellobiose, or acetate as sole source of carbon. The number of colony forming units (CFUs) growing on each plate was counted. The purpose of this experiment is to acquire an estimation of the number of individual bacterial cells in the termite gut fulfilling the role of utilizing common carbon sources found in the termite gut. Cellobiose is representative of cellulose, although it is smaller, and phenol is representative of the abundance of aromatic compounds in the termite gut derived from lignin monomers. Acetate is a common metabolic product in the termite gut and is the main carbon source for the termite. Our hypothesis is that CFUs will differ on phenol and cellobiose based on the diet of the host, but will not differ on acetate. The results from this ongoing study will be discussed in detail.

Immunology and Virology - Selected Abstracts

S.9. CXCL5 Downregulates IL-17A to Enhance Susceptibility to MRSA Induced Pneumonia

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Background: Community-acquired-methicillin resistant *Staphylococcus aureus* (CA-MRSA) is endemic in the U.S. and causes severe life threatening necrotizing pneumonia with high mortality and morbidity. Despite continuous effort, the detailed pathophysiology of CA-MRSA pneumonia still remains elusive. Lipopolysaccharides induced chemokine CXCL5 (aka LIX) is a potent chemoattractant for neutrophil migration in the lungs during acute bacterial infections. However, its role in CA-MRSA-induced pneumonia has not been established.
Methods: We used 8-12-week-old, female, wild-type and LIX knockout (LIX KO) mice to induce pneumonia by intratracheal inoculation of $5x10^7$ colony forming units (CFUs) of MRSA per mouse. Mice were sacrificed at 12 and 24 hours post-infection to enumerate bacterial burden, assess cellular recruitment, and perform cytokine analysis. For blocking IL-17A, LIX KO mice were injected intratracheally with 1µg per mouse of IL-17A antibody one hour prior to infection with MRSA. Mice were euthanized 24 hours post-infection to compare cellular recruitment and bacterial burden in lungs and bronchoalveolar lavage fluid (BALF).
Results: LIX KO mice were protected from MRSA-induced pneumonia having significantly less bacterial burden in lungs and BALF compared to their wild-type counterparts. Total leukocytes and neutrophil recruitment into the air spaces was significantly higher in KO mice. However, proinflammatory cytokines, such as IL-1β, IL-6, and TNF-α were significantly higher in WT mice compared to that of KO mice. In contrast, IL-17A was significantly higher in KO mice. Blocking of IL-17A in LIX KO mice increased the bacterial burden in the lungs

and BALF without altering the neutrophil recruitment to the alveolar space suggesting that IL-17A plays and important role in bacterial clearance.
Conclusions: Our findings together demonstrate that LIX negatively regulates IL-17A-mediated immunity during MRSA-induced pneumonia and blocking LIX could be a potential therapeutic approach to treat MRSA-infected pneumonic patients.

S.10. Defining Tissue-Specific Requirements for Viral Replication in Chronic Gammaherpesvirus Infection

Arundhati Gupta and J. Craig Forrest, *University of Arkansas for Medical Sciences*

Gammaherpesviruses (GHVs) are DNA tumor viruses that establish lifelong chronic infections associated with lymphomas and other malignancies. GHVs exhibit a biphasic replication cycle characterized by lytic replication or persistence as a latent episome. However, roles for acute replication and periodic reactivation from latency in long-term GHV pathogenesis are unclear. To address this question, we developed a viral genetic strategy that enables conditional ablation of conserved immediate-early viral proteins that are essential for viral replication. Using murine gammaherpesvirus 68 (MHV68) infection of mice, we evaluated the impact of conditional deletions of genes encoding RTA (ORF50) or MTA (ORF57) using a Cre-lox strategy in a small animal model of GHV infection. Viruses in which ORF50 or ORF57 were flanked by loxP sites replicated as efficiently as wild-type (WT) MHV68 and exhibited WT latency establishment and reactivation from latency following infection of C57BL/6 mice, demonstrating that insertion of loxP sequences did not have deleterious off-target effects. Induction of Cre expression following infection of cultured fibroblasts led to deletion of ORF50 and ORF57 genes, loss of RTA and MTA protein, and inhibition of downstream viral protein production, which confirmed that the system for conditional gene deletion was functional. Infection of CD19-Cre knock-in mice, which produce Cre recombinase in B cells, the major cell type in which MHV68 establishes latency, resulted in WT levels of latency establishment by viruses with floxed ORF50 or ORF57, but a severe defect in reactivation from latency. These data demonstrate that RTA and MTA are critical in B cells for MHV68 reactivation and highlight the importance of the B cell compartment to viral reactivation. Furthermore, these findings suggest that lytic replication in B cells does not contribute to MHV68 latency establishment.

S.11. A Recombinant Varicella Vaccine Expressing Simian Immunodeficiency Virus (SIV) Antigens Stimulates Humoral and Cellular Immune Responses Against SIV Following a Prime-Boost Immunization Strategy

Micheal Hohl[1], Wayne Gray[1], Bapi Pahar[2], and Vicki Traina-Dorge[2], *[1]University of Mississippi and [2]Tulane National Primate Research Center*

Background: A vaccine is urgently needed to control the worldwide HIV-AIDS epidemic. We have developed a live, attenuated varicella vaccine expressing the simian immunodeficiency virus (SIV) antigens. In this study, we show that the recombinant vaccine efficiently expresses SIV *gag* and *env* antigens and induces antibody and cell-mediated immune responses to these SIV antigens in immunized nonhuman primates.
Methods: A recombinant varicella vaccine was constructed by inserting the SIV *gag* and *env* genes within the glycoprotein C region of the simian varicella virus (SVV) genome (rSVV-SIV*gag/env*). A prime- boost immunization strategy was used to vaccinate nonhuman primates with a combination of the rSVV-SIV*gag/env.* Antibody responses to SVV and SIV antigens were evaluated by neutralizing or IgG binding assays. Cellular immune responses to the SIV antigens were determined by intracellular cytokine analysis.
Results: rSVV-SIV*gag/env* infected Vero cells were confirmed to efficiently express the SIV *gag* and *env* antigens by immunoblot and immunofluorescence assay. Immunization of nonhuman primates with a prime-boost strategy employing rSVV-SIV*gag/env* induced antibody responses to the SVV and SIV antigens. In addition, the immunization induced $CD4^+$ and $CD8^+$ polyfunctional T-cell cytokines responses to the SIV antigens.
Conclusion: This study demonstrates that a prime-boost strategy employing a recombinant varicella vaccine effectively induces humoral and cellular immune responses against SIV *gag* and *env* antigens in immunized animals. The ability of this approach to induce immune protection in immunized animals following challenge with pathogenic SIV will be evaluated. The results of this study may provide a foundation for development of an effective recombinant varicella vaccine against HIV-AIDS.

S.12. Rotavirus NSP1-Mediated Inhibition of IRF3 and NF-κB Nuclear Translocation

Samantha K. Murphy and Michelle M. Arnold**,** *Louisiana State University Health Sciences Center - Shreveport*

Background: Rotavirus is a leading cause of severe gastroenteritis in young children worldwide. The rotavirus nonstructural protein 1 (NSP1) inhibits production of type I interferon (IFN) to promote viral spread in an infected host. NSP1 induces degradation of IFN regulatory factors (IRF3, IRF5, IRF7) or β-transducin repeat-containing protein (β-TrCP), which is required for NF-κB activation. The pattern of IRF and β-TrCP degradation varies among different rotaviruses, but in general animal strains induce degradation of IRFs whereas human strains induce degradation of some IRFs and β-TrCP. We hypothesize that NSP1 proteins that induce IRF degradation but not β-TrCP degradation may block NF-κB nuclear translocation by another mechanism. Similarly, NSP1 proteins that induce β-TrCP degradation but not IRF degradation may block IRF nuclear translocation by another mechanism. Therefore, the aim of this study is to identify the subcellular localization of NF-κB when NSP1 proteins from different rotavirus strains are expressed in cells.

Methods: To determine the localization of endogenous IRF3 and NF-κB in cells expressing a constitutively active form of RIG-I (Flag-2CARD), subcellular fractionation was used. A plasmid expressing Flag-2CARD was transfected into human 293T cells that were then fractionated to determine if IRF3 and NF-κB were localized to the cytoplasm or nucleus. Next, cells were transfected with plasmids encoding Halo-tagged NSP1 from the SA11-4F rotavirus strain (induces IRF degradation), the OSU rotavirus strain (induces β-TrCP degradation), or the WI61 rotavirus strain (induces IRF and β-TrCP degradation). Transfected cells were treated with human TNF-α for 15, 30 or 60 minutes to induce NF-κB nuclear translocation and cells were fractionated and analyzed for levels of NF-κB in the nucleus.
Results: When Flag-2CARD was expressed, IRF3 and NF-κB were localized to the cytoplasmic and nuclear fractions of transfected cells. However, the levels of IRF3 and NF-κB in the nuclear fractions were low, indicating a stronger stimulus was needed to induce efficient nuclear translocation of IRF3 and NF-κB in 293T cells. The levels of NF-κB in the nuclear fractions of cells expressing WI61 or OSU NSP1 were lower after TNF-α stimulation when compared to cells expressing empty vector after TNF-α stimulation. Experiments to determine the level of NF-κB in the nuclear fractions of cells expressing SA11-4F NSP1 after TNF-α stimulation are ongoing.
Conclusion: Our results show that OSU and WI61 NSP1 reduce nuclear translocation of NF-κB during TNF-α stimulation. The reduction in NF-κB levels in the nucleus was likely observed because OSU and WI61 NSP1 promote degradation of β-TrCP, which sequesters NF-κB in the cytoplasm during TNF-α stimulation. If SA11-4F also prevents NF-κB nuclear translocation during TNF-α stimulation, this suggests NSP1s that do not promote degradation of β-TrCP prevent nuclear translocation of NF-κB through a mechanism that does not involve degradation. Future studies will determine localization of endogenous IRF3 in the presence of Halo-NSP1 from SA11-4F, OSU and WI61 during stimulation of IRF3 nuclear translocation.

S.13. Human Papillomavirus E7 Oncoprotein as a Regulator of Transcriptional Complexes

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Background: Failure to clear human papillomavirus (HPV) infection is the leading cause of cervical cancer worldwide and contributes to other anogential cancers. The viral oncoprotein E7 drives the cell into S phase to support viral DNA replication, and also regulates host genes involved in the immune response. How E7 regulates host gene expression to promote persistent infection remains incompletely understood, but it is known that E7 regulates host and viral gene expression by binding host transcriptional regulators. One such regulator is cyclin dependent kinase 8 (CDK8), a nuclear serine-threonine kinase that regulates transcription as a component of the Mediator complex. We showed that E7 associates with CDK8 and CDK8 is needed for the expression of late viral transcripts. We sought to understand how E7 and CDK8 may regulate host gene expression in the context of HPV infection.
Methods: We performed chromatin immunoprecipitation-sequencing (ChIP-seq) from human foreskin keratinocytes (HFKs) and HPV16-containing HFKs during the early or late stage of the viral life cycle. ChIP-seq analysis identified cellular loci occupied by E7 and CDK8. RT-qPCR was performed to measure the gene expression of loci occupied by E7 and CDK8.
Results: We have discovered that many host loci occupied by E7 and CDK8 are transcriptionally activated or suppressed in cells that contain HPV16 genomes. Loci occupied by E7 and CKD8 have various biological functions important to HPV, such as innate immunity, cell cycle regulation, and cell growth and proliferation.
Conclusion: We have made the novel discovery that E7 interacts with CDK8 and associates with host chromatin. This novel interaction between E7 and host chromatin suggests E7 may be a chromatin protein involved in regulation of host gene expression. We are currently testing the hypothesis *that E7 associates with the host chromatin to alter the activity of transcriptional regulatory complexes*.

S.14. Differential Delivery of Genomic dsRNA Dictates Reovirus Serotype-Specific IRF3 Activation

Johnasha Stuart and Karl W. Boehme, *University of Arkansas for Medical Sciences*

Early events in mammalian orthoreovirus (reovirus) infection activate innate immune responses, including the type 1-interferon (IFN) pathway. Following attachment to cell surface receptors, reovirus virions are taken up into cellular endosomes where particles are disassembled by cellular proteases to enable the transcriptionally active viral core to enter the cytosol and initiate viral replication. Although serotype 1 (T1) and serotype 3 (T3) reoviruses use similar mechanisms to enter host cells, the two serotypes differ in their capacity to induce IFN. T3 reoviruses elicit substantially higher levels of IFN production than T1 strains. We found that in SV-40 immortalized endothelial cells (SVECs), differences in IFN induction between T1 and T3 reoviruses correlates with the capacity to activate interferon regulatory factory 3 (IRF3), the key transcription factor for IFN production. T3-induced IRF3 activation uses the RIG-I/MDA5–MAVS axis independent of de novo RNA synthesis. We found that rsT1L does not impair IRF3 activation by causing degradation of RNA sensors or blocking IRF3-activating pathways. Transfected rsT1L and rsT3D RNA elicit IRF3 activation, indicating that genomic RNA from both strains can activate the IFN pathway. Further, transfection of rsT1L and rsT3D in vitro-generated virus core particles induced comparable levels of IRF3 activation. Together, our results suggest that differences in IRF3 activation by T1 and T3 reoviruses in SVECs does not result from variability in RNA structure or degradation of IFN induction signaling molecules. Instead, differential delivery of genomic RNA into the cytosol by T1 and T3 reoviruses leads in differences in IFN activation.

S.15. Regulation of Interferon kappa by Transforming Growth Factor Beta in HPV infection

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Background: Persistent high-risk human papillomavirus (HPV) infection is the major causal factor of cervical and other anogenital cancers. Because there are currently no existing therapeutics capable of preventing neoplastic progression of HPV infections, understanding the mechanisms of HPV-mediated persistence is a major research priority. Transforming growth factor beta (TGFβ), a key player in regulating immune responses and inhibits expression of early viral transcripts from integrated genomes. However, the mechanism of TGFβ-induced inhibition has not been characterized. Recent research has found that interferon kappa (IFNκ), a keratinocyte-specific, constitutively-expressed cytokine, is targeted by multiple viral proteins and possesses anti-viral activity, although the effect of IFN-κ on the viral life cycle remains unknown. We sought to better understand the effect of TGFβ on HPV and the relationship between TGFβ and IFNκ.
Methods: We utilized recombinant TGFβ treatment, lentiviral shRNA knockdown, siRNA transfection, and RT-qPCR to assess the effects of TGFβ on HPV and the IFNs. In order to assess the methylation status of the IFNκ promtoer, we utilized 5-aza-2'deoxycytidine and bisulfite sequencing.
Results: We have discovered that TGFβ1 can upregulate IFNκ, specifically in the context of episomal viral infection, and this upregulation could contribute to the anti-viral activity of TGFβ1. Upregulation of IFNκ by TGFβ requires the presence of the virus, by reversing HPV-induced hypermethylation of the IFNκ promoter. We believe that TGFβ1 induces active demethylation of the promoter through the recruitment of thymine DNA glycosylase enzyme (TDG), which is involved in base excision repair. We have also observed that HPV can repress TGFβ1-mediated upregulation of IFNκ through a passive mechanism that likely requires cellular replication.
Conclusion: This novel interaction between growth factor and innate immune signaling may shed light on the mechanisms of HPV persistence.

S.16. The Human Cytomegalovirus Tropism Modulator UL148 Induces Endoplasmic Reticulum Quality Control Compartments during Infection

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Background: Human cytomegalovirus (HCMV) infection induces endoplasmic reticulum (ER) stress and has been reported to activate and regulate the unfolded protein response (UPR). During over-expression of misfolded glycoproteins, a subset of cellular factors involved in glycoprotein folding and in ER associated degradation (ERAD) have been described to coalesce at ER quality control (ERQC) compartments, which are thought to play roles in adaptation to ER stress. We previously identified a viral ER resident protein, UL148, that contributes to HCMV cell tropism by modulating the expression of alternative viral glycoprotein complexes involved in cell entry. Here, we demonstrate that UL148 is required for the formation of prominent ERQC compartments during HCMV infection, and furthermore, that its expression is sufficient to induce the ERQC structures in non-infected cells.
Methods: Human foreskin fibroblasts (HFF) were infected with wild-type (WT) strains TB40/E or TR (e.g. TB_WT, TR_WT), *UL148*-null mutant derivatives of the same viruses (e.g, TB_148$_{STOP}$), strain TB40/E harboring an HA tag on UL148 (TB_148^{HA}), or expressing an HA-tagged version of rhesus cytomegalovirus homolog of UL148, Rh159, instead of UL148 (TB_159^{HA}). Confocal microscopy was used to image the ER markers calnexin and Hrd1 that participate in ERQCs, other ER markers (PDI and BiP/Grp78), the HCMV glycoprotein H (gH), and HA-tagged UL148 or Rh159. ARPE-19 epithelial cells that ectopically express of UL148^{HA} and Rh159^{HA} upon doxycycline treatment were similarly imaged by confocal microscopy. Expression of ATF4, total and phosphorylated eIF2α, HA-tagged proteins, and loading controls were monitored by western blot. Splicing of *Xbp-1* mRNA was assayed by RT-PCR. siRNA pools targeting cellular proteins involved in ER stress signaling (PERK, ATF4, ATF6, IRE1) were used in studies to interrogate the signaling requirements for ERQC formation.
Results: We observed that the ER marker calnexin formed prominent structures resembling ERQC compartments during WT but not *UL148*-null mutant HCMV infection. The ERQC markers calnexin and Hrd1 co-localized with UL148 at oblong, semi-globular structures distinct from the cytoplasmic viral assembly compartment, while BiP and PDI did not. Ectopic expression of UL148 was sufficient to induce ERQC structures that resembled those seen during infection, and led to splicing of Xbp-1, phosphorylation of eIF2α, and ATF4 expression, all of which are consistent with UPR induction. Results from siRNA studies suggest that ERQCs induced by UL148 require the ER stress sensor kinase PERK. Taken together, our results demonstrate that UL148 is necessary to induce ERQC compartments during HCMV infection, and sufficient to induce these structures in non-infected cells. Our findings may have implications for understanding the mechanisms by which UL148 influences HCMV cell tropism. In particular, we hypothesize that the induction of ERQC compartments is required for the effects of UL148 on HCMV cell tropism.

Pathogenic Microbiology - Selected Abstracts

S.17. The Stress-Response Protein *DDR48* is Required for Adequate Cellular Stress Response in the Pathogenic, Dimorphic, Fungus *Histoplasma capsulatum*

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Histoplasma capsulatum (Hc) is a systemic, dimorphic, fungal pathogen. *Hc* grows as a multicellular mold at environmental temperatures (25°C) whereas, upon inhalation into a human or other mammalian host (37°C), it transforms into a unicellular, pathogenic yeast. Our research aims to elucidate the numerous stress response pathways (e.g., oxidative stress and DNA damage) that *Hc* utilizes to survive in the ever-changing environment. Specifically, we are characterizing the DNA damage-responsive protein *DDR48*, an *Hc* homolog sharing sequence similarity to *C. albicans DDR48p*. Previously in our lab an allelic replacement deletion-mutant was generated (*ddr48*Δ) to elucidate the function of Hc*DDR48*. Upon analysis, we found that *DDR48* is required for resistance to numerous cellular stressors such as oxidative stress, DNA damage, heat shock, and antifungal drugs. RNAseq analysis of the deletion mutant showed that a majority of down-regulated genes are involved in cell signaling of the various cellular stress response pathways. Interestingly, many genes that were up-regulated in the deletion mutant are involved in cysteine/sulfur metabolism as well as carbohydrate and nitrogen metabolism. Research is ongoing to unveil the intricate role *DDR48* is playing in sensing and responding to cellular stress.

S.18. The Role of Phosphodiesterase PdeD in Regulating Intracellular Concentrations of Cyclic di-GMP under Anoxic Conditions in *Listeria monocytogenes*

Sally J. White[1], Daniel M. McClung[2], Hossam Abdelhamed[2], and Janet R. Donaldson[1], *[1]The University of Southern Mississippi, [2]Mississippi State University*

Background: The ability of the foodborne pathogen *Listeria monocytogenes* to survive exposure to biological stressors encountered throughout the gastrointestinal tract is critical to disease progression. Previous work from our laboratory has shown anaerobic conditions increase survival of *L. monocytogenes.* Therefore, the hypothesis of this study is that oxygen availability impacts the intracellular regulation of cyclic di-GMP, which results in altered regulation of virulence factors.
Methods: To examine the regulation of c-di-GMP, the phosphodiesterase *pdeD* gene was deleted from the *L. monocytogenes* 4b strain F2365 and confirmed through gene sequencing. The effects of the mutation on survival, cyclic di-GMP levels, NADH:NAD+, cellular invasion potential, and gene expression were examined following exposure to bile and acidic conditions under aerobic or anaerobic environments.
Results: The F2365Δ*pdeD* showed higher survival in comparison to the wild-type F2365 strain following bile exposure and under acidic, anaerobic conditions. NADH:NAD+ ratios remained constant for F2365 across the tested conditions, while F2365Δ*pdeD* ratios following exposure to anoxic conditions increased in comparison to aerobic conditions. The data also indicate that F2365 invaded similarly between both aerobic and anaerobic conditions, but the F2365Δ*pdeD* mutant displayed increased invasion under aerobic conditions. Gene expression of various putative oxygen sensors was increased under anaerobic conditions in *L. monocytogenes* strains.
Conclusion: Together, these data suggest that *L. monocytogenes* utilizes phosphodiesterases such as PdeD to regulate intracellular cyclic di-GMP concentrations to influence many cellular functions, including those related to pathogenicity. Variations in growth, invasion potential, and gene expression between aerobic and anaerobic conditions suggest a connection to oxygen availability. This could indicate that PdeD, as part of a two-component system responding to an oxygen sensing histidine kinase, could influence the progression of disease.

S.19. *msaABCR* Operon Regulates Antibiotic Resistance Maintaining Cell Wall Integrity in *Staphylococcus aureus*

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Background: *Staphylococcus aureus* is an important human pathogen in both community and health care settings. One of the key problems with *S. aureus* as a pathogen is the acquisition of antibiotic resistance. Previously, we have shown that deletion of the *msaABCR* operon effects cell wall thickness and vancomycin resistance in *S. aureus*. In this study, we investigated the nature of the cell wall defect in the *msaABCR* operon mutant as a first step to define the role of the operon in cell wall structure integrity.
Methods: We performed crude peptidoglycan hydrolysis with lysozyme and whole cell autolysis assay with lysozyme and lysostaphin. Also, we performed HPLC analysis of muropeptides obtained from muramidase hydrolysis of peptidoglycan from the *msaABCR* mutant, *msaABCR*/protease double-deletion mutant and wild type *S. aureus*. Zymographic analyses of the cell-wall-bound fractions of murein hydrolases were performed for these strains. We also estimated teichoic acid content in these strains.
Results: We found that the *msaABCR* mutant cells and its respective purified PG are more susceptible to lysozyme enzyme compared to wild type strains. However, the *msaABCR* mutant cells were more tolerant to lysostaphin treatment. Based on HPLC analysis, the *msaABCR* mutant has reduction in the proportion of oligomeric muropeptides that eluted from the HPLC column with retention times longer than 100 min compared to wild type *S. aureus*. Moreover, Increased processing of the major autolysin Atl and other murein hydrolases

were observed in the *msaABCR* mutants. Interestingly, these phenotypes were reversed back to wild type in *msaABCR*/protease double-deletion mutant. Also, *msaABCR* mutant cells have less amount of cell wall teichoic acid compared to wild type.
Conclusion: In this study, we showed that *msaABCR* mutant cells had increased *extracellular proteases* activity leading to increased processing of murein hydrolases/autolysis causing defective crosslinking in cell wall promoting cell wall lysis, which was further favored by presence of less teichoic acid content in the cell wall. We will further characterize mechanism of regulation of cell wall biosynthesis and other cell envelope related stresses by *msaABCR* operon.

S.20. Bacterial Lipases Differentially Affect the Pore-Formation Activity of Cholesterol-Dependent Cytolysins

Patrick S. Gellings and David J. McGee, PhD, *Louisiana State University Health Sciences Center*

Background: Cholesterol-dependent cytolysins (CDC) constitute a family of pore-forming toxins predominately produced by Gram-positive bacteria with a reliance on membrane cholesterol to mediate pore-formation. The exposed hydroxyl group of cholesterol serves as the receptor site for CDCs while having the potential to interact with neighboring phospholipids such as phosphatidylcholine and sphingomyelin. The interaction between cholesterol and these phospholipids effectively creates an umbrella-like covering over cholesterol and occludes the ability of CDCs to interact with cholesterol. Removal of the extracellular portion of phosphatidylcholine or sphingomyelin displaces the phospholipid-cholesterol interaction and increases the membrane pool of free-cholesterol. Analysis of the genomes of CDC-producing bacteria suggests these bacteria also produce and secrete a variety of phospholipases that utilize either sphingomyelin or phosphatidylcholine as substrates. We hypothesized that CDC-producing bacteria use phospholipases to remove the head groups of membrane-bound sphingomyelin and phosphatidylcholine to increase the pool of accessible cholesterol within a host membrane, leading to enhanced CDC pore-formation.
Methods: Using a 6-His purification system, we purified sphingomyelin-specific phospholipases from *Arcanobacterium haemolyticum* and *Bacillus anthracis* (PLD and SPH, respectively), the phosphatidylcholine-specific phospholipase from *Bacillus anthracis* (PC-PLC) and arcanolysin (ALN), a CDC also produced by *A. haemolyticum*. The purified proteins were used in a variety of hemolysis assays to measure the impact various lipase activities have on CDC pore-formation in human erythrocyte membranes.
Results: Treating human erythrocytes with sphingomyelin-specific phospholipases subsequently increases the hemolytic activity of ALN in both time- and concentration- dependent manners. In addition, sphingomyelinase-deficient PLD loses its ability to promote ALN-mediated hemolysis in any capacity. Contrary to our initial hypothesis, treating erythrocytes with purified PC-PLC decreased the hemolytic activity of ALN.
Conclusion: Altering the lipid profile of eukaryotic membranes via bacterial lipases affects the pore-forming activity of CDCs. Specifically, the elimination of sphingomyelin increases pore-formation while the elimination of phosphatidylcholine decreases pore-formation activity. Previous studies reported that cholesterol has a higher affinity for sphingomyelin than phosphatidylcholine. Based on our data, we hypothesize decreasing the population of phosphatidylcholine by PC-PLC increases the pool of free cholesterol until membrane sphingomyelin interacts with and more securely sequesters the recently free cholesterol. Shifting the population of sequestered cholesterol away from phosphatidylcholine-cholesterol interactions to sphingomyelin-cholesterol interactions would decrease the population of CDC-accessible cholesterol and decrease CDC-mediated hemolysis. Future studies will continue to investigate the complex relationship among bacterial phospholipases, CDCs, and host cell membranes and how this relates to host-pathogen interactions.

S.21. Characterization of Type VI Secretion System in *Edwardsiella ictaluri*

Safak Kalindamar, Hossam Abdelhamed, Adef O. Kordon, JingJun Lu, Salih Kumru, Hasan C. Tekedar, Lesya M. Pinchuk, and Attila Karsi, *Mississippi State University*

Background: *Edwardsiella ictaluri* is a Gram-negative rod in *Hafniaceae*, and it has been an important pathogen of farm-raised catfish. *E. ictaluri* deploys Type III and Type VI Secretion Systems (T3SS and T6SS) for survival in the host environment. Although the role of T3SS in *E. ictaluri* pathogenesis has been studied, T6SS have not been explored completely.
Methods: T6SS mutants were constructed, and their roles in *E. ictaluri* pathogenesis were evaluated. First, we also tested adhesion and invasion of the mutants in catfish epithelial cells. Second, survival and replication of the mutants were determined in catfish peritoneal macrophages. Third, adaptation and survival of the mutants under stress were explored. Finally, persistence and virulence of the mutants in catfish fingerlings and fry were evaluated.
Results: Deletion of the T6SS genes caused lower cell attachment and invasion, decreased replication within macrophages, sensitivity to stress, and attenuated virulence.
Conclusion: Our data suggest that T6SS is essential for *E. ictaluri* survival, invasion, and persistence in catfish.

S.22. *msaABCR* Operon is Involved in the Oxidative Response of *Staphylococcus aureus*

Shanti Pandey, Gyan S. Sahukhal, and Mohamed O. Elasri, *The University of Southern Mississippi*

Background: *Staphylococcus aureus* is a notorious human pathogen capable of causing acute to chronic and recurrent infections. *S. aureus* has a complex regulatory network that controls multitude of defense mechanisms to withstand the deleterious effects of the oxidative stress inside host immune cells leading to persister formation and recalcitrant infections. We characterized *msaABCR* operon that

regulates virulence, biofilm development and antibiotic resistance and tolerance. Transcriptome of the operon deletion mutant shows downregulation of more than 10 genes involved in oxidative stress that led us to hypothesize that the operon play role in defending oxidative stress and intracellular persister formation.
Method: Stationary phase cells were prepared in Tryptic Soy Broth (TSB), pelleted, washed with phosphate buffer saline and diluted to OD_{600}0.1 in 5 mL TSB. 25mM hydrogen peroxide (H_2O_2) was added to each tubes and growth was monitored for 24 hours.
Results: Growth of *ΔmsaABCR* was abolished by 25 mM H_2O_2 while wild type and complementation strains could grow as comparable to the unstressed cells. Chromatin Immunoprecipitation (ChIP) assay revealed that MsaB protein directly binds the promoter region of OsmC/Ohr family protein (SAUSA300_0786) that is involved in the oxidative stress. Furthermore, significantly downregulated transcript of SAUSA300_0786 in *ΔmsaABCR* suggests MsaB as an activator of this protein.
Conclusion: These results suggest that *msaABCR* operon is involved in oxidative-stress-defense mechanism possibly via regulation of OsmC/Ohr family protein facilitating intracellular persister formation and recurrent infections. Further, we plan *in-vivo* study for understanding this mechanism underlying intracellular persister development and consequently overcome treatment failures of staphylococcal infections.

S.23. Relative Role of *Staphylococcus aureus* SarA and SaeRS Regulators in the Pathogenesis of Osteomyelitis

Aura Ramirez, Karen Beenken, Daniel Meeker, Weston Mills, Christopher Walker, and Mark Smeltzer, *University of Arkansas for Medical Sciences*

Background: *Staphylococcus aureus* is the most common cause of osteomyelitis. Mutation of the *saeRS* two-component regulator system or the staphylococcal accessory regulator *sarA* has been shown to attenuate the virulence of *S. aureus* in a murine osteomyelitis model, and in the case of *saeRS* this has been attributed to the increased production of extracellular proteases. Here we confirm that mutation of *sarA* results in an even greater increase in the production of these proteases than mutation of *saeRS* and that this increase is directly reflected in several potential critical phenotypes
Methods: We used the wild-type LAC strain along with 5 mutants derived from it differing in their *sarA* and *saeRS* functional status. We measured the effect of these combined genotypes on accumulation of extracellular proteins, biofilm formation, cytotoxicity for osteoblasts and osteoclasts, and in a murine osteomyelitis model.
Results: The observed increased production of extracellular proteases is directly reflected in several potential critical phenotypes including the reduced accumulation of extracellular proteins, reduced biofilm formation, and reduced cytotoxicity for both osteoblasts and osteoclasts. The exception was internalization by osteoblasts, which was essentially abolished in a *saeRS* mutant but largely unaffected in a *sarA* mutant. Most importantly, our preliminary experiments strongly suggest that mutation of *saeRS* limits virulence in a murine osteomyelitis model to a much greater extent than mutation of *sarA.*
Conclusion: These results suggest that factors other than the increased production of extracellular proteases play a predominant role in defining the pathogenesis of *S. aureus* in osteomyelitis and that the production of these factors is primarily controlled by the activity of *saeRS*. They also suggest that the inability to invade osteoblasts may be of particular importance in this regard.

S.24. Role of Mycolactone in Suppression of *Staphylococcus aureus* Virulence Genes

Laxmi Dhungel and Heather Jordan, *Mississippi State University*

Background: Buruli ulcer disease (BUD) is a necrotizing skin disease caused by *Mycobacterium ulcerans* (MU). The disease is referred to as a 'mysterious disease' due to its unknown mode of transmission, ecology and pathology. BUD has been reported in over 33 countries worldwide and has highest prevalence in West Africa. The major virulence factor of MU is mycolactone, a lipid toxin which is encoded by a plasmid pMUM001. Mycolactone has been found to suppress immune cells, which is responsible for BUD painless characteristics. Additionally, pathogens such as *Staphylococcus aureus*, *S. epidermidis* and *Pseudomonas aeruginosa* have been isolated from BU ulcers, but without typical pathology associated with those pathogen's colonization. This raises the question whether mycolactone plays any role to provide fitness advantage to MU in natural environments and during skin infection.
Hypothesis: The central hypothesis of our research is that mycolactone attenuates the quorum sensing and virulence genes of other bacteria to outcompete those bacteria in natural environment and during skin infections.
Method: To test our hypothesis, *S. aureus* was cultured with mycolactone or ethanol (control) and incubated at 37^0 C. Following this, growth and hemolytic activity and gene expression of *S. aureus* global regulators *saer* and *agr* and virulence gene *hla* were measured after 6 hours and 22 hours of incubation.
Results: Results showed that there was reduction trend in hemolytic activity of *S. aureus* with mycolactone at both timepoints compared to control, however, it was statistically not significant. The *saer* gene was found to be significantly downregulated at 22 hours.
Conclusion: Results suggest that mycolactone suppresses *S. aureus* virulence, while not inhibiting growth. Though more work is underway, these findings aid to understand the role of mycolactone in providing fitness to MU in polymicrobial environments and also aid in determining possible treatment outcomes of BU following antibiotic treatment.

Poster Session Abstracts

P.1. Pangenome Analysis Methodology Comparison Using *Salmonella*

Kaleb Abram, Visanu Wanchai, Se-Ran Jun, Intawat Nookaew, and David Ussery, *University of Arkansas for Medical Sciences*

Background: Next generation sequencing (NGS) has drastically lowered the cost of whole genome sequencing (WGS). As the cost dropped, the number of genomes in public databases skyrocketed. These numbers continue growing every day, increasing the ability and need to do large pangenome analyses. Pangenome analyses allow us to better understand what makes a certain bacterium that bacterium. Three different pangenome analysis methods were compared on 4,367 genomes of Salmonella. Around 2,000 *Salmonella enterica subsp. enterica serovar Typhi* and 500 *Salmonella enterica subsp. enterica serovar Typhimurium* genomes were included in the analysis.
Methods: All genomes were downloaded from NCBI originally and analyzed with a variety of tools to create a private database. Gene predictions were obtained using Prodigal and the resulting .faa files were used to carry out three different pangenome analysis methods: a k-mer based method (FindMyFriends), an alignment based method (USEARCH), and a Pfam based method. Once protein families were obtained from the pangenome analyses, the proteins in *S. Typhimurium* str. LT2 were extracted from these families.
Results: All three methods produced somewhat similar results. Both USEARCH and FindMyFriends had similar results with a core of around 3,400 protein families. The Pfam core was the most conservative with around 3,000 protein families. These Pfam domains mapped to around 5,000 proteins in the type strain including around 2,000 proteins that correspond to Pfam domains conserved across all of bacteria.
Conclusion: Each of these methods have their own strengths and weaknesses but produce similar results. A combination of these comparative methods should be used to produce a more complete view of the biology of an organism and to minimize any bias that may be introduced.

P.2. Impact of Impaired Polyamine Biosynthesis on *Streptococcus pneumoniae* TIGR4 Gene Expression

Moses B. Ayoola[1], Mark A Arick II [2], Leslie A. Shack[1], Edwin Swiatlo[3], and Bindu Nanduri[1], *[1]Mississippi State University, [2]Southeast Louisiana Veterans Health Care System*

Background: *Streptococcus pneumoniae (Spn, pneumococcus)* are gram-positive, encapsulated bacteria that asymptomatically inhabit human nasopharynx, and can cause invasive infections such as pneumonia, septicemia, otitis media and meningitis. High morbidity and mortality rates due to pneumococcal infections and increasing prevalence of antibiotic resistance necessitate the development of novel treatment and prevention strategies. Polyamines are ubiquitous, aliphatic hydrocarbons that are positively charged at physiological pH and can bind to RNA and proteins and regulate a number of cellular processes. Previous work from our laboratory has shown that deletion of spermidine synthase (speE, polyamine biosynthesis) in *Spn* results in an attenuated phenotype in a murine model of pneumococcal pneumonia. In this study, we utilized RNA-Seq to identify pneumococcal gene expression that is dependent on intact spermidine synthesis. We also determined the impact of impaired spermidine synthesis on capsular polysaccharide, a key *Spn* virulent factor.
Methods: *S. pneumoniae* TIGR4 and Δ*speE* were cultured in Todd Hewitt Yeast extract medium and total RNA and total capsular polysaccharide were isolated from bacteria collected from mid-log phase of growth (n=4). Illumina's KAPA Stranded RNA-Seq kit was used for library preparation and sequencing with HiSeq 3000. Read alignment, aligned reads/gene count and significant changes in gene expression were performed using Bowtie2, HT Seq-count and edgeR respectively. Immunoblots using capsular polysaccharide serotype 4 specific antibodies were performed to measure capsule in wild type and Δ*speE*.
Results: RNA-Seq analysis identified 412 upregulated and 434 downregulated genes in Δ*speE* compared to wild type. We did not see significant changes in genes encoded by the capsule biosynthesis operon. This was confirmed by immunoblot assays that did not detect any difference in the total capsule between TIGR4 and Δ*speE*. Expression of *prtA*, a gene coding for serine protease, an important enzyme capable of killing *Spn* is upregulated 8-fold in Δ*speE* compared to TIGR4. Two component regulatory system (TCS) 12 and 13 that have been linked to competence and virulence of *Spn* were downregulated in the Δ*speE*. Taken together, these results could explain capsule independent attenuation mechanisms in Δ*speE*.
Conclusion: Our results indicate that polyamine mediated attenuation of pneumococcal virulence includes capsule independent pathways.

P.3. Biological Phosphorous Removal from Impacted Water

Carina M. Jung[1], G. Alon Blakeney, Matt Carr[1], and Jed O. Eberly[2], *[1]US Army Corps of Engineers, Engineer Research and Development Center, [2]Montana State University*

Background: Nutrient input into waterways from agriculture runoff, sewage, and other sources is a major cause of water pollution and cultural eutrophication. Nutrients such as nitrogen and phosphate are growth-limiting substrates that when present in high concentrations allow for unchecked growth of cyanobacteria, resulting in reduced water quality and the potential for proliferation of harmful algal blooms

(HABs). Physical removal of these nutrients from waterways is needed. Immobilized phosphorous accumulating bacteria could be used as a phosphate biofilter that could, absorb phosphate and then be collected and removed from the impacted water.
Methods: The research presented herein demonstrates the immobilization or encapsulation of phosphate accumulating bacteria in a silica sol-gel matrix, which is encased in a porous, rigid container that can be suspended in a high phosphate water system and retrieved at predetermined times to collect excess phosphate.
Results: Growth and PO_4 uptake was monitored in free-living, pure cultures of the phosphate hyperaccumulator *Microlunatus phosphovorus*, which accumulated phosphate at a rate of 3.3 ppm PO_4 /h (1.11 mmol P/h). Sol gel immobilized cells were able to sequester phosphate at a rate of 80 mmol P/g cell dry wt/h and were found to be metabolically active even after 53 days of encapsulation.
Conclusions: Application of immobilized cells to phosphate impacted waters for uptake and removal of phosphate would allow for large-scale removal of phosphate, thus reducing the eutrophication effect and limiting the potential for HABs.

P.4. Leveraging Nature's Horizontal Gene Transfer (HGT) Pathways to Enhance *in situ* Bioremediation Potential

Carina M. Jung, G. Alon Blakeney, and Karl J. Indest, *US Army Corps of Engineers, Engineer Research and Development Center*

Background: Horizontal gene transfer (HGT) is the lateral movement of genetic material between bacteria, which contributes to genome plasticity and allows for rapid adaptation. Many HGT studies are based on *in silico* deductions or are highly focused on dissemination of antimicrobial resistance and pathogenicity factors with limited, and often conflicting HGT studies in the environmental arena. The Environmental Microbiology Team at ERDC has demonstrated *in vitro* HGT of the pGKT2 plasmid of *Gordonia* sp. KRT9 which contains *xplAB* responsible for degradation of the explosive RDX.
Methods: Plasmid and chromosomal genes involved in maintenance of the RDX-degrading plasmid, pGKT2 were identified by transcriptome studies via microarray analysis with wild-type and transconjugants. Phenotypic growth and survivability comparisons were performed in minimal medium and rich medium and in the presence of stressors such as starvation and desiccation to assess overall bacterial fitness costs of maintaining this plasmid. Biogeochemical parameters that stimulate HGT of pGKT2 were identified through various growth conditions in soil slurry batch mating experiments.
Results: Overall, there was a fitness cost to transconjugants harboring the transferred plasmid. However, in starved conditions the added metabolic capabilities conferred by the plasmid may have conferred an advantage to transconjugants. DNA replication and modification enzymes were upregulated while signal transduction, transport, fatty acid and nitrogen metabolism were downregulated in transconjugants.
Conclusions: There is a large knowledge gap between knowing that HGT occurs in environmental bacteria and being able to predict or manipulate HGT events. Understanding the fundamental contributions of the biotic and abiotic factors governing HGT would benefit current and ongoing bioaugmentation efforts where beneficial bacteria with transferrable elements have been reintroduced into contaminated settings.

P.5. Seasonal and Nutrient Drivers on Contaminant Degradation in Alaskan Soils

Matthew Carr, Gary A. Blakeney, Carina Jung, and Fiona Crocker, *U.S. Army Engineer Research and Development Center*

Background: Management of recalcitrant military contaminants like energetics, heavy metals, and PCBs in Arctic/subarctic soils has been limited due to a lack of knowledge of the fate and transport of these contaminants in cold climates and soils. Effects of freeze/thaw on soil structure, nutrient availability, and microbial activity, as well as the linkages of these processes to nutrient cycling and contaminant biodegradation are poorly understood. The objective of this study is to quantify explosive biodegradation rates as a function of soil geochemistry, environmental fluctuation, and nutrient amendments in subarctic soils.
Methods: Two silty subarctic soils, one from a discontinuous permafrost site with no prior history of exposure to explosives and the other soil from an active training range firing range site without permafrost were used in this study. Microcosms were used to investigate the influence of temperature, moisture, and nutrient amendment on microbial activity in Alaskan soils. Since the costs of transporting equipment and supplies to remote Arctic regions can be expensive, a locally abundant and inexpensive waste product, crab shells, was used as the nutrient amendment. Microbial activity was measured as basal respiration, potential nitrification, and explosive biodegradation rates.
Results: In general, hexahydro-1,3,5-trinitro-1,3,5-triazine (RDX) was poorly biodegraded in the two soils at all temperatures. In contrast, the rates of biodegradation for 2,4-dinitrotoluene (DNT) were significantly higher. Amendment of the soils with chitin increased biodegradation and basal respiration rates at 22°C and 12°C, but not at 2°C.
Conclusion: There is a large knowledge gap concerning biogeochemical factors that affect microbial community composition and function in Arctic and subarctic soils, especially with respect to military relevant contaminants. An understanding of the biogeochemical factors that contribute to contaminant biodegradation would reduce uncertainty in risk assessment and management of military training ranges in these cold climates.

P.6. Exploring the Capacity of Fungi and Algae to Form Mutualisms

Michael Clear, Erik Hom, *University of Mississippi*

Background: Mutualisms are ubiquitous throughout the natural world and can shape the evolutionary trajectory of their communities. In this study, we probed for potential mutualisms between endophytic fungi isolated from the natural environment and the model algae, *Chlamydomonas reinhardtii* and *Chlorella vulgaris*. Our aim was to validate our screening methods and provide an initial dataset analyze for phylogenetic patterns of mutualism formation.
Methods: Mutualism formation was determined by comparing the cumulative biomass of monocultures to co-culture biomass. Additionally, we measured the resultant pH and nitrate concentrations in the culture medium and imaged cultures to screen for physical interactions.
Results: There are no clear phylogenetic patterns of mutualism formation across different fungal families. However, there are significant differences in mutualistic tendencies between different fungal-algal pairings.
Conclusion: Preliminary data show significant differences in growth between different fungal-algal pairings and low variability between replicates. These results confirm the utility of our mutualism screening assay as a first-pass screening of potential mutualisms between fungi and algae. We will continue to add to our dataset in order to search for potential patterns of mutualism formation.

P.7. Ribosomal Proteins for Better Resolution in Microbial Taxonomy

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Background: 16S ribosomal RNA (rRNA) is the known standard for taxonomic classifications within the Microbial community. A lot of times the reeds are too short to obtain adequate full length16S sequences. However, even when the full 16S sequences are present and used to build microbial trees they only showing species level information. They are still unable to differentiate multiple genomes within the same species, like pathogenic Escherichia coli O157:H7 and Shigella or a commensal strains of E. coli and a probiotic strain. Furthermore, with the level of information provided by the standers method of 16S sequencing how can one correctly classify rapidly growing organisms, like vibrio, which have multiple divergent copies of their 16S rRNA. Where these genomes end up being classified is solely dependent on which one of the many different 16S sequence is chosen.
Methods: Bacterial genomes were downloaded from GenBank, and initially the GenBank annotations were used to extract the ribosomal proteins. In parallel, for the same genomes, PfamA domains corresponding to the ribosomal proteins were used to automatically extract the ribosomal proteins for each genome. These were carefully compared, and determined to yield the same results. We then used the automated approach for all subsequent work. For each genome, a unique set of ribosomal proteins was extracted and used to build the phylogenetic tree, based on alignment, using ClustalW for alignments.
Results: More than 10,000 bacterial genomes in the NCBI database do not have full length 16S rRNA sequences; this is due to the resolution obtained from second generation sequence reads. On average each bacterial genome contains a full complement of about 50 ribosomal protein sequences. We genomes from bacterial type strains for comparison of genomes from closely related species; we find agreement with known taxonomy, and further, we can get strain level separation using ribosomal proteins.
Conclusion: We find that ribosomal proteins can be used to build phylogenetic trees for bacterial genomes; using high throughput computing this can be extended to more than a hundred thousand genomes currently available. Ribosomal protein trees can give strain-level resolution for bacterial genomes within the same species.

P.8. An Examination of the Effluent Quality of Aerobic Treatment Unit Wastewater

Sarah Deeb, Karoline Macias, Tamylles Souza da Costa, Zahaan Eswani, and Christopher G. Struchtemeyer, *McNeese State University*

Background: Aerobic treatment units (ATUs) are commonly used to treat wastewater in rural areas. In many cases, the effluent from ATUs does not undergo chemical disinfection and is discharged into ditches and ravines that empty directly into major water bodies. In spite of their widespread use, very little is known about the effectiveness of ATUs. The goal of this work was to examine the microbial quality of ATUs by quantifying numbers of *Escherichia coli*, fecal coliform bacteria, total bacteria, and antibiotic resistant bacteria in effluent samples that were collected in southwest Louisiana.
Methods: The concentrations of *E. coli*, fecal coliform bacteria, total bacteria, and amoxicillin resistant bacteria were monitored in effluent samples from 30 ATUs in Lake Charles, LA. *E. coli* and fecal coliforms were quantified in triplicate using pour plate assays, which were conducted with CHROMagar ECC medium. Total and amoxicillin resistant bacteria were quantified using three-tube most probable number dilutions, which were performed in half strength tryptic soy broth (TSB). The TSB that was used to quantify amoxicillin resistant bacteria was also amended with 32 mg/L amoxicillin.
Results: The numbers of *E. coli*, fecal coliforms, total bacteria, and amoxicillin resistant bacteria in the ATU effluent samples that were studied ranged from 0-7.3 x 10^5 CFU/ml, 1.1 x 10^3-1.4 x 10^6 CFU/ml, 1.1 x 10^3-1.1 x 10^7 MPN/ml, and 4.6 x 10^4-5.5 x 10^6 MPN/ml, respectively.
Conclusions: This study shows that ATUs release large numbers of bacteria into the environment. In most cases, the ATUs released concentrations of *E. coli* and fecal coliform bacteria that exceeded federal and state regulations. The detection of antibiotic resistant

bacteria in ATUs is significant since these microbes have never been quantified in ATU effluent. The results of this work also show that additional disinfection steps are needed to ensure the proper function of ATUs.

P.9. Monitoring the Fate of Microbial Contaminants from Aerobic Septic Systems

Zahaan Eswani, Tamylles Souza da Costa, Sarah Deeb, Navdeep Thind, Robert Rutz, Tallen Cavenah, Andre Davis, and Christopher G. Struchtemeyer, *McNeese State University*

Background: Aerobic septic systems are used to treat wastewater in rural areas of the United States. Many of these systems discharge effluent directly into ditches and ravines, which rely on sunlight for disinfection purposes. In spite of this practice, very few studies have examined whether this effluent is adequately disinfected by sunlight or if it negatively impacts the water quality of ditches and ravines that serve as discharge points. The goal of this work was to assess the environmental impacts of aerobic septic system effluent by: 1) Determining if aerobic sewer system effluent was disinfected by sunlight exposure and 2) Monitoring the microbial quality of water from ditches and ravines where aerobic sewer system effluent was discharged.
Methods: The disinfection capabilities of sunlight were evaluated by comparing the concentrations of heterotrophs, fecal coliforms, and *E. coli* in aerobic sewer system effluent samples that were incubated either in the presence or absence of sunlight. The microbial quality of water from several ditches and ravines that served as discharge points for aerobic sewer system effluent was also assessed by measuring concentrations of heterotrophs, fecal coliforms, and *E. coli*. Heterotrophs were enumerated in this study using three-tube most probable number dilutions, which were performed in half strength tryptic soy broth (TSB) that was incubated at 37°C. Concentrations of *E. coli* and fecal coliforms were quantified in triplicate during this study using pour plate assays, which were performed with CHROMagar ECC medium that was incubated at 44°C.
Results: Sunlight did not appear to disinfect any of the aerobic septic system effluent samples that were collected in this study. This observation was further supported by the fact that all of the ravine and ditch samples that were monitored in this study contained high concentrations of heterotrophs (2.1×10^4-1.1×10^7 MPN/ml), fecal coliforms (1.3×10^2-greater than 15,000 CFU/ml), and in some cases *E. coli* (0-8.35×10^2 CFU/ml).
Conclusions: This study shows that aerobic septic systems release large numbers of bacteria, which will likely impact the environment. The results of this work also show that additional disinfection steps are needed to ensure the proper function of these systems.

P.10. Necrobiome Changes in a Secondary Wild Boar Mortality Event in the Absence of a Soil Microbiome

Lindsay Harrison and Heather Jordan, *Mississippi State University*

Background: The size and number of mass mortality events across the globe being reported are increasing as climate change continues to create more severe weather events. The impact of these closely spaced mortality events creates unknown ecological consequences. Changes in the necrobiome (i.e. microbes associated with decomposing remains) of mass mortality events and the associated soil communities may impact the rate of decomposition in future mortality events. As part of a comprehensive study investigating the effects of large nutrient pulses caused by mass mortality events, we showed that microbial diversity in a soil-free system decreases over time, and becomes more homogenous between skin and internal communities and is not impacted by the size of an initial mass mortality event.
Methods: Samples were collected at three timepoints from the skin and internal cavities of six wild boar carcasses placed inside individual plastic bins. Each bin was left open and positioned beside one of six initial mass mortality events with biomasses of 25, 60, or 725 lbs of wild boar carcass in the active stage of decomposition. DNA was extracted, 16s rRNA sequenced, and microbial diversity analyzed by swab sampling location (skin versus internal), timepoint, and initial biomass.
Results: An initial difference in microbial diversity between skin and internal communities was observed, but was not distinguishable after three hours or at four days. After four days, microbial diversity had significantly decreased for all sample types when compared to the initial and three hour timepoints. A difference in overall microbial diversity was not observed between pigs placed at different initial mass mortality biomasses.
Conclusion: In this study we observed an initial difference in microbial communities of the skin and internal cavity that decreased to an insignificant level after only three hours, and whose community persisted after four days. Overall microbial diversity decreased after four days, most likely due to development and out-competition by a few predominant species. Transfer of microbes from the existing mortality event did not seem to have an impact on microbial diversity between the three different biomass treatments, and show decomposition driven by the host microbial community. These results underscore the importance of determining the overall effects of microbial communities to decomposition of mass mortality events and facilitate the need for further investigation.

P.11. A Preliminary Investigation into the Antibacterial Properties of Venom from the Wolf Spider, *Rabidosa rabida*

Brandon Hogland, Amber Hug, Nicole Lacina, and Ryan Stork, *Harding University*

Background: The global increase in antibiotic resistance has prompted the World Health Organization (WHO) to encourage scientists to discover new antibacterial agents. In addition, the World Health Organization has identified 12 priority pathogens in desperate need of novel antibiotics. Historically, antibiotics have been identified in both fungi, arthropods and even other bacteria. From previous studies, spider venom proteins have shown antimicrobial activity, yet the antibacterial activity of *Rabidosa rabidosa* venom and digestive fluids has

not been explored. In our study, we compared the antibacterial activity of spider venom and digestive fluids against a panel of 12 bacterial isolates.
Methods: We collected and extracted venom and digestive fluid from 300 spiders over a two-week period. The venom/digestive fluid mixture was stored at -20°C. The fluids were filtered using a 0.2-micron filter to remove any contaminants picked up during the extraction process. Once enough fluid was collected, we made two-fold dilutions. To test our dilutions, we grew bacteria overnight to achieve log phase growth. Bacteria were spread out over LB plates. Five microliters of each of the venom/digestive fluid dilutions were then added to the plates and zones of inhibition were measured for each organism. A panel of currently used antibiotics was performed using the Kirby-Bauer plate method to show susceptibility patterns of the organisms tested. Zones of inhibition were measured and compared to standard charts.
Results: Only *Klebsiella pneumoniae* exhibited susceptibility against whole venom/digestive fluid and the first dilution containing one half the original venom/digestive fluid mixture and LB broth. The other organisms tested exhibited resistance to the whole venom/digestive fluid and subsequent dilutions.
Conclusion: Proteins from venom and or digestive fluid from *R. rabida* may be a novel antibiotic against *K. knuemoniae and* other *Klebsiella* species. Further research is needed to identify specific proteins in the venom and digestive fluids that may have antimicrobial properties.

P.12. Effects of Military Relevant Chemical Contaminants on a Reptilian Model Species' Microbiome: Can Microbiome Composition Serve as a Biomarker for Chemical Specific Exposure?

Karl J. Indest, Carina M. Jung[1], Steven Everman, Sandra Newell, and James Lindsay, *US Army Corps of Engineers, Engineer Research and Development Center*

Background: While mammalian microbiome-based studies have become ubiquitous in the medical literature, there have been limited gut microbiome studies focused on ecologically relevant vertebrate models like reptiles. Because of their relatively small home range, fast maturation, and high fecundity, lizards are an excellent reptilian terrestrial indicator species. For this study we used the green anole, *Anolis carolinensis,* as our model lizard. Anoles are commonly found on DoD installations in the southeastern US, and as a result have been used to assess toxicity of military relevant contaminants. We hypothesize that predictable changes in lizard gut microbiome composition, as a result of contaminant exposure, may serve as an easily assayed, noninvasive biomarker for a chemical exposure.
Methods: Fourteen day sub-acute exposures were conducted with 2,4,6-Trinitrotoluene (TNT) at a dose of 60 mg/kg of body weight. Anoles (n=7) were orally gavaged daily using corn oil as a carrier with controls only receiving soybean oil. Body weights and food consumption were monitored and fecal samples were collected for high-throughput 16S DNA sequencing and analytical chemistry at days 0 and 14. At the end of the study, organs were harvested for body burden data. Microbial community sequence analysis was accomplished using the QIIME pipeline.
Results: Significant changes in lizard weight loss (~6%) were observed in the TNT dosed anoles at completion of the experiment. Chemical analysis confirmed accumulation of TNT and TNT transformation products in tissue and fecal samples. PERMANOVA analysis of control and TNT bacterial fecal communities revealed significant differences at the end of the study with members of the Enterobacteriacea enriched for in the TNT dosed lizards.
Conclusions: Previously, members of the Enterobacteriacea have been shown to transform TNT as a result of nitroreductase activity. Such activity may have enriched for these organisms in the fecal microbiome. Additional transcriptome studies are planned to confirm bacterial nitroreductase activity. Predictable changes in lizard gut microbiome composition could offer an easily assayed, noninvasive biomarker for a specific chemical exposure providing enhanced scientific support to risk assessments on military installations.

P.13. Third Generation Sequencing Reveals Complete Genomic and Transcriptional Landscape in *Saccharomyces cerevisiae*

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Background: Obtaining a complete genome of eukaryote can be a difficult task, due to a combination of highly repetitive sequences along the chromosomes and short read lengths obtained from second-generation sequencing. *Saccharomyces cerevisiae* strain CEN.PK113-7D is a widely used as a model organism and as a cell factory, and was selected for this study to demonstrate the superior capability of very long sequence reads.
Methods: Long reads were generated using both Oxford Nanaopore (ONT) and Pacific Biosciences (PacBio) technology and short reads obtained using Illumina sequencing. The combined long reads of ONT and PacBio were *de novo* assembled using Canu software. Furthermore, we generated long reads using direct RNA sequencing with ONT to investigate transcriptional landscapes and quantification.
Results: We obtained complete sequences for all 16 yeast chromosomes, as well as the mitochondrial chromosome. Further, three types of DNA methylation (5mC, 4mC and 6mA) were identified. Comparison between the reference strain S288c and strain CEN.PK113-7D identified chromosomal rearrangements, against a background of similar gene content between the two strains. Full-length transcripts were identified through a novel approach of direct RNAseq. This method provides accurate identification of transcriptional landscapes, including

untranslated regions as well as differential gene expression quantification. About 94% of the predicted could be detected. Direct RNAseq identified many polyadenylated non-coding RNAs, including rRNAs, telomere-RNA and a long noncoding RNA.
Conclusion: This work demonstrates a strategy to obtain complete genome sequences and transcriptional landscapes that can be applied to other eukaryal organisms.

P.14. MCP-1 Modulates Neutrophil Function and Pyroptosis during Carbapenem-Resistant *Klebsiella pneumoniae*

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Background: Monocyte chemoattractant protein-1 (MCP-1) is important for monocyte recruitment to the lungs in response to bacterial infection. MCP-1 is also essential for protective neutrophil recruitment to the lungs during *Escherichia coli* infection. Carbapenem-resistant *Klebsiella pneumoniae* (CRKP) infection has been rapidly growing as a life-threatening nosocomial disease in many countries. Little is known regarding the role of chemokines and early cellular immune responses in protective immunity to pulmonary infection with CRKP. In the current study, we investigated the role of MCP-1 in pulmonary innate immunity against CRKP in C57Bl/6 and MCP-1$^{-/-}$ mice.
Methods: We used 8-12-week-old female, wild-type and MCP-1 knockout mice to induce pneumonia by intratracheal inoculation of 10^7 CFUs of CRKP per mouse. We sacrificed the mice 24 and 48 hours post infection to estimate bacterial burden in different organs and enumerate neutrophil recruitment into the alveolar space.
Results: MCP-1 appears to be essential for restricting bacterial outgrowth and death in mice. Compared to the C57Bl/6 mice, MCP-1$^{-/-}$ mice showed reduced influx of neutrophils in the airways and lung parenchyma, as assessed by nucleated cell concentrations in BALF, and myeloperoxidase activity (MPO) in lung tissue. The bacterial burden in the lung and BALF was significantly more in MCP-1$^{-/-}$ mice compared to that of wild-type mice. Moreover, the neutrophil extracellular traps (NETs) induced by CRKP were reduced in MCP-1$^{-/-}$ mice either in vitro or in vivo experiments. Intriguingly, subsequent to CRKP infection, MCP-1$^{-/-}$ mice demonstrated increased pyroptosis through higher expression IL-1β and cleaved gasdermin-d in the lung.
Conclusions: These findings enhance our understanding of the critical role of MCP-1 in modulating neutrophils function and pyroptosis in CRKP infection.

P.15. OifA (YtfB), an Oapa Domain Containing Protein, is a New Cell Division Protein in *Escherichia coli*

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Background: Bacterial cell division in the Gram-negative bacterium *Escherichia coli* is mediated by the septal ring, a multiprotein complex composed of more than thirty proteins, which simultaneously constricts the three layers of the cell envelope: the inner membrane, peptidoglycan (PG) cell wall, and outer membrane. In a search for novel PG binding domains, we identified OifA (formerly YtfB), a bitopic inner membrane protein whose notable feature is a C-terminal OapA domain that is annotated to be a LysM-like domain. Importantly, LysM domains are known to bind PG and mediate septal localization in Gram-positive bacteria.
Methods: We assayed the morphological effect of overproducing OifA, monitored its subcellular localization, determined its placement in the order of recruitment to the septal ring, and searched for synthetic phenotypes with known division proteins. In addition, we localized the OifA-derived OapA domain in cells with or without septal PG.
Results: Overproduction of OifA from a plasmid produced filamentous cells, which suggested it might directly affect cell division. Interestingly, a fluorescent fusion to OifA accumulated at the septal ring and required the initiating division protein FtsZ but not downstream members. While loss of OifA had no discernable impact on cell division, a mutant lacking both OifA and DedD grew as filamentous cells. The OapA domain from OifA independently localized to sites of division, and localization depended on formation of septal PG, suggesting the OapA domain binds to PG.
Conclusion: We conclude that OifA is a *bona fide* cell division protein that is required for proper constriction of the cell. Furthermore, the results indicate that the OapA domain may localize by binding to a transient form of PG found at sites of constriction.

P.16. Effect of Bacterial Supplementation on Black Soldier Fly Growth

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Background: The need for sustainable agricultural practices is ever growing as the world's population increases exponentially and with it, the demand for food. Black Soldier flies (*Hermetia illucens)* are non-pest, non-biting flies that are not known to vector disease agents. Its larvae occur in organic environments ranging from decaying food scraps, to trash bins, and even decomposing bodies. Aside from its uses in composting and waste management, the fly larvae have been used as feed in aquaculture. With their relatively low production cost, easy rearing, and non-mechanized harvesting, dried larvae are a prime candidate to supplement existing feeding routines for both fish and poultry. However, nutritional requirements for optimal production of protein and oil from BSF have not been refined, nor is it known whether probiotic amendment will aid to enhance BSF digestion, nutrition, and conversion.
Methods: We have addressed this question by supplementing a grain-based diet with two different oleaginous bacterial strains on which black soldier fly larvae were reared. The microbes were selected for their high lipid content with predictions that it would increase larval

weight and nutritional density. We fed 100 or 300 BSF larvae (in triplicate) a grain-based diet supplemented with two strains of oleaginous bacteria, and measured growth and fatty acid compositions, as compared to the no-supplement control.
Results: Results showed BSF larvae fed with probiotics led to a significant increase in larval weight and faster development compared to control. Larvae fed with bacterial supplementation also metabolized more of the diet that when fed diet alone. Furthermore, analysis of fatty acids from the fist microbial strain revealed bacterial supplementation increased the concentration of short chain fatty acids, and reduced the amount of unsaturated fatty acids, and showed detectable levels of vaccenic and eicosatrienoic acids, which are unsaturated fatty acids implicated in health benefits.
Conclusion: Altogether, these data showed that food waste supplementation with oleaginous microbes has the potential to increase black soldier fly larval lipid and protein content, feeding efficiency and waste conversion. Results of this work contribute to the understanding of the microbial contribution to BSF waste conversion and nutrition, and will provide a foundation for manipulation strategies to enhance larval feeding rates, and protein and oil production highly impacting food security, environmental quality, waste management, and sustainable energy.

P.17. 16S rRNA Sequencing of the Wolf Spider Microbiome Reveals Potential for Spread of Disease to Mammalian Hosts

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Background: The National Microbiome initiative has encouraged researchers to study all organisms relevant to human health. Many arthropods are known to carry microorganisms important to human health, yet not all groups have been focused on. Previous studies on arthropods indicate the presence of a bacterial microbiome contributing to disease. However, microbiome data on spiders is lacking. Spiders are the most numerous terrestrial predators on the planet and often come into contact with people occasionally causing bites that become infected yet we know very little about their microbiome. Previous biochemical and serological research indicated that wolf spiders such as *Rabidosa rabida* carry *Staphylococcus* species and other microorganisms that grew in culture yet could not be initially identified. We hypothesized that *R. rabida* would have a microbiome consisting of common microbes found in soil due to its natural habitat of fields and low grasses. We also hypothesized that *R. rabida* could potentially have microorganisms living in and on its surface, that could be pathogenic to humans.
Methods: Initial samples were obtained from the hemolymph, abdominal cavity/thorax, sucking stomach, and surface of *Rabidosa rabida,* a common wolf spider. Utilizing aseptic technique 47 different samples from 9 spiders were isolated, then pure cultures were obtained and sent to GeneWiz LLC to be sequenced via 16S rRNA analysis. BLAST Searches of the resulting gene sequences were performed using the NCBI database.
Results: Our analysis revealed that wolf spiders have a microbiome consisting of both nonpathogenic and potentially pathogenic organisms. The nonpathogenic organisms included: *Agrobacterium sp., Arthrobacter sp., Aureimonas sp., Bacillus velezensis, Curtobacterium sp., Microbacterium hominis, Providencia vermicola, Pseudomonas fulva, Rhodococcus sp.,* and *Sphingobacterium paludis*. The opportunistic pathogens include: *Acinetobacter sp., Bacillus toyonensis, Cellulosimicrobium sp., Enterobacter sp., Flavobacterium sp., Lelliottia amnigena, Pesudomonas aeruginosa,* and *Stenotrophomonas maltophilia*. Organisms of unknown pathogenicity include: *Staphylococcus kloosii* and *Lysinibacillus fusiformis*.
Conclusion: After analysis of the gene sequences, it is clear that *R. rabidosa* is a potential mechanical vector in transferring organisms to mammalian environments. Due to their ubiquitous presence in the environment we have to recognize their medical importance. With this initial data, we can begin to gain a better understanding of the potential zoonoses of spiders in transmitting bacteria to mammalian hosts.

P.18. Exploring the 'Core' Microbiome of Kefir Grains

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Background: Kefir is known as an excellent source of probiotics with potential benefits that include improved immune and digestive health, with antimicrobial, antitumor, and antioxidant properties. As the fermentation starter of kefir, kefir grains are comprised of diverse bacteria and yeast, which thrive together to contribute to these nutritional and health benefits. We are interested in identifying and isolating common species in kefir grains of different origins to determine whether there is a 'core' functional community that can characteristically define "kefir."
Methods: We are currently optimizing culture and fermentation conditions for various heirloom milk and water kefir grains in preparation for metagenomic and culture-based analyses of community composition. This includes establishing standard procedures for maintenance and culturing of these grains.
Results: We are in the early stages of this project and will present details of our efforts to date and describe our future plans for culture-dependent and culture-independent experiments to define kefir grain community composition.
Conclusion: This work will establish a foundation for understanding and further dissection of the kefir grain microbiome. Ultimately, we would like to develop kefir into a model laboratory system to study questions in microbial and synthetic ecology on the path towards designing improved probiotics.

P.19. A Missense Mutation in the Undecaprenyl Diphosphate Synthase Gene *uppS* Alters Cell Shape in *Escherichia coli*

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Background: Peptidoglycan (PG) is an exoskeleton that functions to shape bacteria and to protect against force due to turgor. PG intermediates are assembled onto the isoprenoid lipid carrier undecaprenyl phosphate (Und-P). Und-P is synthesized first as undecaprenyl pyrophosphate (Und-PP) by UppS before being dephosphorylated to Und-P. Here, we show that a UppS mutant results in misshapen cells and is unable to synthesize isoprenoids *in vitro* as efficiently as wild type UppS.
Methods: We constructed different combinations of mutants affecting isoprenoid synthesis in two strains of *Escherichia coli* CS109 and MG1655, and visualized morphological differences between the constructs. To quantify the isoprenoid products produced by the UppS mutants, purified proteins were incubated with a fluorescent analog of FPP and a nonfluorescent IPP, the substrates for Und-PP synthesis, and the resulting products were analyzed by HPLC.
Results: Growing CS109 at 42°C induced cell shape defects not observed at lower temperatures. In contrast, MG1655, which contains the wild type version of *uppS*, produced normal shapes at 42°C. CS109 containing wild type *uppS*$^{+}$ maintained a normal cell shape, while MG1655 expressing the mutant *uppS31* did not maintain normal cell shape, causing cells to produce shapes similar to those of CS109 grown at 42°C. Wild type UppS produced isoprenoids with 8-10 added isoprene units to the GPP analog to make Und-PP. However, UppS31 activity was much lower with the FPP analog producing less product, and shorter product length.
Conclusion: Our results suggest that UppS31 is responsible for the cell shape defects observed at 42°C and that UppS31 is unable to synthesize isoprenoids as well as native UppS. Thus, UppS function is critical for maintaining normal cell shape.

P.20. Identification of Soil Bacterial Biodiversity in Central American Rainforest Ecosystems

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Background: The rainforest is best known for being one of the most diverse ecosystems for flora and fauna. However, very little is known about the microbial diversity associated with this region. Therefore, the goal for this project was to analyze the microbial diversity associated with soil in a tropical rainforest in Belize.
Methods: Four soil samples were collected from a tropical rainforest ecosystem in Western Belize. Bacteria were isolated from the soil by culturing on Tryptic Soy Agar plates. Isolates were then selected, genomic DNA isolated, and 16S rRNA gene sequences were obtained by PCR amplification. Sequences were analyzed using NCBI BLAST for classification.
Results: There were a total of twenty-nine unique colony morphologies isolated from the soil samples. Of the twenty-nine colonies, twenty successfully lead to the amplification of a 500 base pair fragment of 16S rRNA genes using Bac27F and Univ1492R as universal primers. After sequencing, ten of the fragments were classified by NCBI BLAST to nine bacterial genera including the following: *Pseudomonas, Vibrio, Bacillus, Amphibacillus*, *Paenibacillus, Psychrobacillus, Streptomyces, Brenneria,* and *Hippea.*
Conclusion: Ten different bacterial species were identified from soil samples collected in Belize. Of the bacteria isolated, ecological roles were identified ranging from providing antifungal compounds, to nitrogen fixation, to being pathogenic. These species play vital roles in supporting plant life and controlling plant populations of the tropical rainforest. Understanding the diversity of these soil microorganisms could also potentially provide new insight for novel antibiotic compounds.

P.21. Changes in the Gut Microbiome Induced by Resistant Starch Supplementation in the Rat Model of Chronic Kidney Disease: Comparison of Metaproteomics and 16S rRNA Sequencing Methods

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Background: Changes in the human gut microbiota have been linked to a variety of diseases including Chronic Kidney Disease (CKD). Diet can affect the composition of the gut microbe community. Resistant starch (RS) serves as prebiotic that is metabolized by the gut microbiota. Previous research in a rat model has shown that a RS-enriched diet can alleviate CKD symptoms. In this study, we compare two methods to assess changes in microbiome composition upon RS supplementation: metaproteomics and 16S rRNA sequencing.
Methods: Previously, 16S rRNA sequencing was used to infer taxonomy of the bacteria present in the cecum samples of CKD rats (group of 9) with and without a RS-enriched diet (group of 9). We used a mass spectrometry-based metaproteomics approach to identify host and microbiome-related proteins in the same set of samples that was used for 16S rRNA sequencing (Kieffer et al 2016). Next, we performed label-free quantification using, in parallel, spectral counting and intensity-based absolute quantification (iBAQ). Differentially-abundant proteins were determined using moderated t-test and correction for multiple testing. Finally, using a taxonomy map from the non-redundant NCBI protein database, we grouped the identified proteins into operational taxonomic units (OTUs) using summation either over spectral counts, or over iBAQ values. Differentially abundant OTUs were determined using the moderated t-test.
Results: We found 1198 differentially abundant bacterial proteins. 359 proteins were down regulated upon the addition of resistant starch. Conversely, 839 bacterial protein were up-regulated. 585 distinct bacterial species were identified with 193 being differentially abundant (adjusted p-value <0.01). Taxonomic information derived from metaproteomics data surpasses previous 16s rRNA analysis and reaches

species resolutions for moderately abundant taxonomic groups. In particular, the *Ruminococcaceae* family becomes well resolved. Butyrate producers and a mylolytic species such *as R. bromii* were clearly visible and significantly rose, while fibrolytic species such as *R. flavefaciens* significantly declined with RS. The observed differentially abundant (DA) proteins are clearly consistent with CKD phenotype. Several known host CKD-associated proteins and biomarkers of impaired kidney function were significantly reduced with resistant starch supplementation.
Conclusions: This study shows the taxonomic resolution of the CKD rat gut microbiome by metaproteomics is better than the resolution obtained from traditional 16S rRNA sequencing.

P.22. The Use of Lichens to Kill Giardiasis

John P. Neal and Srinivas Garlapati, *University of Louisiana at Monroe*

Background: *Giardiasis* also known as beaver fever is a parasitic disease that causes diarrhea and abdominal pain in humans. *Giardiasis* is one of the leading causes of waterborne diseases in the United States. The most effective way to prevent this diseases is good hygiene, however if someone is infected with giardiasis a doctor could prescribe them with antibiotics. As these antibiotics become less and less effective, scientist must find another solution to cure this disease. Thus the idea of using compounds from lichens as an alternative to cure *Giardiasis*.
Method: We used cell counting to test if the lichens do kill the parasite. The lichens were finely grounded and suspended in ethyl acetate for two days, and centrifuged to separate the insoluble material.
Results: We tested five different lichen extracts and only two isolated lichens where able to kill *Giardia* parasites.
Conclusion: This study shows some lichens do kill *Giardiasis*, however, not all of them work to kill *Giardiasis*. This could because that not all lichens are the same, and some compounds have more of one type of protein the other lichens. In conclusion the lichens do work on the *Giardiasis*, however, more research must be done to figure out the compound in lichen extract that has the activity.

P.23. K-mer ALignment of Intervals (KALI): a Fast Alignment Free Method for Sequence Comparison

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Background: Sequence comparison is a fundamental method in bioinformatics for decades. Recently, a k-mer based method for sequence comparison has been gaining an interested due to its simplicity and speed. Typical k-mer based method compare sequences composition by compare frequency of present substrings at a respected length (“k”). However; k-mer frequency based method does not consider a structure of sequence which plays a significant role biological function, which ultimately impact an overall accuracy. To address this problem, we use an adjacent distance of k-mer (ad-kmer), which represents a relative structure of sequence.
Methods: KALI create an index database of sequence by calculate intervals of distance in each specific k-mer. For each k-mer of specific length, distances between all adjacent k-mer were calculated in all location of considered genome sequence. Then the interval distances were store by overlap window of 3 in length of distance array. The comparison between two indexes can be done by a score of mean of Jaccard distance through Minhash algorithm.
Results: A 170 complete bacterial genomes of type strains were retrieved from National Center for Biotechnology Information (NCBI). An ad-kmer size of 4 for each genome were calculated and store as database for this study. We then benchmark it by random sampling a sequence of the 170 bacteria genomes with a size of 2, 4, 10 Kbp and query back to the database. The average accuracy for mapping back to the correct genome are 90.59, 94.11 and 97.64 in order.
Conclusion: KALI is a new method enables a quick comparison between genome with surprisingly accurate result. This allows an application such as identify a sequence in metagenome sample or read mapping to reference sequence. Still an improvement such as overall performance is needed to tackle a data at large scale.

P.24. Temporal Patterns in Diversity of the Lower Mississippi River Microbiome

Jason Payne, Justin J. Millar, Colin R. Jackson, and Clifford A. Ochs, *University of Mississippi*

Background: Large river ecosystems of the north temperate zone are characterized by substantial temporal variation in physical and chemical conditions. The life cycles of many riverine taxa are calibrated with this variation, resulting in temporal patterns of change in the composition and diversity of many aquatic communities. However, compared to studies of relatively long-lived multicellular taxa such as insects or fish, there has been little research on the dynamics or drivers of variation in community composition of aquatic bacteria of large rivers. This is a significant gap in our knowledge of large river ecology, because of the importance of large rivers as conduits of materials to the sea; because, as in other environments, bacteria are the most versatile, and presumably the most important, catalysts of most forms of biochemical transformation; and because bacteria can reproduce rapidly and their community composition respond to environmental changes on a short-term basis. In this study, we assessed temporal patterns of change in the structure of the bacterioplankton community (microbiome) of the largest (by discharge) river in North America, the Lower Mississippi River (LMR). We hypothesized that the degree of change in the microbiome over short time periods (days) would be less than variation in composition measured over longer time frames (among weeks, months or seasons).

Methods: To evaluate rates of change in community structure over time, measurements were made on a daily to monthly basis over an annual cycle. Bacterioplankton communities were sampled from mid-river (0.5 m depth) on a daily and weekly basis over summer, and on a monthly basis over a year at a single location on the LMR. Using next-generation methods, the V4 region of 16S rRNA genes were sequenced from samples using dual-index barcoded primers with the Illumina MiSeq platform, and sequences classified into operational taxonomic units (OTUs). Differences in relative abundances of OTUs between sample dates, or beta diversity, were evaluated using the Bray-Curtis dissimilarity index. In addition, on each sample day we measured environmental factors potentially associated with, or driving, changes in the microbiome. Relationships of community composition to environmental factors were assessed by non-metric multidimensional scaling (NMDS).
Results: Relative abundances of different bacterial OTUs (i.e. community composition) were stable among samples collected over short time periods (days), while considerable variation in microbiome structure was observed over longer time frames. Community dissimilarity scores plotted against differences between sample dates exhibited an arc-shaped pattern, with maximum dissimilarity between communities sampled six months apart. Changes in composition and diversity were most highly associated with temperature, but shifts were also observed following the period of high river discharge in spring and low discharge in late summer.
Conclusion: There is a great deal of variation in the LMR in temperature, rates of flow, and nutrient status that elicit responses in its biotic composition over time. We conclude that the LMR microbiome is structured based on seasonal changes in temperature, and also by factors related to the river's hydrograph including the transfer of bacteria from terrestrial environments into the river system. The bacterioplankton microbiome is an essential component of large river ecosystems, and temporal variation in composition of these communities is likely to impact ecological processes in the LMR.

P.25. Investigating the Role of the Paf1 Complex in Meiotic Recombination in *Schizosaccharomyces pombe*

Reine U. Protacio, Mari K. Davidson, and Wayne P. Wahls, *University of Arkansas for Medical Sciences*

Background: The Polymerase-Associated Factor 1 complex (Paf1C), composed of several conserved proteins, was first identified through its interaction with RNA Polymerase II. Many studies have since characterized its function in different aspects of transcription, such as elongation, mRNA processing and the modulation of certain histone modifications. Recent findings have also indicated it may regulate the distribution of double-strand breaks (DSBs) during meiosis. This project explores the role of Paf1C in meiosis using the fission yeast *Schizosaccharomyces pombe* as a model system.
Methods: Strains harboring deletions in the genes encoding the components of Paf1C, namely Paf1, Leo1, Prf1, Cdc73 and Tpr1, were constructed and used to measure meiotic recombination at the well characterized hotspot *ade6-M26* and its corresponding control allele *ade6-M375*. In addition, the viability of meiotic products produced by each mutant strain was quantified. The level of histone H2B monoubiquitination (H2Bub1), known to be influenced by Paf1C, was also determined by western blotting. In addition, Prf1, which is the subunit containing the histone modification domain, was tagged with a myc epitope at the C-terminal end (Prf1-myc). Both anti-myc and anti-H2Bub1 antibodies were used to carry out preliminary chromatin immunoprecipitation (ChIP) studies.
Results: Deletion of different members of Paf1C impacted meiotic recombination and meiotic product viability to various extents, ranging from slight to pronounced. The magnitude of each effect is correlated to the known role of the components in influencing H2B monoubiquitination. Experiments detected both Prf1-myc and H2Bub1 at the *act1* locus, a highly expressed housekeeping gene, indicating that Prf1-myc and monoubiquitinated H2B can be successfully localized by ChIP. Strains expressing Prf1-myc behave like wild-type with respect to cell morphology and growth rate, suggesting that the myc tag does not significantly affect Prf1 function.
Conclusion: Our results indicate that the Paf1 complex plays a role in meiotic recombination in *S. pombe*. Deletion of specific components decreases recombination levels at the *ade6* test locus and may be a consequence of the impairment of H2B monoubiquitination. Future studies include ChIP experiments to determine the genomic location of Paf1C and H2Bub1 during meiosis, as well as characterization of meiotic events in the deletion strains.

P.26. Evaluation of Virulence and Antimicrobial Resistance Elements in Incompatibility Group FIB Plasmid-positive *Salmonella enterica* Isolates

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Background: *Salmonella enterica* is one of the most common food-borne bacterial pathogen in the United States. Many *Salmonella* strains contain plasmids, including incompatibility group (Inc) FIB plasmids that can carry virulence, antimicrobial resistance, and/or transfer genes which allow them to adapt in diverse environments. This study evaluated IncFIB-positive *Salmonella* isolates from food and food animal sources to characterize antimicrobial resistance, virulence and the transferability of the plasmids among different
Methods: Forty IncFIB-plasmid positive *Salmonella* isolates were evaluated for antimicrobial resistance, plasmid replicon type profiles, the ability to transfer resistance plasmids and genotype using *Xba*I PFGE. The DNA was extracted and analyzed using PCR to determine the presence of virulence (*ccdB, vagD, cvaC, sitA, iutA, iroN, iss*), antimicrobial resistance (*strA, tetA, sul1, sul2, aphAI, blaTEM*) and various transfer-associated (*traG*, *traV, traX, traN, traM, traC, traI*) genes identified in previously sequenced IncFIB plasmids. Furtherthe strains were evaulated for their ability to invade and persist for 48hr in human intestinal epithelial (Caco-2) cells.

Results: The IncFIB-positive *Salmonella* displayed a diversity of genotypes by PFGE and PCR, with over 80% of the isolates containing host addiction genes *ccdB* and *vagD*; and approximately half the strains containing iron acquisition genes *sitA* and *iutA.* Multiple plasmid replicon types were detected in 29 (71%) and 24 (59%) were resistant to at least five antimicrobials. Plasmids from 7 (17%) were able to conjugally transfer antimicrobial resistance to the recipient strain. While each of the tested *Salmonella* strains demonstrated an ability to infect the Caco-2 cells, greater variability was observed in persistence dynamics, with 16 (40%) increasing, while the others decreased.
Conclusions: Most of the IncFIB positive strains carried multiple virulence genes and were able to infect and persist in intestinal epithelial cells with variable levels. The ability to transfer antimicrobial resistance and virulence genes on a single plasmid may serve as a vehicle of creating more virulent and resistant organisms in a single genetic event.

P.27. Systematic Identification of Proteins and Histone Modifications that Regulate Meiotic Recombination

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Homologous recombination is induced in meiosis to promote genetic diversity and, by establishing physical connections between homologous chromosomes, to ensure their faithful segregation in the first meiotic division. Meiotic recombination is clustered at hotspots that regulate its frequency and distribution throughout the genome. DNA binding proteins and post-translational modifications (PTMs) of histones help to regulate hotspots by recruiting the basal recombination machinery (e.g., Spo11/Rec12). However, few of the ~500 known histone PTMs and chromatin remodeling enzymes have even been interrogated for a potential role in recombination. We developed a mass spectrometry-based approach for the unbiased, systematic discovery of hotspot-specific (potentially regulatory) proteins and histone PTMs using the well-characterized, DNA sequence-dependent hotspot *ade6-M26* of the fission yeast *Schizosaccharomyces pombe*. We constructed small (4.2 kbp) minichromosomes (MiniCs) harboring hotspot and negative control alleles that differ at only two base pairs. Chromatin immunoprecipitation (ChIP) of recombination-initiating dsDNA breaks revealed that the hotspot is active in MiniCs. MiniCs were purified from meiotic cultures using the high-affinity interaction between LacO DNA sites in the MiniCs and a prA-LacI fusion protein conjugated to beads. Enrichment levels of 20,000-fold (relative to single copy loci) were routinely observed by gel electrophoresis and quantitative PCR. Mass spectrometry of the MiniCs identified 1023 proteins, 71 of which were enriched specifically in the hotspot sample. Fifteen different histone PTMs were identified, along with 7 histone modifying enzymes and 37 recombination-related proteins. The DNA repair proteins Dmc1 and Rad50 were highly enriched in the hotspot sample, further validating the sensitivity and accuracy of this approach. We constructed strains lacking hotspot-enriched proteins, conducted genetic crosses, and measured recombinant frequencies. This revealed that several, distinct chromatin remodeling complexes each regulate hotspot activity. Mapping of chromatin structure at the level of mononucleosomes, and ChIP of ubiquitinated histone H2B, revealed that the mechanisms involve meiotically induced histone PTMs and the eviction of a single nucleosome at the hotspot.

P.28. Characterization and Host Range Determination of Novel *Agrobacterium tumefaciens* Bacteriophages

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Background: The genus, *Agrobacterium* contains species of rod-shaped, Gram-negative, pathogenic bacteria found in the soil. Infection by *Agrobacterium tumefaciens* (*Rhizobium radiobacter*) causes crown gall disease, which can limit the yield in infected plants. Over 40 families of plants are susceptible which include apples, grapes, roses, tobacco, and soy beans. Infection of a plant by *Agrobacterium* induces a production of plant hormones, which causes an upregulation of cell division and growth leading to the production of galls.
Methods: Ten novel phages were isolated, purified, and characterized using wildtype *Agrobacterium tumefaciens* strain #15955 (*Rhizobium radiobacter*). Host range was then determined for each bacteriophage, using other species of *Agrobacterium* and an unrelated *Rhodococcus* by performing both spot tests and infections on the lawns of the bacteria. Spots were done using all 10 bacteriophages on *Agrobacterium tumefaciens* strains C58 and B6, *Agrobacterium rhizogenes* strains, #100, #117, #15834, and *Rhodococcus fascians,*. If spots were produced at a dilution of 10E-3 or higher, infections were performed for dilutions of 10E-2, 10E-3, and 10E-4. DNA restriction enzyme digests were also performed in an effort to determine relatedness between phages.
Results: As expected, no phages were able to infect *R. fascians.* Four phages were found to infect *A. tumefaciens* strain B6, while 6 infected C58. All other phages tested were negative, showing no broad range infection capabilities across multiple species. Restriction digests of the phage DNA is still ongoing to determine if there are similar taxonomic groups.
Conclusion: This study shows that none of our Agrobacteriophages could infect a broad host range (across the genus). Four phages were found to infect multiple strains of *A. tumefaciens.* Determination of phage taxonomic grouping is still ongoing. Our hope is to be able to determine relatedness between the phages that did show some host range capabilities.

P.29. What is Life? Conserved Functional Domains Across All Life

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Background: The definition of life remains an elusive topic in modern biology. Many studies have found that not a single protein is conserved across three domains of life or even in the bacteria superkingdom. In this study, we look at functional domains that conserved in bacteria, archaea, and eukaryotes.

Methods: We downloaded approximately 103,000 genomes available from all three domains of life from the National Center for Biotechnology Information database (NCBI). A quality score was applied for each genome to filter the good quality set of genomes for comparison. We identified core functional domains by the profile of presence and absence of functional domains.
Results: We find 503 conserved functional domains within more than a hundred-thousand bacterial genomes, and 393 core domains in Archaea. When combined with a set of 21 finished eukaryotic genomes, we find a set of 132 core functional domains conserved across all three kingdoms. We compare the core set of bacterial protein functional domains with the sets of essential genes from transposon mutagenesis experiments, and find nearly all of the 'essential genes' have core domains. Finally, we review functions of conserved functional domains with basic cellular functions. We can categorize core functional domains into three general biology functions: metabolism, DNA repair/structure, and transcription/translation.
Conclusion: Although no full-length amino acid sequence of a protein is conserved across all of life, hundreds of functional domains are conserved. Thus, the 'core set of functions for life' can be derived. These core functional domains may estimate to minimal functions for early cellular life on this planet, and could be useful in designing minimal cells for synthetic biology.

P.30. Chromatin Remodeling Enzyme Fft3 and Histone Chaperone Hir1 Regulate Meiotic Recombination Hotspots

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Background: Meiotic recombination hotspots are important for the proper segregation of homologous chromosomes in meiosis and for generating genetic diversity upon which natural selection can act. Previous mass spectrometry data from our laboratory identified several histone chaperones and chromatin remodeling enzymes that are enriched at the *ade6-M26* hotspot of the fission yeast *S. pombe*. This study sought to determine their roles in hotspot activation.
Methods: We generated strains with deletions of the genes encoding Fft3 (a chromatin remodeling enzyme) and Hir1 (a histone chaperone). We then measured the frequencies of recombination at the *ade6-M26* hotspot and at a basal recombination control, *ade6-M375*. To see if these proteins affect recombination more globally, we also measured the frequency of intergenic recombination between three additional loci. Lastly, we mapped the structure of chromatin at single-nucleosome resolution in meiotic time courses to test the hypothesis that Fft3 and Hir1 remodel chromatin structure at the *M26* hotspot within the *ade6* open reading frame (ORF).
Results: The individual *fft3Δ* and *hir1Δ* deletions each reduced recombinant frequencies at the *M26* hotspot. Basal recombination frequencies were unaffected, indicating that the Fft3 and Hir1 contribute specifically to hotspot activation independently of DSB repair. The double deletion, *fft3Δ hir1Δ*, essentially abolished *ade6-M26* hotspot activity, suggesting that Fft3 and Hir1 work in independent pathways to regulate the hotspot. Lastly, nucleosome scanning analysis revealed dynamic remodeling of chromatin at the *M26* hotspot during meiosis, and that this remodeling required Hir1.
Conclusions: Chromatin remodeling enzyme Fft3 and histone chaperone Hir1 activate the *ade6-M26* hotspot via histone turnover and changing the chromatin landscape within the *ade6* ORF. The stronger phenotype of the double mutant, relative to single mutants, suggests that Fft3 and Hir1 have redundant roles in histone turnover and hotspot activation. In summary, this study revealed the importance of histones and associated proteins in activating meiotic recombination hotspots.

P.31. Quantitative Investigation of the Antimicrobial Mechanism of Silver Ions against *E. coli*

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Background: Due to the rise of antibiotic resistance of bacteria in the past decades, alternatives to antibiotics have been attracting broad interest and attention, among which noble metals, especially silver (Ag), have been revisited and found very promising. Ag in various forms can significantly suppress the growth of, and kill, bacteria, which may open a new avenue to fighting against antibiotic resistance of microbes.
Methods: We measured the antimicrobial activities of Ag ions against *E. coli* at the ensemble level using kinetic growth assays and colony-forming-unit assays. Based on these ensemble observations, we developed a quantitative model to account for and predict the actions of Ag ions. To understand the exact antimicrobial mechanism, we quantitatively investigated the response of *E. coli* to treatment of Ag ions at the molecular level in single cells by examining the expression and spatial organization of a global negative regulator (histone-like nucleoid-structuring protein, or HNS) using super-resolution fluorescence microscopy in combination with biochemical assays.
Results: It was found that Ag ions caused elongation of lag time in the bacterial growth, while the max growth rate remained unaffected. Fractions of *E. coli* were killed exponentially by Ag ions. Based on these experimental observations, a quantitative model was established to describe the antimicrobial activity of Ag ions. Predictions from the model agree well with the experimental results. In addition, parameters in our model as well as their dependence on the concentrations of Ag and bacteria were determined experimentally. It was found that the expression of HNS proteins was significantly enhanced by Ag ions. In addition, super-resolution fluorescence microscopy with a spatial resolution ~ 20nm was used to localize and count individual HNS proteins inside single cells, confirming the enhancement of the expression of HNS proteins in single *E. coli* cells. Furthermore, we found that the spatial organization of HNS proteins inside bacteria was changed from dispersed small clusters to one or several large aggregates in the presence of Ag ions, which likely results in DNA condensation at the center of bacteria.

Conclusion: We developed a quantitative model at the ensemble level to understand the antimicrobial actions of Ag ions against *E. coli* bacteria. It is expected that our quantitative model and the associated parameters provide an alternative means to minimum inhibitory concentration values for characterizing the antimicrobial activities of Ag ions. Furthermore, our studies of Ag ions' effect on bacteria at molecular level in single cells suggests a new acting mechanism of Ag ions against bacteria through the interactions of Ag with DNA binding proteins, which in turn causes DNA condensation and inactivation.

P.32. Gut Microbiome Composition of the Unionidae Family of Mussels is Determined by Host Species and Environment

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Background: Bivalves such as mussels recruit gut microbiota that are distinct from the overlying water column. We found that four co-occurring mussel species had significantly different gut microbiomes and that a species' microbiota was also correlated with physicochemical characteristics of its habitat. We also identified a potential symbiont which has previously been observed in the marine surgeonfish.
Methods: Individuals of mussel species *Fusconaia cerina, Lampsilis ornata, Obovaria unicolor,* and *Quadrula asperata* were collected from six sites along a 50 km stretch of the Sipsey River in Western Alabama, USA. Gut microbiome DNA was extracted, amplified, and Illumina MiSeq used to sequence the V4 region of the 16S rRNA gene. We used ordination to correlate site and mussel species with the proportions of major bacterial taxa and to water/sediment physicochemistry.
Results: The overall effects of both mussel species ($p < 0.001$) and site ($p < 0.001$) were significant. Surface nitrite, pore ammonia, and pore orthophosphate were the principal differences in site chemistry. Despite environmental and species variation, *Epulopiscium sp.*, an unusually large bacterium previously identified in the marine surgeonfish, was the most abundant bacterium found in all samples. Nitrogen-fixing taxa, including *Bradyrhizobium, Hyphomicrobium,* and *Methylosinus* were also major gut constituents across all mussel species.
Conclusion: This study demonstrates that freshwater mussels accumulate different gut microbiota according to species and overlying water conditions. The consistent presence of *Epulopiscium sp.,* a known symbiont in fish, as well as the frequent occurrence of nitrogen- fixing bacterial taxa suggests that the mussel microbiome plays a role in host metabolism, and potentially in nitrogen cycling of the surrounding water.

P.33. Novel Protein-Protein Interactions Between Translation Initiation Factors in *Giardia lamblia*

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Background: Cap binding protein eIF4E is an important factor for translation initiation and regulation in eukaryotic cells. It interacts with the 5'cap structure of mRNA and eIF4G which also serves as an important binding protein for other translation initiation factors and recruits the 43S pre-initiation complex (PIC) to mRNA. Homologs of eIF4E have been identified in Giardia but not eIF4G.This raises a fundamental question of how the 43S pre-initiation complex (PIC) is recruited to the 5' end of Giardia mRNA. eIF4E may have expanded functions in 43S PIC recruitment in Giardia.
Method: Thirteen Giardia initiation factors: GleIF1A, GleIF2alpha, GleIF2beta, GleIF2gamma, GleIF3i, GleIF4A, GleIF4E2, GleIF5, GleIF5A, GleIF1, GleIF3B, GleIF3F, GleIF3H were tested for protein-protein interaction using a yeast-two hybrid assay (Y2H).
Results: Indicate that GleIF4E2 interacts with only GleIF2beta. Other results of the Y2H revealed a novel interaction between GleIF4A and GleIF3i.
Conclusion: Further investigation into these interactions may explain how the 43S PIC is recruited to the 5'end of Giardia mRNA.

P.34. Rapid Genome-Based Surveillance for the Mumps Virus

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Background: Mumps is a highly contagious disease caused by the mumps virus, a member of the genus *Rubulavirus* (Paramyxoviridae). The mumps virus is a single-stranded, non-segmented, negative-sense RNA virus, which contains 7 genes encoding for nucleocapsid (N), phosphoprotein (P), matrix (M), fusion (F), small hydrophobic (SH), hemagglutinin-neuraminidase (HN), and large (L) proteins. The virus spreads via the respiratory route and presents with parotitis, while complications include meningitis and encephalitis. For prevention of mumps, a highly protective, two-dose vaccination is recommended. However, the last decade has witnessed major resurgences of mumps outbreaks in the US including one in Arkansas, in 2016, which spread in highly vaccinated populations. These sporadic large-scale mumps outbreaks renewed our interest in the mumps virus as a re-emerging pathogen that may escape vaccine-induced immunity, a hypothesis that we tested using a bioinformatics approach.
Methods: We obtained buccal swap samples collected by the Arkansas Department of Health (ADH) from patients who tested mumps-positive by RT-PCR of the SH gene during the 2016 outbreak in AR involving about 3,000 cases. Viral RNA was extracted from the samples and RT-PCR was conducted with tailor-made primers (5 overlapping primer pairs which cover 15 kb of the genome) targeting most of the mumps genome. For whole genome sequencing, portable third-generation Nanopore sequencing was employed. Sequencing data was processed with base-calling, reference-based genome assembly, and consensus-based error correction of assembled product

with a focus on understanding of the advantages and disadvantages of Nanopore sequencer. We performed comparative genomes analysis for genome-based genotyping using all publicly-available complete mumps genomes, including 35 vaccine strains plus three Arkansas mumps genomes sequenced through this study. To test the hypothesis of vaccine-induced immunity escape, we applied immunoinformatics to predict B cell and T cell epitopes at the genome scale using neural network-based machine learning while applying two thresholds (strong and weak thresholds). We then investigated antigenic differences between genomes from different genotypes.
Results: PCR-based whole genome sequencing was feasible with the Nanopore technology, as demonstrated with four samples from the AR outbreak. Mumps comparative genomes analysis revealed that the Arkansas mumps strains belong to genotype G, a group of strains that is phylogenetically the most distant from the representative vaccine strain, Jeryl Lynn (JL), of all strains included in our comparison. Genome-based genotyping based on maximum likelihood phylogenetic grouping showed better resolution than grouping based on the SH gene only, a procedure that is currently used in epidemiological investigations. Potential epitopes were identified in all seven viral proteins, including in surface glycoproteins F and HN with a strong threshold. Furthermore, we observed antigenic differences along the genomes from different genotypes as well as differences in the major antigen HN protein.
Conclusion: Genome sequences provide a unique and reliable marker for pathogen identification and molecular epidemiological investigations. Here we present experimental and computational protocols for a rapid genome-based surveillance of the mumps virus. We show that by implementing a cutting-edge sequencing technology along with whole-genome comparison and immunoinformatics, we can accurately amplify, genotype and predict the immunogenicity of the mumps virus from clinical samples at the genome scale. The protocols are currently being optimized and will be tested for application to other infectious agents.

P.35. From Past to Present: A 20-year Timeline of Select Major Viral Disease Outbreaks

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Background: Within the last twenty years, researchers with the World Health Organization have reported information on their website (www.who.int) about viral disease outbreaks around the world. Using Disease Outbreak News (DON), we reviewed select viral diseases occurring since 1996 to show a potential correlation between human transmission and biomes.
Methods: Each outbreak was categorized and analyzed by country, continent, region, and biome. Disease Outbreak News and other sources were used to review information about each disease outbreak and its distribution. A timeline was constructed to show the relationship between each disease outbreak and the biomes in which they occurred.
Results: The select viral diseases reported by DON include Ebola, Marburg, Lassa fever, measles, polio, enteroviruses, hepatitis E, hantaviruses, coronaviruses (less Middle East respiratory syndrome), and Nipah virus. The five most reported diseases were Ebola, polio, Marburg, severe acute respiratory syndrome (SARS), and Lassa fever. The majority of the five diseases occurred in tropical and temperate regions. Ebola, Marburg, and Lassa fever occurred mainly in tropical humid regions; whereas, polio mainly occurred in tropical semi-arid regions and desert (tropical) regions. Severe acute respiratory syndrome mainly occurred in temperate humid regions. Many of the diseases were reported from Africa and Asia. The case fatality rates for the five most reported diseases are included.
Conclusion: The majority of the select viral disease outbreaks occurred in temperate and tropical biomes. Ebola and SARS were the most reported viral outbreaks. Understanding viral disease transmission in relation to biomes provides further information for controlling and preventing the spread of disease outbreaks.

P.36. Middle East Respiratory Syndrome (MERS): An Emerging Coronavirus Disease

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Background: Five years ago, Middle East respiratory syndrome (MERS) emerged as a significant human viral respiratory illness on the Arabian Peninsula. Middle East respiratory syndrome is caused by a coronavirus (MERS-CoV). The World Health Organization has reported MERS outbreaks on its website (www.who.int) via Disease Outbreak News (DON) since 2012. Herein, we review MERS outbreaks and their occurrences throughout the world, with reference to ecological features that may affect disease transmission.
Methods: Each MERS case was categorized and analyzed by country, continent, region, and biome. Disease Outbreak News and other sources were used to review information about outbreaks of this disease and its distribution. A timeline was constructed to show the relationship between each disease outbreak and the biomes in which they occurred. A country-specific review of cases, including a regional evaluation, also was conducted.
Results: A review of the literature indicated that one of the first outbreaks occurred in Jordan. Most MERS outbreaks have been reported from the Arabian Peninsula. Throughout the brief history of MERS, outbreaks which did not occur on the Arabian Peninsula were due mainly from individuals contracting MERS-CoV while traveling from/visiting this area. The Arabian Peninsula is mainly desert temperate; while Yemen, a country on the Arabian Peninsula, is considered desert tropical.
Conclusion: The majority of MERS cases have been from the Arabian Peninsula, mainly in the region of Saudi Arabia. Since MERS-CoV transmission is not fully understood, further information regarding human-human/animal-human interactions in specific biome units may aid in preventing and controlling the spread of this disease.

P.37. Development of an *ex vivo* Human Tissue Platform to Study Pneumonic Plague

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Background: *Yersinia pestis*, the causative agent of plague, is one of the most lethal killers in nature's repertoire of infectious microbes afflicting humans. When transmitted via aerosols and delivered directly to the lungs, *Y. pestis* causes primary pneumonic plague. The virulence of *Y. pestis* is largely attributed to the delivery of *Yersinia* effector proteins (Yops) into the cytosol of target cells via the Ysc type 3 secretion system (T3SS). The initial interaction of the Yops with target host cells facilitates the pre-inflammatory stage of infection during which the pathogen evades and/or suppresses host innate immune responses and replicates to significant bacterial burdens in the lung. The sudden onset of a hyper-inflammatory response follows, culminating in death of the host. A detailed understanding of the initial host/pathogen interactions that drive disease progression is lacking, and the roles of the Yops in the progression to a hyper-inflammatory response in the lungs are unknown. A murine infection model has been instrumental to understanding plague pathogenesis. Unfortunately, important differences impacting disease in mice compared to humans have been revealed. This is a general theme, as the applicability of many animal models to human disease has been of recent concern to the NIH, as countless findings have not translated during human trials.
Methods: We are currently developing an *ex vivo* human tissue infection platform for the analysis of pulmonary infection with *Y. pestis* using human precision cut lung slices (hPCLSs) from human donor lungs. HPCLSs are viable in culture, maintain ciliary motility for ~3 months, and are responsive to infection and treatment with chemical agents. We infected HPCLSs with a *Y. pestis* strain containing a Yop reporter to study initial targets of T3S. Currently, we are using cell-specific immunostaining of tissue slices to identify the targets of Yop injection by confocal microscopy and flow cytometry. We are also analyzing cytokine secretion to evaluate initial host responses in greater detail.
Results: Preliminary results indicate that CD71+ alveolar macrophages are amongst the cells that are initially targeted for injection by *Yersinia* in the human lungs. Also, the initial cytokine response generated by hPCLSs to infection is different compared to THP-1 cells.
Conclusion: These studies will establish a highly relevant and viable model to evaluate *Yersinia* pathogenesis during human infection that may be applicable to other important pathogens.

P.38. MsaB and CodY Nutrient-Dependent Regulatory Interactions in *Staphylococcus aureus*

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Background: *Staphylococcus aureus* has developed a complex regulatory network for the control of virulence factors. One of *S. aureus* major virulence factors, the capsule polysaccharide, is controlled by several regulators in response to various environmental stimuli. We have described MsaB, of the *msaABCR* operon, as binding the *cap* promoter in a growth-phase or nutrient dependent manner. Several other regulators have also been shown to bind this region. Here, we examine the interactions between MsaB and two other nutrient-sensing regulators (CodY and CcpE) binding the *cap* promoter.
Methods: To explore the nutrient-dependent interactions between MsaB CodY, and CcpE, we constructed single mutations of *codY* and *ccpE* and double mutations of *msaABCR/codY* and *msaABCR/ccpE.* We compared the capsule phenotype of these mutants to *msaABCR* mutant under different nutrient conditions. We also explored binding of MsaB to the *cap* promoter in these mutants under rich and nutrient-depleted conditions.
Results: Under rich growth conditions MsaB binds to and activates *cap* in late-exponential/stationary phases. In the *codY* mutant we found that MsaB binds to *cap* in all phases yielding capsule production during all phases. Mutation of *ccpE* did not have any effect on MsaB binding. Additionally, mutation of either gene significantly altered transcription of *msaB.* Likewise, mutation of *msaB* significantly altered transcription of both genes, while mutation of any of the genes significantly altered transcription of *cap*.
Conclusions: In this study our results suggest that MsaB and CodY have nutrient-dependent competitive interactions binding to the *cap* promoter. When nutrient concentrations are depleted MsaB binds the *cap* promoter in all phases of growth. These results suggest that CodY represses *cap* transcription under high nutrient conditions by blocking the MsaB binding site, or that MsaB through an undetermined mechanism is sensing low nutrient levels resulting in a change of its binding affinity to the *cap* promoter. Taken together these results suggest that MsaB binding the *cap* promoter contributes to the nutrient-dependent regulation of capsule in *S. aureus.*

P.39. CXCL-1 Mediates Pulmonary Host Defenses in Pneumococcal Pneumonia

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Rationale: *Streptococcus pneumoniae* (the pneumococci) remains the leading cause of community-acquired pneumonia in the U.S. and the world. Understanding the pathogenesis of pneumococcal pneumonia is a prerequisite for designing improved treatment and prevention strategies. Production of chemokine is the first and critical step in the pulmonary immune response that results in the recruitment of leukocytes to the lungs following infection. The mechanism of host defense initiated by the chemokine CXCL-1 against pneumococcal pneumonia is unknown. Here, we used a mouse model using CXCL-1 gene-deficient mice to investigate the role of CXCL-1 in pulmonary host defense against the pneumococcal pneumonia.

Methods: CXCL-1 gene-deficient and their wild type counterparts were infected intratracheally with *Streptococcus pneumoniae*. Survival, leukocyte influx into the airspace, bronchoalveolar lavage fluid chemokines, and bacterial colony forming units (CFUs) in the lungs and extra-pulmonary organs were quantified. Bone marrow chimera experiment was performed to understand the contribution of hematopoietic and non-hematopoietic to the host defense. Recombinant CXCL-2 (aka MIP2) was administered to rescue cell recruitment and bacterial clearance one-hour post infection. Bactericidal function of bone marrow-derived neutrophils was also evaluated.
Results: We demonstrate that CXCL-1 is important for host survival, bacterial clearance and phagocyte killing during pneumococcal infection. In particular, CXCL-1 is essential for restricting bacterial growth in the lungs and its dissemination to extra-pulmonary organs, initiating rapid cellular influx, neutrophil-mediated bacterial killing, and the production of other CXC chemokines, such as CXCL-2 and CXCL5. Bone marrow transplantation experiment revealed CXCL-1 derived from hematopoietic compartment was critical for cellular recruitment and bacterial clearance. Furthermore, another CXC chemokine, CXCL-2 corrected the CXCL-1 deficiency mediated impairment in the host defense.
Conclusions/impact: These novel findings support the key role of CXCL-1 in mediating host immunity during pneumococcal pneumonia through multiple mechanisms, including modulation of neutrophil recruitment, and its functions and cytokine/chemokine production. Thus, modulation of CXCL-1 activity presents a promising avenue for development of therapeutics against pneumococcal pneumonia.

P.40. The Stress-Response Protein *DDR48* is Required for Resistance to Oxidative Stress in the Pathogenic, Dimorphic, Fungus *Histoplasma capsulatum*

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Histoplasma capsulatum is a pathogenic fungus that is the etiologic agent of histoplasmosis, a respiratory disease affecting humans and other mammals. *H. capsulatum* is dimorphic, meaning that it exists as a multicellular mold at 25ºC and a unicellular yeast at 37 ºC. The morphologic shift from mold (environmental) to yeast (tissue form) is critical for the pathogenesis of the organism. Our lab has identified a number of mold-specific and yeast-specific genes in this organism. This study is focused on determining the function of the stress response protein *DDR48*, which shares homology with *C. albicans* and *S. cerevisiae DDR48*. We plated 1.0 x 10^4 cells/µl of wild-type, knock-out (*ddr48*; previously created in our lab via allelic replacement), and complement, in triplicate, on to *Histoplasma* macrophage medium (HMM) supplemented with varying concentrations of 4-Nitroquinoline 1-oxide (4-NQO). The chemical 4-NQO mimics DNA damage caused by ultraviolet (UV) light exposure due to the production of 4-NQO guanidine and 4-NQO adenine adducts. Once plated, the growth of the mold was examined to determine what effect, if any, the loss of the *DDR48* gene had on the organism's resistance to oxidative attack. A significant difference in growth was observed between the wild-type and *ddr48Δ* on plates supplemented with 4-Nitroquinoline 1-oxide. The complement strain restored the resistance to 4-NQO as expected. The deletion of *DDR48* also resulted in increased sensitivity to paraquat, an oxidative stress generator. When *DDR48* expression was restored, oxidative stress resistance was restored as well, to levels near that of the wild type. This indicates that *DDR48* plays a role in the oxidative stress response of *H. capsulatum*, however, more work is required to fully characterize this gene.

P.41. Dissecting the Association of Rotavirus NSP1 with Cul3

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Background: Human rotaviruses cause severe gastroenteritis, leading to over 200,000 deaths in children worldwide. Rotavirus is a non-enveloped virus with a segmented, double-stranded RNA genome. Group A rotaviruses have 11 genome segments, encoding 12 proteins. Gene segment 5 encodes non-structural protein 1 (NSP1), one of the least conserved rotavirus proteins. Rotavirus NSP1 helps the virus evade host immune responses by inducing the degradation of proteins required to stimulate the production of type I interferon (IFN).
It has been hypothesized that NSP1 associates with cullin RING E3 ubiquitin ligases via the Cul3 scaffolding protein in order to induce degradation of specific protein targets involved IFN activation. Ubiquitination is a post-translational modification that targets proteins for degradation or altered cellular localization. An E1 activating enzyme activates ubiquitin, which is then transferred to an E2 conjugating enzyme. An E3 ubiquitin ligase then facilitates the transfer of ubiquitin from an E2 conjugating enzyme to the target substrate. NSP1 has been shown to associate with Cul3 by mass spectrometry, and direct binding of the two proteins was observed using purified proteins. Interestingly, NSP1 was still able to induce degradation of target proteins in the absence of Cul3. This study aims to define the regions of Cul3 necessary for the NSP1-Cul3 interaction.
Methods: The NSP1 amino acid coding sequence from rotavirus strains SA11-4F and OSU were cloned into pHT-N mammalian expression vectors to generate Halo-tagged NSP1 proteins. Different domains of the gene encoding human Cul3 were cloned into p3X-Flag expression vectors to generate Flag-tagged Cul3 proteins of varying lengths. Full-length Cul3 is 770 amino acids in length. Two N-terminal domains of Cul3 were generated spanning amino acids 1-418 and 1-381, and two C-terminal domains of Cul3 were generated spanning amino acids 419-770 and 382-770. A HaloTag pulldown using HaloLink resin was performed to capture proteins that transiently interacted with NSP1. Protein complexes were analyzed by immunoblotting for Halo and Flag after SDS-PAGE. PCNA and GAPDH were used as loading controls.
Results: NSP1 from rotavirus strains SA11-4F and OSU were detected after a HaloTag pulldown with HaloLink resin. Full-length Cul3 protein and the N-terminal domains of Cul3 were detected after a HaloTag pulldown. Interestingly, both C-terminal domains of Cul3 were

also detected in association with NSP1. This result was unexpected, and additional N- and C-terminal deletions are being constructed to identify a region of Cul3 that does not associate with NSP1 as a negative control.
Conclusion: This study shows that NSP1 appears to bind both the N and C termini of Cul3 in addition to full-length Cul3. Further studies must be performed to determine if NSP1 binds activated or inactivated Cul3. NSP1 is believed to bind Cul3 to aid the virus in evading the IFN response; work is ongoing demonstrate the significance of this interaction.

P.42. Evaluating the Effects that the Cell Membrane Lipid Composition has on the Resistance to Bile Induced Damage in Avirulent Strains of *Listeria monocytogenes*

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Background: *Listeria monocytogenes* is a gram-positive, facultative anaerobic foodborne pathogen that causes listerosis. When this bacterium enters the gastrointestinal tract, it encounters many different stressors including bile. Previous results from our lab have shown that exposure to bile decreases the thickness of the cell membrane of the avirulent strain HCC23, but not the virulent strain EGDe. Additionally, we have shown that fatty acids were exogenously incorporated into the membrane into the avirulent strain HCC23. Therefore, the goal of this project was to determine whether incorporation of fatty acids into the cell membrane from bile reduced bile survival among avirulent strains.
Methods: The three *L. monocytogenes* avirulent strains HCC23, LO28 and 15313 and the avirulent strain *L. innocua* were analyzed in this study. For each of these strains, the fatty acid profiles were determined after exposure to either 0% or 0.3% bile using Fatty Acid Methyl Esters (FAME) analysis. Under aerobic and anaerobic conditions, the cultures were pretreated with a lipid mixture (Sigma L0288) were grown to mid-logarithmic phase and then exposed to 0% and 5% bile for 1 hour, after which survival was determined through viable plate counts. The rigidity of the membrane was measured using anisotropy due to changes in the fatty acid composition using a florescent dye, DPH (1,6-diphenyl-1,3,5-hexatriene). A minimum of three replicates was performed for all assays.
Results: FAME analysis showed that each of these four avirulent strains incorporated oleic acid and stearic acid from bile into the membrane and that there was an increase in the content of palmitic acid when exposed to bile. There was a significant increase in the survival for these strains after bile exposure when pretreated with the lipid mix, thus indicating a trend that these fatty acids improved the survival of these strains after bile exposure under aerobic conditions.
Conclusion: This study showed that the incorporation of fatty acids improves the survival of avirulent strains when exposed to bile. The results suggest that avirulent strains may incorporate fatty acids to survive bile, but that this may come at the expense of a loss in fitness in other stressors. Further research is needed to determine if this mechanism of bile survival impacts the expression of efflux pumps involved in the resistance to stressors.

P.43. Understanding the Regulatory Mechanism of PecS in *Vibrio nigripulchritudo*

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Background: The shrimp pathogen *Vibrio nigripulchritudo* encodes PecS, a multiple antibiotic resistance regulator (MarR) protein; the gene encoding PecS is divergently oriented to the *pecM* gene encoding an efflux pump PecM. The PecS protein is predicted to bind the intergenic DNA region between *pecS* and *pecM*, thereby controlling expression of both genes. In *Dickeya dadantii*, a knockout of *pecS* causes an upregulation of the *pecM* gene to efflux indigoidine (blue pigment), an antioxidant that protects the bacteria from reactive oxygen species (ROS) generated by infected plants. In other plant pathogens, urate acts as a PecS ligand to prevent its DNA binding, thereby increasing bacterial virulence as it evades host defenses. Notably, a gene encoding an enzyme predicted to produce indigodine, *bpsA,* is found downstream of *pecS in V. nigripulchritudo.* This interesting gene arrangement suggests that PecS also regulates production of indigoidine in this species.
Methods: The *pecS* gene was cloned in the expression vector pET28b in order to express and purify the PecS protein using Ni-His tag Affinity Chromatography. *V. nigripulchritudo* colony morphology on marine agar plates containing different concentrations of ligands such as urate and xanthine or various oxidants was monitored to gain insights into the *bpsA* gene expression patterns. A *pecS* gene knockout is being constructed for further gene expression studies.
Results: The purified PecS protein (~20 kDa monomer) is a dimer. The colony morphology studies suggest that urate, xanthine and oxidants are ligands for PecS as evidenced by their presence altering the expression of *bpsA* (development of color).
Conclusion: The purified PecS protein shows similar characteristics as other MarR proteins in terms of subunit composition. Analysis of colony morphology suggests that the tested ligands alter the conformation of PecS leading to an increase in *bpsA* expression, thereby enhancing the survival of bacteria under oxidative stress. Gene expression studies using qRT-PCR of the *pecS* knockout strain and the wild-type in the presence of oxidative stress would help to further understand the bacterial virulence mechanism.

P.44. *In vivo* Competitive Fitness Between Susceptible and Resistant Strains of *Staphylococcus aureus* using *Galleria mellonella* as a Host Model

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Background: *Staphylococcus aureus* is a common cause of infection in both community and hospital settings. The aim of this study was to exploit a wax moth larva model, *Galleria mellonella* in order to explore *in vivo* competitive fitness of clinical isolates SCCmec type II (MRSA) and SCCmec minus (it's genetically knockout mecA element-MSSA) individually and when both of these isolates are mixed in equal ratio.
Methods: We constructed a *mecA* deleted mutant in BH1CC strain. The *in vivo* and *in vitro* competitive fitness of *Staphylococcus aureus* SCCmec type II (MRSA) isolate and SCCmec minus (MSSA) and both strains mixed in 1:1 ratio on the survival of *G. mellonella* over 5 days was studied. 10 μg/ml of oxacillin was used to distinguish MRSA and MSSA from the mixed culture and further validated using patching and PCR methods.
Results: The survival of SCCmec minus-inoculated caterpillar was significantly decreased to ~68% and the survival of SCC*mec* type II was ~84%. Interestingly, when the caterpillar were inoculated with SCC*mec* type II+ SCCmec minus (1:1 ratio), the survival was significantly increased to ~88%. Thus, validating the fitness of SCCmec type II. In *in vitro* competition assay, the ratio between MSSA and MRSA when mixed in 1:1 ratio was 1.1+-0.3 from day 0 to 5. Whereas, the MSSA strain had outcompeted MRSA when injected in to larva when mixed in 1:1 ratio, as the time increased the ratio between MSSA: MRSA increased from 1.0 ± 0.2 to 6.9 ± 0.3.
Conclusion: Therefore, the observed reduced fitness in an in vivo Galleria model of the MRSA relative to MSSA can be helpful to determine the virulent determinants in *Staphylococcus aureus*. This was a first report to assess the *in vivo* competitive experiment using caterpillar model. Further experiments include assessing the fitness cost between MSSA and MRSA at ratio's 1:10, 1:100 and 1:1000.

P.45. Characterizing a Novel Platform to Study *Staphylococcus aureus* Pulmonary Infection

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Staphylococcus aureus is a Gram-positive, opportunistic pathogen capable of causing severe pulmonary disease such as necrotizing pneumonia. Upon entry into lung tissue, *S. aureus* encounters various cell types, from epithelial cells to phagocytic alveolar macrophages. Conflicting reports in the literature provide opposing data on the ability of *S. aureus* to survive and replicate within macrophages. Due to the diversity of strains used for comparison, and vastly different macrophage sources, a disease relevant platform is needed for studying *S. aureus* infection. Here, we present two novel systems to study the interaction between *S. aureus* and the human lung, first using primary human alveolar macrophages (hAMs). hAMs were infected with two strains of *S. aureus*, UAMS-1 (methicillin sensitive) and LAC (methicillin resistant), a community-acquired strain of particular health concern. Both strains of *S. aureus* infected hAMs, but did not replicate efficiently. Next, we assessed infection of human Precision-Cut Lung Slices (hPCLS) to determine the cell type preference of *S. aureus* within human tissue. When introduced to hPCLS, *S. aureus* entered and survived within the epithelium of the hPCLS, but was not present in the hAMs. Upon infection of alveolar or airway epithelial cell lines, the pathogen again survived, but did not replicate. Finally, cytokine analysis was performed to determine the inflammatory response of each cell type following infection. Establishment of the human lung infection platform for *S. aureus* now allows us to further assess differential toxin production and consequent changes in host cell integrity in a disease relevant setting.

P.46. Characterizing *Coxiella burnetii* Infection of Human Lung Epithelial Cells

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Coxiella burnetii is an intracellular pathogen that causes human Q fever. Q fever can present with acute flu-like symptoms or progress to chronic, severe endocarditis. After inhalation, *C. burnetii* travels to the alveolar spaces and is engulfed by macrophages. The pathogen transits through the phagolysosomal maturation pathway and resists acidic pH upon fusion with lysosomes to form a parasitophorous vacuole (PV) for replication. Previous studies showed that *C. burnetii* replicates efficiently in primary alveolar macrophages in *ex vivo* human lung tissue. Although *C. burnetii* replicates in most cell types *in vitro*, the pathogen does not grow in alveolar epithelial cells in human lung tissue. Here, we assessed whether *C. burnetii* replicates in pulmonary epithelial cells apart from the lung environment. We found that *C. burnetii* infected, formed a PV, and replicated in airway, but not alveolar, human immortalized epithelial cell lines. However, growth curve analysis showed that *C. burnetii* replication was less efficient in airway cells compared to macrophages. Additionally, the cytokine profiles of infected epithelial cells were compared to macrophages to define cell-specific innate responses. Our results suggest alveolar epithelial cells do not support *C. burnetii* growth outside the lung environment, providing a new cell-specific context for defining anti-*C. burnetii* activity. Additionally, airway epithelial cells may represent an uncharacterized replication niche for the pathogen. Collectively, this study defined parameters of infection for future use in characterizing *C. burnetii* interactions with primary human epithelial cells.

P.47. Proteomic Analysis of Human Epithelial Cells Infected with *C. muridarum*

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Background: Chlamydiae are a group of biphasic intracellular bacterium that are responsible for the most common sexually transmitted disease worldwide. To date, it is unknown as to why some women develop severe upper genital tract pathology while others have mild or even silent infections. Further characterization of host cell responses during infection is certainly needed to elucidate the mechanisms of infection that might dictate pathological outcome. Our laboratory has previously observed changes in miRNA and mRNA processing proteins, suggesting chlamydiae's ability to hijack normal cellular processes for continued survival under the host's radar.
Methods: In order to investigate this phenomenon, we analyzed proteomes of human epithelial cells infected at 1 multiplicity of infection of *C. muridarum* by using tandem mass tag (TMT) six-plex isobaric labeling followed by mass spectrometric analysis on an Orbitrap Fusion Tribrid mass spectrometer (Thermo). Proteins found to have a false discovery rate (FDR) adjusted p-value < 0.05 by student's t-test and a fold change > 2 were considered significantly differentially expressed and were analyzed by Ingenuity Pathway Analysis.
Results: Of nearly 2,710 total host proteins identified, 1,229 proteins were differentially expressed during chlamydial infection. Of these proteins, 21 were upregulated, while 185 were significantly down-regulated 2-fold compared to uninfected cells. Functional analysis using IPA software identified eIF2, eIF4, mTOR, Spliceosomal Function, and Colanic Acid Biosynthesis pathways were most affected.
Conclusion: A few of the top proteins upregulated during infection were CNKSR2 (scaffolding protein in the MAPK cascade), MX1(GTP metabolizing protein involved in antiviral response), and CCDC114 (coiled coil domain of the dynein docking complex). Conversely, ZBED9 (nucleic acid binding protein), RPL27A and RPL18A (both of which encode proteins in the 60s ribosomal subunit) represent a few of the top downregulated proteins identified in this study. Furthermore, 345 bacterial proteins were identified in an attempt to pinpoint bacterial virulence factors that influence pathology. Future investigations are needed to confirm these targets via western blot, as well as analysis of bacterial proteins to further explore the host-bacteria relationship.

P.48. Which Way to Go? HCMV-induced Signaling Through EGFR Navigates the Trafficking Route in Monocytes

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Background: Human Cytomegalovirus (HCMV) displays a unique, extended intracellular trafficking and nuclear translocation pattern in monocytes when compared to that observed in fibroblasts, endothelial cells, and human progenitor cells. Our lab has shown that HCMV-induced signaling through c-Src is required during viral entry to promote efficient intracellular trafficking (to early endosomes, the trans-Golgi network, and recycling endosomes) and to avoid sorting into late endosomes. In this study, we began to investigate the role of the other major signaling pathway activated in monocytes by HCMV during viral binding: the epidermal growth factor receptor (EGFR) pathway. We found that EGFR signaling is required throughout the duration of the trafficking events to promote proper viral intracellular trafficking and nuclear translocation.
Methods: We utilized the specific EGFR inhibitor AG1478 to block EGFR signaling at specific time points during HCMV infection in primary human monocytes. We examined the effect of EGFR inhibition on viral trafficking and nuclear translocation by immunofluorescence microscopy and PCR.
Results: Inhibition of EGFR signaling at important transition points during the viral trafficking process led to decreased nuclear translocation and the mislocalization of the virus from the expected intracellular vesicles. Whole cell PCR to detect the viral genome demonstrated that the virus is not being degraded when EGFR signaling is blocked. Together these data suggest that EGFR signaling is important during the entire intracellular trafficking process and appears to direct the virus along the proper route to the nucleus.
Conclusion: Our new data suggests that EGFR signaling promotes key events in the viral trafficking pathway leading to proper routing and timing of nuclear translocation, thus enabling the virus to establish a productive infection in monocytes.

P.49. Impact of *Coxiella burnetii* Infections on Host Gene Expression in THP-1 Cells

Christa Jackson and Joel Funk, *John Brown University*

Background: *Coxiella burnetii* is an intracellular bacterium that replicates within its host cell by inducing the development of a parasitophorous vacuole (PV). C. *burnetii* commonly infects livestock, but also causes Q fever when infecting humans. The bacteria not only survives in the acidic and hydrolytic environment of the lysosome-like PV, but actually requires these conditions for replication. The purpose of this research is to further understand changes in gene expression during *C. burnetii* infections of THP1 cells, especially genes involved in protein kinase C (PKC) cell signaling.
Methods: *C. burnetii*-infected cells were harvested over a 96 hr time course. Cell lysates were processed for western blot analysis, and extracted RNA samples was analyzed using real-time PCR.
Results: For the PKC substrate Myristoylated Alanine-Rich C-Kinase Substrate (MARCKS), gene expression levels, protein levels and the phosphorylated form of MARCKS all sharply increases at 24 hours post infection (p.i.). A similar response was seen from a related protein, MARCKSL1. However, the host protein ABCA1 responded later to the infection, with the greatest increase in gene expression and protein expression at 48 hr p.i.

Conclusion: This study shows that the host response to *C. burnetii* infections varies temporally, depending on the gene. The more immediate response of MARCKS and MARCKSL1 compared to ABCA1 indicates a specific sequence of gene expression in response to the infection, and an indication of the ability for *C. burnetii* to manipulate the host cell.

P.50. Development of a Recombinant Varicella Vaccine Expressing Dengue Virus Antigens

Wayne Gray and Michael Hohl, *University of Mississippi*

Background: Dengue fever caused by dengue virus (DV) causes an estimated 390 million primary infections per year, especially in tropical countries. Subsequent infection with a different DV serotype causes Dengue Hemorrhagic Fever, a severe, life-threatening disease. An effective DV vaccine will require immunization against each of the four DV serotypes. In this study, we have developed a recombinant varicella vaccine vector that expresses the immunogenic EDIII domain of each of the four DV serotypes.
Methods: A gene encoding the EDIII domain (glycoprotein E) of each of the four DV serotypes was chemically synthesized. The EDIII gene was inserted within the glycoprotein C region of the simian varicella virus (SVV) genome (rSVV-DVEDIII). Expression of the DVEDIII antigen was confirmed by immunofluorescence assay.
Results: A SVV cosmid recombination genetic system was used to inset the DVEDIII gene into the SVV genome. PCR analysis confirmed that the DVEDIII gene was properly inserted within the SVV gC open reading frame. Infection of Vero cells with the SVV-DVEDIII revealed expression of antigenic epitopes of DV serotypes 1, 2, 3, and 4 as determined by immunofluorescence analysis. The rSVV-DVEDIII replicated as efficiently in Vero cells as does wild-type SVV confirming that insertion of the DVEDIII does not alter viral growth. Using a similar approach, a recombinant varicella-zoster virus (VZV) vaccine expressing the DV EDIII is also currently being construction.
Conclusion: A recombinant varicella vaccine expressing antigenic EDIII epitopes of four dengue serotypes was constructed. A subsequent study will evaluate the ability of this rSVV-DVEDIII vaccine to immunize and protect experimental animals against DV infection. A safe and effective recombinant varicella vaccine would be beneficial to prevent global outbreaks of Dengue Fever and Dengue Hemorrhagic Fever.

P.51. Lack of Hyaluronidase Activity from *Staphylococcus aureus* Grown in a Chemically-Defined Medium Supplemented with Hyaluronic Acid

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Staphylococcus aureus is a gram-positive bacterium known to cause a wide variety of diseases in humans ranging from minor skin infections to life-threatening diseases such as osteomyelitis, infectious endocarditis, pneumonia, and toxic shock syndrome. This organism is predominately found on the skin and mucosal membranes and becomes a problem when the host immune system is compromised. *Staphylococcus aureus* produces a diverse group of virulence factors such as hemolysins, proteases, superantigens, and various adhesion proteins that contribute to some aspect of disease. Proteomic and virulence studies have indicated a role for staphylococcal hyaluronidase in disease. This enzyme degrades hyaluronic acid, a large linear unsulfated, glycosaminoglycan polymer made up of β1-4 alternating units of D-glucuronic acid-[1-3] - β-N-acetylglucosamine. This polysaccharide is a major component of the extracellular matrix of many human tissues including the skin. While studies have demonstrated a role for hyaluronidase in staphylococcal disease its role, apart from modulating biofilm formation and dispersal, is not clearly known. One possibility is that *S. aureus* uses hyaluronic acid as a carbon and energy source. In this study, *S. aureus* strains and their isogenic hyaluronidase mutants were grown in a chemically-defined medium (CDM) with and without hyaluronic acid. Spectrophotometric growth of each strain over a period of twelve hours demonstrated that there was no difference in growth among strains containing the genes for hyaluronidase and those without the genes when hyaluronic acid was used as carbon source. Concentrated spent media from various times in growth were assessed for hyaluronidase activity using a qualitative plate assay containing hyaluronic acid. Results from these studies indicated little to no detectable hyaluronidase activity for strains grown in CDM and CDM supplemented with hyaluronic acid as compared to CDM supplemented with glucose. Collectively, these data indicate that the lack of optimal growth is due to insufficient amounts of hyaluronidase when *S. aureus* is grown in the presence of hyaluronic acid. A lack of detectable levels of hyaluronidase may clearly be a lack of cell mass or may represent an effect on hyaluronidase expression by an unknown regulatory mechanism. The views presented in this presentation do not necessarily reflect those of the U. S. Food and Drug Administration.

P.52. Purification and Enzyme Activity of *Giardia lamblia* eIF4A

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Background: Translation initiation factor eIF4A, a DEAD-box helicase, is required for unwinding secondary structures in the 5'UTR of mRNAs during translation initiation in higher eukaryotes. It exists as the eIF4F complex with two other initiation factors eIF4G (scaffold protein) and eIF4E (5' Cap binding protein). However, in *Giardia lamblia*, a primitive protozoan eukaryotic parasite, the mRNAs have very short (0-6 nucleotides) 5'UTRs. Preliminary results indicate that eIF4A is crucial for *G. lamblia* cell survival. Bioinformatics analysis of Giardia genome database suggests that *G. lamblia* may not have the eIF4F complex as it lacks eIF4G. Interestingly, yeast two hybrid assays have demonstrated that eIF4A complexes with eIF3 instead. Since this eIF4A appears to have a role highly unlike that in other eukaryotic organisms, this study is designed to determine the structure and function of eIF4A in *G. lamblia.*

Methods: The 6XHis tagged eIF4A helicase was cloned and purified using Ni-NTA column chromatography. Future methods include an ATPase assay and a helicase activity assay to determine eIF4A function. We will also establish a homology enzyme model using the *Homo sapiens* eIF4A to create a representative structure for the *G. lamblia* eIF4A. Through the model, we will create a dominant negative mutant of the *G. lamblia* eIF4A via site-directed mutagenesis.
Results: The *G. lamblia* eIF4A was cloned and purified to 90%. Purification will continue to achieve 100% purity before beginning activity assays and homology modeling.
Conclusion: Currently, the eIF4A protein has been purified to 90%. Once a higher purity is achieved, we will commence the ATPase and helicase activity assays, as well as the modeling to determine the *G. lamblia* eIF4A structure and possible role.

P.53. The M2 Gene Influences Reovirus Virulence and Myocarditis

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Attachment of mammalian orthoreovirus (reovirus) to host cells is mediated by engagement of glycans and the protein junctional adhesion molecule-A (JAM-A) on the cell surface. Previous studies indicate that the M2 gene-encoded outer capsid protein μ1, which is crucial for mediating viral membrane penetration during entry, also modulates reovirus binding to JAM-A. A reassortant reovirus containing the M2 gene from strain T3D in an otherwise strain T1L genetic background (T1L/T3DM2) utilizes JAM-A more efficiently than T1L. T1L normally produces a non-lethal infection when inoculated into neonatal mice. However, replacing the native T1L M2 gene with the T3D M2 allele dramatically increased virulence, as the majority of mice succumbed to T1L/T3DM2 infection. Similarly, T1L/T3DM2 was markedly more virulent than T1L following intracranial inoculation. T1L/T3DM2 was detected in the spleen, heart, liver, and brain at earlier time points compared to T1L, indicating that T1L/T3DM2 disseminates more efficiently than T1L. Hearts from mice infected with T1L/T3DM2 contained purulent lesions characteristic of myocarditis. In contrast, no lesions were observed in mice infected with T1L. Together, our findings indicate that the T3D M2 gene enhances reovirus dissemination and myocarditis induction, possibly by enhancing interactions with the reovirus receptor JAM-A.

P.54. Identifying Host Factors Contributing to *Yersinia pestis* Effector Translocation using CRISPR/Cas9

Samantha Huckuntod, Shalynn Mills, and Roger Pechous, *University of Arkansas for Medical Sciences*

Background: *Yersinia pestis*, the bacterium responsible for plague, escapes early elimination by host innate immune cells by targeting them for effector protein translocation via a type 3 secretion system (T3SS). The T3SS forms a needle-like complex on the bacterial cell surface that allows proteins to travel from the bacterium directly into the cytosol of target host cells. The secreted effectors known as *Yersinia* Outer Proteins (or Yops) function to inhibit host inflammatory responses and phagocytosis. The targeting of host cells via T3S is essential to *Y. pestis* virulence. In this study, we develop a screen to identify host factors contributing to Yop translocation in the human monocytic THP-1 cell line.
Methods: A CRISPR RNA library was transfected into macrophage-differentiated THP-1 cells expressing Cas9 to disrupt more than 8,000 human genes. After transfection and differentiation, cells were infected with a *Y. pestis* reporter strain which expressed a Yop protein tagged with β-lactamase. Following infection, cells were washed and incubated with a FRET-based substrate that emits blue fluorescence upon cleavage by β-lactamase as a readout of Yop translocation. Relative blue fluorescence units were then measured using a plate reader to compare Yop translocation in CRISPR RNA-treated cells and untreated cells.
Results: We have optimized a screening technique that utilizes CRISPR/Cas9 to knock down genes contributing to the targeting of macrophage-differentiated cells for Yop secretion. After knockdown, infected cells undergoing decreased T3S will be observed, and specific host genes facilitating translocation can be identified.
Conclusion: This screening system will help to identify host factors associated with *Y. pestis* secretion in a relevant cell type.

P.55. Proteogenomic Mapping of Bovine Herpesvirus 1 Reveals a Previously Unknown Coding Region in Genome

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Background: Bovine herpesvirus type 1 (BoHV-1) is an important agricultural pathogen that infects cattle and other ruminants worldwide. Acute infection of the oral-respiratory tract leads to immune suppression and can allow for secondary infection with commensal bacteria in the lower respiratory tract. It is therefore one of a few viruses that initiates the condition known as Bovine Respiratory Disease (BRD). BoHV-1 latently infects the host for life, with recurrent stress events re-initiating BRD, translating into high morbidity and large economic losses. As a member of the alphaherpesvirus family, BoHV-1 consists of an encapsulated double stranded DNA genome surrounded by tegument proteins and a host cell derived lipid envelope. Though BoHV-1 was first sequenced and annotated over twenty years ago as a composite of various strains, the Cooper strain, used in this study, was fully sequenced more recently (2012).
Methods: Using proteomics, we aimed at characterizing the protein content of BoHV-1 infected cells. In addition to peptides representing the currently annotated proteins, we found uncharacterized viral peptides that map between annotated coding regions of the genome. Using strand specific primers, we amplified transcripts via RT-PCR. Furthermore, rapid amplification of cDNA ends (RACE) was used to characterize the complete sequence of an intergenic transcript.

Results: Amplification via RT-PCR was achieved when using primers specific for a target region on the strand complementary of the BoHV-1 U_L50 gene. RACE allowed for the amplification of the 5' end of this transcript, while custom primers were needed to amplify the 3' end.
Conclusion: This study identifies a potential novel viral gene. We are currently working on the time frame of expression for this transcript.

P.56. Microbiome Comparison of Breast Cancer and Lung Cancer Tissues

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Background: Breast and lung cancers are two common cancers worldwide. Among women, breast cancer is the main cause of cancer death, affecting 1 in 8 women in the United States. More than a million deaths each year are from lung cancer, which has a low five-year survival rate compared to other cancers. Recently it has been shown that bacterial microbiota can cause carcinogenesis in animals and human beings. There are knowledge gaps about the role of microbiota in carcinogenesis in organs such as lung and breast. In this study, we aim to investigate the common microbiota pattern shown in both lung and breast cancer tissues and distinguish microbiota unique to each disease, allowing for potential new methods for screening and diagnosis.
Methods: We extracted and sequenced the microbial DNA from Formalin-Fixed, Paraffin-Embedded (FFPE) lung cancer and breast cancer tissues. In the lung cancer tissues, we focused on the adenocarcinomas (AC) and squamous cell carcinomas (SCC) subtypes. Qiime and R packages were used to characterize the diversity and taxonomic composition of the microbiota in the cancer tissues.
Results: At phylum level, the results showed that *Acidobacteria* significantly enriched in SCC compared to AC. *Thermi* and *Acidobacteria* significantly enriched in SCC and AC compared to breast cancer (BC). *Thermogae* is significantly enriched in BC compared to SCC. *Firmicutes* are slightly but significantly enriched in BC compared to AC.
Conclusion: Different microbiota community structures between lung cancer subtypes or subtypes of lung cancer and breast cancer were observed in our study. These results can be used to identify antimicrobial regimens for cancer treatment.

P.57. Virulence Evaluation of Incompatibility Group 1 (IncI1) Plasmids Containing *Salmonella enterica*

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Background: *Salmonella enterica* is leading foodborne pathogen in the US. Mobile genetic elements like plasmids can potentially increase their ability to infect and persist in to hosts. IncI1 plasmids are widely distributed in food animal sources and associated with clinically important strains. We have evaluated the putative virulence-related genes and potential virulence of *Salmonella* isolates from human and animal sources using colicin-associated inhibition and the Caco-2 tissue culture model.
Methods: PCR was carried out on thirty IncI1 containing *Salmonella enterica* isolates, using primers designed based on reference genome for the putative virulence-related genes including *ccdA, cib, imm, pilS*, *traL*, and *col1*. Colicin inhibition assays of *Salmonella enterica* isolates on *E. coli* J53 strain was conducted using plate-mating method. They were evaluated for ability to infect and persist in the Caco-2 human intestinal epithelial cell line. The Caco-2 cells were grown to confluence, and were infected with *Salmonella* and incubated for both one and 48 hours for the invasion and persistence assays respectively.
Results: Most of the isolates were positive for *traL* (96%, 29/30), while nearly half of them contained *pilS* (63%, 19/30), *imm* (46%, 14/30), and *ccdA* (60%, 18/30). Colicin-associated inhibition of *E. coli* J53 strain was observed among 66% (20/30) of isolates tested. All of the isolates infected Caco-2 cells after one hour incubation and most were able to persist in the cells at 48 hrs. In general, persistence assay cell count was noted be one log higher than invasion assay cell count in most of our isolates.
Conclusions: In this study some similarity between the presence of putative virulence-related genes and virulence shown by the isolates was observed. However additional work needs to be done to refine the contribution of the specific plasmid-associated genes to virulence.

P.58. Whole-Genome Shotgun Metagenomics of Multiple Mammalian Species

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Background: Whole-genome shotgun metagenomics is widely used to explore the role of the gut microbiota in human health. Stool samples offer a diverse microbial community and are easily available specimen for the study of metagenomics. Recent studies provide a strong evidence of using stool-microbiome-derived metagenomic signature to detect some specific diseases in human. We believed that in other mammalian species, a set of multiple samples may be used to study group relationships of the samples and to understand dissimilarity between samples. The objective of this study is to detect microbial diversity and gut microbiota-derived signature directly from the stool samples from mice, dog, and human.
Methods: In this study, stool samples were collected from different mice, human, and dog. Zymobiomics Kit was used for DNA extraction from the stool samples. DNA quantity and quality check was performed by Qubit, Nanodrop add gel electrophoresis. Library preparation for sequencing was done using Nextera XT DNA library prep kit that fragments the DNA and adds adaptor sequences onto the DNA template. Fragment analyzer was used to detect the quality of the short fragments made during library preparation. Fragments of good quality were used for sequencing using Illumina sequencing.

Results and conclusion: Sufficient DNA were retrieved from all the samples and the DNA library preparation were of good quality for Illumina sequencing. Preliminary results suggested the presence of different microbial communities in all fecal samples. Metagenomic sequencing of stool samples could provide an effective tool to detect and characterize diverse microorganisms from mammalian stool samples. This data could be used for future analysis to find gut microbiota-derived signature in each organism.

P.59. The Loss of Function of MS95 Inhibits Mold-Phase Growth of the Pathogenic Fungus *Histoplasma capsulatum* under Conditions of Oxidative Stress

Lauren Kennedy, *The University of Southern Mississippi*

Background: *Histoplasma capsulatum* is a pathogenic fungus that is the etiologic agent of the respiratory disease histoplasmosis in mammals. *Histoplasma* undergoes a morphogenic shift from mold to yeast which is crucial to the pathogenesis. To study this M-Y dimorphism we have identified a number of mold-specific and yeast-specific genes. This study is aimed at elucidating the function of the mold-specific gene*, MS95*, which shares homology with the S. cerevisiae DDR48 gene which is involved in DNA repair and resistance to oxidative damage.
Methods: We plated 1.0 x 10^4 cells µL of G186AS (MS95+), G186AS (Δms95) G186AS (ms95/MS95) strains, in triplicates, on *Histoplasma* macrophage media (HMM) with varying concentrations of the chemicals 4-Nitroquinoline N-oxide (4-NQO) and Paraquat dichloride, which create oxidative stress. The loss-of-function mutant was created via allelic replacement previously in this lab. Once plated, growth of mold was monitored to determine the effect of loss of function in the MS95 gene in resistance to this oxidative attack.
Results: A significant difference in growth was observed between the MS95+ and Δms95 strains on plates containing 40uM, 60uM, 80uM and 100uM concentrations of paraquat dichloride. No significant difference was seen in 4-NQO plates. Complementation of the knockout mutant with a functional copy of MS95 mostly restored the resistance to paraquat.
Conclusion: Deletion of the *MS95* gene resulted in paraquat sensitivity. Restoration of MS95 activity (confirmed by RT-PCR) restored paraquat resistance to near wild type levels indicating that *MS95* plays a role in resistance of *H. capsulatum* to oxidative stress. Work is ongoing to determine if *MS95* also plays a role in DNA repair.

P.60. Comparative Genome Analysis of *Aeromonas veronii*

Salih Kumru, Hasan C. Tekedar, Mark L. Lawrence, and Attila Karsi, *Mississippi State University*

Background: *Aeromonas veronii* is a Gram-negative rod commonly isolated from soil, water, food, and human or animal environments. *A. veronii* can cause diarrhea, wound infections, or septicemia in immunocompromised people. It is also the causative agent of bacterial hemorrhagic septicemia in fish, which is becoming a major economic problem in aquaculture.
Methods: Comparative genome analysis was performed on four completed *A. veronii* genomes (*A. veronii* B565, *A. veronii* TH0426, *A. veronii* CB51, *and A. veronii* AVNIH1) isolated from soil, fish, and human, as well as one draft genome (ML09-123) isolated from channel catfish. Secretion systems (Type I-VI), Average Nucleotide Identity (ANI), and integrons were evaluated.
Results: All genomes have Type I secretion system (T1SS), Type II secretion system (T2SS), Type IV pili (T4P), and flagellum secretion operons. Type III secretion system (T3SS) presented only in strains TH0426, CB51, and ML09-123. The strains TH0426 and ML09-123 have type VI secretion system (T6SS), but they lack the Tad secretion system. The strains B565, AVNIH1, and CB51 have Tad secretion system, but they lack the T6SS. The genomes that have T3SS have two flagellum operons, but others have only one flagellum operon. The strain AVNIH1 genome has a plasmid with three complete integrons, while other strains do not have any plasmid and integrons. Interestingly, genomes of the US catfish strain ML09-123 and China catfish strain TH0426 are very similar with an ANI of 99.95%.
Conclusion: Secretion system and secreted proteins are necessary for bacterial adaptation to environment and pathogenicity. Comparative genome analysis of *A. veronii* strains from different sources is expected to lead a better understanding of their virulence mechanisms.

P.61. Detection of Peptidoglycan Recognition Proteins and N-Acetylmuromyl-L-Alanine Amidase Activity in Corneal and Retinal Epithelial Cells

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Background: Mammals have four peptidoglycan (PGN) recognition proteins PGRPs. One mammalian PGRP, PGLYRP-2 secreted by the liver, has been reported to be an inducible N-acetylmuramoyl-L-alanine amidase that hydrolyzes bacterial peptidoglycan and reduces its proinflammatory activity. Liver cell PGLYRP-2 mRNA was reported to be stimulated by 6h incubation with LPS and expressed in corneal epithelial cells. Since the decreased susceptibility to bacterial infection may be dependent in part upon the presence of L-Ala amidase, PGRP activity in human corneal and retinal cells were compared to rabbit kidney (RK) cells (Nod 2 ligands cause RK cell, but not HCoC or RPE, apoptosis).
Methods: The L-Ala amidase activity in human corneal epithelial cells (HCoC), human retinal pigmented epithelial (RPE), and RK cells was identified and quantified by agarose gel electrophoresis of cleaved FITC-MDP and by reduction of cytotoxic activity in RK cells. For PGRP induction, cells were stimulated with 1-20ug/mL Nod 1 ligands (C-iE-DAP, PGN *E coli*, PGN *Staph. aureus*) and Nod 2 ligands (MDP and MDP Control peptide) then terminated after 6 h incubation by the addition of lysis buffer. To identify the activity, anti-PGRP specific

antibodies were used to inhibit PGRP activity. The relative amounts of cleavage FITC and control FITC-MDP were determined densitometrically. An immunofluorescent antibody assay was used to visualize PGRPs in ethanol fixed HCoC, RPE and RK cells on slide cultures.
Results: Enzymatic cleavage of FITC-MDP by L-Ala amidase activity was detected in all cell lysates. Constitutive expression of PGRP-2 was detected in HCoC, RPE and RK cells, but was not significantly increase by NOD ligand incubation for 6h. The L-Ala amidase activity/mg protein was greater in RK than in HCoC and less in RPE cells. Antibody to PGRP identified PGRP-1 & 2 positive cells and inhibited the PGRP L-Ala amidase activity in HCoC, RPE and RK cells (PGRP-2 predominantly).
Conclusion: PGLYRP-1 and -2 and L-Ala amidase activity were detected in all cells. These results support PGRP/L-Ala amidase activity as a constitutive enzyme that can putatively limit the innate immune response to local bacterial infection.

P.62. Drug-Delivery Nanoparticle Immunological Effects on Induction of Pro-inflammatory Responses to *Candida albicans*

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Background: Vaginal candidiasis is a common inflammation of human vulvovaginal tissues during infection with the fungus *Candida albicans*. This study evaluates the effects of the drug delivery nanoparticles, polyethylene glycol-conjugated poly(DL-lactic-*co*-glycolic acid) (PLGA-PEG), on VK2 vaginal epithelial cell (VEC) responses to *C. albicans* infection. Recently, using an *in vitro* model of *C. albicans* infection of VECs, we demonstrated that *C. albicans* exposure suppressed apoptotic gene expression, however; it induced oxidative stress and pyroptosis in the cells. The PLGA-PEG nanoparticles induced cytotoxicity by activating apoptosis, endoplasmic reticulum stress, oxidative stress, and DNA damage repair responses alone and, in some cases with *C. albicans*.
Methods and Results: In the current study, we determined the underlying cell signaling pathways for these cell stress responses and showed that *C. albicans* infection inactivates the Sonic Hedgehog (SHH) signaling pathway in human VECs by suppressing the expression of transcription factors *GLI1*, *GLI2*; *SHH* and its receptors patched 1, 2 (*PTC1*, *PTC2*). We hypothesized that this causes increased Bcl2 expression that suppresses apoptosis, even in the presence of nanoparticle induced inflammation. These *in vitro* studies were validated *in vivo*, where CBA/J mice were challenged with PLGA-PEG nanoparticles for 2 hours followed by intra vaginal challenge with *C. albicans* strain B-311. Presence of nanoparticles and *C. albicans* in vaginal washings and in isolated VECs were observed at 2, 6 and 18 hours post infection. Immunofluorescence staining showed co-localization of nanoparticles with VECs and endothelial cells. Kinetic data showed increased levels of inflammatory cytokines IL1β and TNFα over time. Levels of IL1α, IL1β, IL18 and TNFα were further analyzed in the serum and vaginal washings of mice challenged with either PLGA or *C. albicans* or PLGA challenge followed by *C. albicans* infection. Significant induction of IL1β was observed with both PLGA and *C. albicans* challenge.
Conclusion: These results clarify our understanding of how *C. albicans* modulates host cellular responses during an infection and will be applicable to the effects of intravaginal nanomedicines. In addition to intracellular signaling pathways for pro-inflammatory and cell stress responses, we will also investigate vaginal cell growth, autophagy and apoptosis responses regulated by *C. albicans* through the host SHH pathway and how drug-delivery nanoparticles affect this pathogenic mechanism.

P.63. Studying Temperature-Dependent Gene Regulation in the Relapsing Fever Spirochete, *Borrelia turicatae*

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Background: Tick-borne relapsing fever (TBRF), characterized by repeated bouts of febrile episodes, is globally distributed and represents an imminent public health concern. *Borrelia turicatae* is one of three TBRF *Borrelia* spirochetes that are transmitted by *Ornithodoros* ticks in the US. During the enzootic cycle of TBRF spirochetes, the bacteria alter their gene expression to colonize vector and host environments. We hypothesize that temperature is a key stimulus that TBRF spirochetes sense in the tick and mammal.
Methods: A proteomic analysis was conducted to identify proteins differentially produced at mammalian body temperature (37°C) and a temperature representative of the unfed tick (23°). BTA121, a protein maximally produced at 23°C and not produced at 37°C was chosen as a model to study differential gene regulation. Luciferase-based transcriptional reporters were then utilized to define regions of DNA required for temperature-dependent regulation, and DNA affinity chromatography was used to identify potential transcriptional regulators involved.
Results: Results suggested a transcriptional repressor may bind to the region 95- to 265- bp upstream of the *bta121* open reading frame at 37°C. DNA affinity chromatography identified two proteins that bound this region: an ortholog of BadR (*Borrelia* host adaptation Regulator) in *Borrelia burgdorferi*, and an ortholog of BosR (*Borrelia* oxidative stress Regulator) in *B. burgdorferi*. Analysis of the DNA sequence 95- to 265-bp upstream of *bta121* revealed potential binding sites for *B. burgdorferi* BadR and BosR.
Conclusion: *B. turicatae* orthologs of BadR and BosR, two global regulators in the Lyme disease spirochete *B. burgdorferi*, bind a region required for temperature-dependent regulation of *bta121*. These results suggest a role for BadR and BosR orthologs in host and vector adaptation in TBRF spirochetes.

P.64. Using Riboswitch to Control Internal Ribosome Entry Site Mediated Translation Initiation in *Giardiavirus*

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Background: Riboswitches are sequences of RNA capable of binding a ligand, inducing a conformational change that affects translation of a gene. The discovery of riboswitches has aided molecular biology research as a means of controlled expression of a gene of interest. This project aims to engineer *Giardia lamblia* virus (GLV) with a riboswitch in the internal ribosome entry site (IRES) that will allow control of viral gene expression in the host *Giardia lamblia*.
Methods: Several riboswitches were chosen as possible candidates for successful GLV gene regulation by using ligands tetracycline, neomycin, and theophylline. The viral construct pC631Luc contains a reporter gene Fluc driven by viral IRES. Using site-directed mutagenesis we have replaced a key stem-loop structure in the IRES element with a selected riboswitch. The rationale is that a riboswitch assumes a stable secondary structure when it binds to its cognate ligand and it will then facilitate binding of ribosome to the IRES element to initiate translation. Currently, we are transfecting Giardia lamblia cells with *in vitro* synthesized viral RNA constructs using electroporation followed by luciferase assay to check for reporter gene expression.
Results: We have obtained five riboswitch-engineered pc631Luc models that are in the process of being tested for regulation effectiveness in Giardia. Transfection of viral RNA into Giardia cells and subsequent luciferase assays will allows to determine the efficacy of these riboswitches.
Conclusion: Although we have the riboswitch models, much testing is still to come to determine the best riboswitch for regulating gene expression of *Giardia lamblia* virus. Many variables of RNA transfection, cell lysis, and future follow-up experiments still require optimization before we obtain reproducible results.

P.65. Detecting Episomal or Integrated HPV-16 Using an Exonuclease V - qPCR Based Assay

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Background: Though not required for transformation, integration of the HPV16 viral genome into the host genome is commonly observed in HPV-positive oral and anogenital tumors. Traditionally, Southern blots are used to determine the viral genome configuration within HPV-infected cells, but requires microgram quantities of DNA that is not achievable with small tissue biopsies. To reduce dependence on DNA quantity, we developed an exonuclease based assay to determine the HPV16 genome configuration in tissue and cell lines.
Methods: We established the assay using genomic DNA from UMSCC47 cells, a carcinoma cell line carrying 18 integrated copies of HPV, and HPV16-infected 293TT cells carrying episomal HPV DNA. 100ng of DNA was either digested with exonuclease V or left undigested in mock reactions. DNA levels were measured by qPCR with primers that amplified HPV16 E6, human mitochondrial DNA, and human 18S ribosomal DNA. Human 18S ribosomal DNA served as an internal linear control, while human mitochondrial DNA was an internal episomal control and a marker of DNA integrity. The percent of DNA resistant to exonuclease V digestion was then calculated relative to undigested DNA for determination of episomal or integrated HPV16 status.
Results: The assay reliably detected episomal or integrated HPV16, and reproduced results with DNA that had undergone up to 6 freeze/thaw cycles. As a correlate for HPV-positive tissues, we tested the HPV state of keratinocyte cell lines harboring integrated or episomal HPV16 after growth in organotypic raft culture. The exonuclease based assay accurately called the HPV genome state in the raft tissues and allowed exclusion of tissue samples with poor DNA integrity.
Conclusion: The exonuclease assay was an accurate and rapid assay to distinguish episomal from integrated HPV16 using reduced quantities of DNA. The reduced amount of DNA required for the assay supports analysis of HPV episomal and integrated states in tissue biopsies, allowing for rapid screening of HPV integration in the progression of HPV-associated cancers.

P.66. Evaluation of Impaired Cadaverine Synthesis on Pneumococcal Capsule and Protein Expression

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Introduction: *Streptococcus pneumoniae* (pneumococcus) is the causative agent of community-acquired pneumonia, meningitis, septicemia, and otitis media; resulting in significant mortality and morbidity worldwide. The ability of pneumococci to colonize, invade, and damage host tissue is attributed to virulence factors such as: capsule, pneumolysin and adhesins. Polyamines such as putrescine, spermidine and cadaverine, are poly cationic aliphatic hydrocarbon compounds that are ubiquitous in all living cells where they regulate a variety of vital functions. Previous work in our laboratory has shown that deletion of lysine decarboxylase *(cadA),* a gene that encodes an enzyme that catalyzes the conversion of lysine to cadaverine, a polyamine, resulted in an attenuated phenotype in a murine model of pneumococcal pneumonia. Little is known about the impact of *cadA* deletion on pneumococcal protein expression that ultimately affects virulence.
Methods: In this study we conducted mass spectrometry based expression proteomics with wild type *Streptococcus pneumoniae* serotype 4; TIGR4 and a strain with an impaired cadaverine biosynthesis gene; Δ*cadA*. Pneumococci were cultured (n=4), in Todd Hewitt Yeast extract to mid-log phase and total proteins were isolated trypsin digested and subjected to 1D LC ESI MS/MS. Total capsular

polysaccharides were isolated from mid-log phase of *ΔcadA,* TIGR4, and an un-capsulated strain (T4R), and compared by immunoblots with serotype 4 polysaccharide specific antibody.
Results: Expression proteomics identified 89 proteins that were significantly altered in *ΔcadA* compared to TIGR4, of these; 34 were up regulated and 55 were down regulated. Six proteins involved in polyamine biosynthesis were significantly down regulated; four proteins involved in lysine biosynthesis, one protein involved in putrescine and one protein involved in spermidine biosynthesis. Expression of pneumolysin and lysozyme (-5-fold), known virulence factors was down regulated. There was significant reduction in proteins involved in glycogen, galactose, pyruvate and carbon metabolic pathways. ABC transporter proteins were significantly reduced in Δ*cadA*. Immunoblot assay showed loss of capsule Δ*cadA* compared to the wild type, which could explain the attenuated phenotype in vivo.
Conclusion: Impaired cadaverine biosynthesis is not compensated by synthesis of additional polyamines such as putrescine and spermidine. However, it results in loss of capsule, a major virulence factor. Proteins involved in glycogen, galactose, pyruvate and carbon metabolic pathways are significantly altered; affecting production of important precursors of both polyamines and capsular biosynthesis.

P.67. Functional Analysis of the CA Cluster *Rhodococcus erythropolis* Phage Bonanza and a Comparative Analysis of CA, CB, and Singleton Rhodococcus Phages

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Background: While a large number of Mycobacteriophage genomes have been sequenced as a result of the HHMI-supported SEA PHAGES Program, a limited number of phages have been isolated and sequenced from other Actinobacter hosts. In this study, we report the comparative analysis of *Rhodococcus erythopolis* phages and present evidence of horizontal gene transfer between these isolates and other phages infecting alternative Actinobacter hosts.
Methods: *Rhodococcus erythopolis* bacteriophages were isolated from soil samples. Following purification and DNA isolation, Illumina sequencing was performed. Genome annotation was accomplished through the DNA Master bioinformatics platform and the Phamerator, SplitsTree, and Gepard DotPlot visualization tools were used to perform a comparative analysis of these Rhodococcus vs. other Actinobacter phages.
Results: The eight CA phages range from 46,314 – 46,962 bp in length and have a GC content of 58.7 – 58.8. The left-hand side of the genome is predominantly read from the + strand and represents many structural proteins, while the right-hand side of the genome is largely coded for by the – strand. The S-integrase, read from the – strand, represents the crossover from + → - strand reading frames. Phamerator comparison of these phages indicates that all eight isolates share a considerable degree of sequence homology and exhibit many gene products found only in members of their own cluster. These ORFs code for both hypothetical proteins and known functional proteins and include: tapemeasure protein; holin; tail assembly chaperone; and lysin. There are also a number of functional gene products which CA phages share with members of the A subclusters of Mycobacteriophages. These gene products include: DNA polymerase I; DNA primase; helicase; excisionase; S-integrase; deaminase; capsid; terminase; and the immunity repressor. Additionally, we have employed the SplitsTree, Gepard DotPlot, and Phamerator visualization tools to explore the relatedness amongst all currently-sequenced CA, CB, and Singleton phages that infect *Rhodococcus erythropolis*. These groups represent clusters that exhibit a widely varying degree of sequence homology – highly-conserved CA cluster, moderately-conserved CB cluster, and highly-divergent Singletons. Such analyses provide insight not only into the relationship between *Rhodococcus erythropolis* phages but can point to extended comparisons between other Actinobacter phage group isolates.
Conclusions: This study shows that CA cluster Rhodococcus phages exhibit a high degree of sequence homology to one another and several unique gene products. However, there are also many functional gene open-reading frames that are shared with the A subclusters of *M. smegmatis* bacteriophages indicating possible horizontal gene transfer among the disparate genera of the Actinobacter phages. The CB cluster Rhodococcus phage isolates, however, show a limited amount of sequence homology within their cluster and share some gene products with previously-sequenced Streptomyces phages. The Singleton group of Rhodococcus phage isolates share very little sequence homology with either the CA or CB cluster phages and show additional evidence of horizontal gene transfer with other Actinobacter phages.

P.68. Human Cytomegalovirus UL148 Prevents the ER-Associated Degradation of Viral Glycoprotein O, a Key Determinant of Cell Tropism

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Background: Approximately one-third of proteins expressed by human cells are targeted to the secretory pathway. Nascent proteins either successfully fold within the ER or, failing to do so, are retrotranslocated back into the cytosol for proteasomal degradation in a highly conserved eukaryotic pathway known as endoplasmic reticulum-associated degradation (ERAD). Notably, all enveloped viruses must traffic essential glycoproteins through the secretory pathway. Human cytomegalovirus (HCMV), a ubiquitous pathogen that significantly

threatens the immunocompromised, expresses two envelope glycoprotein complexes known to impact the cell tropism of progeny virions. These are the gH/gL/gO ("trimer") and gH/gL/UL128-131 ("pentamer") complexes. Pentamer is required for entry into epithelial cells and leukocytes while trimer is thought to promote entry into all permissive cell types. We previously found that the viral ER-resident protein UL148 promotes high level expression of gO both in infected cells and on virions. Importantly, we found that deletion of the *UL148* ORF in strain TB40/E significantly increased entry efficiency and replication in epithelial cells, while we observed no effects on replication in fibroblasts. We hypothesize this change in cell tropism is due to a shift in the ratio of trimer/pentamer on virions. Recently, we found that UL148 physically associates with SEL1L, a critical ERAD factor for retrotranslocation of misfolded ER proteins for cytosolic degradation. We therefore designed experiments to investigate: (i) whether gO is targeted by ERAD during HCMV infection and (ii) whether UL148 stabilizes gO expression in the face of ERAD.
Methods: For all experiments, hTERT-immortalized human foreskin fibroblasts (HFF) were infected with strain TB40/E at an MOI of 1 TCID50 per cell. To investigate the role of UL148, we also infected HFF with a recombinant virus, 148_{STOP}, wherein all in-frame start codons of the *UL148* ORF were mutated to stop codons. The ERAD pathway was inhibited using two approaches: (i) siRNA knockdown of SEL1L, or (ii) treatment with the small molecule kifunensine (KIF), which inhibits ER mannosidases that target misfolded glycoproteins for destruction. Steady-state levels of gO during UL148-null infection, with or without ERAD inhibition, were analyzed by western blot. The stability of newly synthesized gO under these conditions was visualized by pulse-chase analysis.
Results: We found that both SEL1L-knockdown and KIF treatment significantly increased steady-state levels of gO in both WT and 148_{STOP} infections. Importantly, we found that gO is significantly less stable during *UL148*-null infection and that SEL1L-knockdown significantly rescues gO stability.
Conclusion: Our results suggest a role for ERAD in the regulation of gO expression during HCMV infection. Furthermore, we show evidence that UL148 prevents the degradation of gO, either by promoting the successful folding and ER-processing of gO or by inhibiting ERAD directly.

P.69. Bacteriophage Classifcation Using a Superimmunity Based Lysogen Library

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Background: Bacterial cultures are termed lysogens when bacteriophage genomes are incorporated into the bacterial genomes as prophages. Lysogens are believed to be immune to phages genetically related to the inserted prophage. This is termed superimmunity. In this study, we aim to classify bacteriophages using immunity patterns determined by creating novel lysogen populations in *Agrobacterium tumefaciens* (*Rhizobium radiobacter*), and subsequent infection of candidate phages. Immunity results will be compared to bacteriophage DNA restriction digests to determine genetic relatedness of the phages.
Methods: Previously isolated bacteriophages were used to infect *Agrobacterium tumefaciens* 15955. The lawns of bacteria were infected with a high titer phage lysate, almost clearing the plate. Regrown colonies on the plate were then chosen as candidate lysogens. Patch and supernatant spot tests were used to determine if the colonies were true lysogens. Restriction digests of bacteriophage DNA were carried out to determine digest patterns.
Results: Preliminary results from the patch and supernatant spot tests confirmed the creation of 4 novel bacterial lysogens of *Agrobacterium tumefaciens*. Positive patch test results were indicated by the presence of clearings when candidate lysogens were plated on lawns of *Agrobacterium tumefaciens* 15955. Positive supernatant spot test results were indicated by clearings when the supernatants of candidate lysogen cultures were spotted on lawns of *Agrobacterium tumefaciens* 15955. Restriction digests of bacteriophage lysates revealed distinct banding patterns.
Conclusion: We expect true lysogenic bacterial cultures to be immune to infection by the phages that created them. If infection does occur, plaques should resemble those of the infecting bacteriophage and not of the prophage which was incorporated into the bacterial genome. The lysogen library created from a compilation of these results will serve as a classification tool to readily determine which bacteriophages belong to the same taxonomic group, without having to sequence each bacteriophage. We expect genetically related bacteriophages to have similar immunity patterns as well as restriction digest patterns, and thus be classified within the same group in the lysogen library.

P.70. The Role of Epigenetic Mechanisms in Antigenic Variation in *Giardia lamblia*

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Background: *Giardia lamblia* is a binucleated, flagellated protozoan parasite that is responsible for the gastrointestinal disease giardiasis. *G. lamblia* trophozoites undergo surface antigenic variation and express one variant-specific surface protein (VSP) on their surface. The *Giardia lamblia* genome contains an estimated 200 different VSP genes with only one VSP being expressed at a time. Recent studies have shown that an RNAi mechanism is involved in down regulating all VSP genes except for one that is expressed on the trophozoite's surface. It has been shown that all VSP transcripts have an antisense RNA strand that binds and degrades the VSP transcripts except for the one to be expressed. These studies were able to demonstrate why only one VSP is able to be expressed while the others are not but how VSP switching occurs still remains unclear.
Methods: This study is investigating epigenetic mechanisms involved in VSP switching. *G. lamblia* trophozoites were endogenously transfected with full-length and truncated VSP genes tagged with a 3x HA-tag at the 3' end and screened for VSP expression via RT-PCR. Antigenic variation is induced using a variety of histone deacetylase inhibitors (HDACi) at 2 μM concentrations.

Results: 3xHA tagged VSP expression was detected via RT-PCR in splitomycin treated cells but not in controls or other cells treated with different HDACi.
Conclusion: This study suggests that histone modifications may have a role in upregulating VSP gene expression and may contribute to VSP selection.

P.71. Defining Cell-Type Specific Functions for Viral Gene Products in Chronic Gammaherpesvirus Infection

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Gammaherpesviruses (GHVs) are DNA tumor viruses that include the human pathogens Epstein-Barr virus and Kaposi sarcoma-associated herpesvirus. GHVs establish life-long, chronic infections that place the infected host at risk for numerous cancers, including Burkitt lymphoma and Kaposi sarcoma. While it is known that GHVs are maintained in B cells during a phase of chronic infection known as latency, how distinct viral gene products function in B cells to regulate latent infection is poorly defined. Utilizing murine gammaherpesvirus 68 (MHV68) infection of mice as a small animal model of GHV pathogenesis, we developed a viral genetic system that enables the conditional deletion of specific viral genes in B cells. We generated conditional deletion viruses in which the genes encoding v-cyclin (encoded by ORF72) or M2, proteins that drive cell-cycle progression and promote differentiation of infected cells, were flanked by loxP sequences (floxed) to facilitate deletion by the Cre recombinase. Insertion of loxP sites did not alter viral replication and latency in mice that do not express Cre, indicating that the presence of loxP sites did not indirectly impact infection. Infection of cultured cells that express Cre recombinase was accompanied by deletion of the floxed viral genes, which demonstrates the feasibility of the approach. Infection of CD19-Cre knock in mice, which produce Cre recombinase in B cells, correlated with a 10-fold reduction in latency compared to WT MHV68 in the spleens of mice infected with a floxed M2 virus and a 100-fold reduction in reactivation from latency. Infection of CD19-Cre mice with floxed ORF72 virus did not affect latency, but correlated with a 10-fold reduction in reactivation from latency. These results demonstrate that M2 functions in B cells to mediate MHV68 latency establishment, while both M2 and v-cyclin are important for viruses that latently infect B cells to reactivate into the productive replication cycle.

P.72. Role of Altered Penicillin-Binding Proteins on the Susceptibility of *Clostridium perfringens* to β-Lactams

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Background: *Clostridium perfringens* causes a variety of mild to severe infections in humans and other animals. A decrease in the affinity of penicillin-binding protein (PBP) transpeptidases for β-lactams is considered one of the mechanisms of β-lactam resistance in bacteria. The effects of different β-lactams on induction of mutation in PBPs and the alteration of the susceptibility of *C. perfringens* to β-lactams were investigated.
Methods: *C. perfringens* ATCC 13124 mutants resistant to penicillin G, cephalothin and ceftriaxone were selected *in vitro*. Etest and microdilution assays were used to detect antimicrobial susceptibility to different β-lactams. PCR and sequencing were used to compare the structures of PBPs of mutant strains resistant to different β-lactams with that of the wild type strain.
Results: *C. perfringens* ATCC 13124 rapidly developed resistance to different β-lactams. Strains resistant to penicillin G had a mutation resulting in the substitution of one amino acid within the central penicillin-binding/transpeptidase domain of CPF_2395, but the ceftriaxone and cephalothin-resistant strains had mutations resulting in the substitution of two amino acids in this region. The cephalothin-resistant mutant also had additional mutations in the CPF_0340 and CPF_2218 genes in this critical region. The recombinant *C. perfringens* strain 3626, carrying a plasmid containing the wild-type CPF_2395 gene, showed higher β-lactam sensitivity than those containing the mutated genes from strains resistant to various β-lactams. Resistance development affected cell morphology and bacterial growth rate.
Conclusions: Exposure of *C. perfringens* to β-lactams resulted in the induction of mutations in the PBPs and resistance development to different β-lactams. The structures of the β-lactams affected the types of mutations induced in PBPs. The largest molecular weight PBP in *C. perfringens* appeared to be the primary target of all three β-lactams. The alteration of this gene may be accompanied by alterations in the other genes affecting cell morphology and growth.

P.73. Deciphering the Role of Fatty acids in Bile Induced Membrane Damage in *Listeria monocytogenes*

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Background: *Listeria monocytogenes* is a Gram positive, facultative intracellular organism responsible for the foodborne disease listeriosis. *L. monocytogenes* must survive a variety of stressors encountered within the gastrointestinal (GI) tract, including variations in pH, oxygen availability, and bile. It is known that changes in the fatty acid profile helps *L. monocytogenes* to survive under extreme cold. Therefore, the hypothesis of this study was that exposure to bile will cause a shift in fatty acid profile in all strains of *L. monocytogenes* by incorporating exogenous lipids and this alteration of the cell membrane improves resistance against bile.
Methods: To determine what effect bile has on the fatty acids in *L. monocytogenes* and if this shift has a direct link to bile resistance, fatty acids from three different strains of *L. monocytogenes* (HCC23, 10403S and F2365) were analyzed under both aerobic and anaerobic conditions, with and without exposure to 0.3% bile. To determine if these fatty acids had a "protective" role, cultures were pre-treated with a lipid mix containing varying concentrations of lipid mix (palmitic acid, oleic acid, stearic acid, linoleic acid and others) and subsequently

exposed to 0% or 5% bile under both aerobic and anaerobic conditions. Cells grown in PBS but without the lipid mix were used as a control. The role of these fatty acids in causing change in membrane fluidity was also assessed by anisotropy where cells after bile exposure were treated with the fluorescent dye 1,6-diphenyl hexa-1,3,5 triene (DPH) for 30 minutes and the emitted signal was recorded using fluorimeter under both aerobic and anaerobic conditions.
Results: Under both aerobic and anaerobic conditions, after exposure to bile, an increase in saturated fatty acids, palmitic acid and stearic acid content and presence of unsaturated fatty acids oleic acid and linoleic acid was detected in all three strains. The lipid mix protected HCC23 against bile induced cell death under aerobic conditions, but not under anaerobic conditions. Change in membrane fluidity was also observed under aerobic conditions.
Conclusions: From this study it can be concluded that alterations to the cell membrane through incorporation of exogenous lipids improves bile survival under aerobic conditions. Additional research is needed to determine the mechanism by which *L. monocytogenes* is resistant to bile induced membrane damage under anaerobic conditions.

P.74. The σ1s Promotes Efficient Reovirus Replication in Antiviral Conditions to Facilitate Viral Dissemination

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Following oral infection, mammalian orthoreovirus (reovirus) disseminates via the blood from the intestine to every organ system in the body, including the CNS. Although hematogenous reovirus dissemination requires non-structural protein σ1s, the mechanism by which σ1s mediates bloodstream spread remains undefined. Previous work indicates that σ1s facilitates reovirus replication in multiple cell lines, including SV-40 immortalized endothelial cells and MEFs, by promoting optimal viral protein production and formation of viral factories. Here, we found that a σ1s-deficient reovirus replicates to the level of wild type virus in MEFs lacking the type 1-interferon receptor (*IFNAR1*$^{-/-}$). The protein synthesis defect for the σ1s-deficient reovirus is largely restored in *IFNAR1*$^{-/-}$ MEFs. The σ1s protein does not function as a direct antagonist to type-1 interferon signaling, as IRF3 activation, IFN-β production and IFNAR1 signaling are comparable between wild type and σ1s-null viruses. In C57BL/6 mice, wild type reovirus spreads systemically to the heart, liver, spleen and brain following oral inoculation, whereas dissemination of the σ1s-null reovirus is restricted. The σ1s-deficient virus disseminated in *IFNAR1*$^{-/-}$ mice, although not to the level of wild type virus. Our results indicate that σ1s functions to promote efficient reovirus replication in antiviral conditions. These results further suggest that type-1 interferon responses act as a barrier to restrict reovirus bloodstream dissemination.

P.75. Periodontal Bacteria Promote the Pathogenesis of Kaposi's Sarcoma-associated Herpesvirus in HIV$^+$ Patients

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Background: The Kaposi's sarcoma-associated herpesvirus (KSHV) is the etiologic agent for Kaposi sarcoma (KS), which commonly arises within the oral cavity of HIV+ patients. However, effective antiretroviral therapy has no apparent impact on KSHV transmission through oral secretions, viral dissemination or oral KS incidence. Therefore, the identification of KSHV interactions with microenvironmental factors including co-infected pathogens may facilitate the treatment or prevention of oral KSHV infection and/or oral KS progression. *However, the interactions between periodontal bacteria and KSHV and their contributions to viral oncogenesis in AIDS patients remain largely unknown.*
Methods: qPCR and qRT-PCR were used to quantify internalized KSHV viral copies and transcripts, respectively. Flow cytometry was used to quantify expression of KSHV receptors. Immunoblots, ELISA, IFA and other assays were used to identify host factors facilitating KSHV infection. The clinical relevance of microenvironmental factors was tested using saliva samples collected from a cohort of HIV+ patients with or without KSHV co-infection.
Results: Our data indicate that both lipoteichoic acid (LTA) from *Staphylococcus aureus* and lipopolysaccharide (LPS) from *Porphyromonas gingivalis* increase KSHV entry and subsequent viral latent gene expression during *de novo* infection. The underlying mechanisms include the up-regulation of one of cellular KSHV receptors, Heparan sulfate proteoglycan (HSPG), increasing Reactive oxygen species (ROS), a co-factor for KSHV entry and activating intracellular signaling pathways associated with viral latency. We found that periodontal bacterial conditioned medium induce KSHV lytic reactivation from latently-infected oral cells, which is potentially through the TLRs-ROS axis. Finally, we found that HIV+/KSHV+ patients had higher levels of salivary LTA, LPS, ROS and host antioxidant factors than HIV+/KSHV- patients.
Conclusion: Our findings provide innovative insights into the mechanisms of periodontal bacteria or their PAMPs promoting KSHV pathogenesis in oral cells, and to correlate clinical data supporting the relevance of bacteria-virus interaction facilitating oral KSHV infection, replication and/or oral KS progression in HIV+ patients.

P.76. Enterotoxin Production in *Clostridium perfringens* is Affected by Bile Acids and Antimicrobial Agents

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Background: *Clostridium perfringens* is the second most common cause of bacterial foodborne illness in the United States, with nearly a million cases each year. Enterotoxin (CPE) produced during sporulation of *C. perfringens* damages intestinal epithelial cells by pore formation, which results in watery diarrhea. *C. perfringens* is exposed to bile acids and antimicrobial agents in food and gastrointestinal tract.

Methods: The effects of low concentration of nisin, norfloxacin and bile acids on sporulation and toxin production were investigated in *C. perfringens* SM101 carrying an enterotoxin gene on the chromosome in nutrient rich medium by growing the cultures in Brain Heart Infusion medium with or without additives.
Results: Addition of bile acids and antimicrobial agents resulted in an increase in the production of enterotoxin in some cultures; bile acids had the most effect. The additives stimulated the transcription of enterotoxin and sporulation related genes and spore production during the early growth phase. They also delayed spore outgrowth. The antimicrobial agents had the most inhibitory affect. Bile acids and nisin also enhanced enterotoxin production in some strains of *C. perfringens* field isolates tested but not in all.
Conclusions: Even low concentrations of bile acids and antimicrobial agents may act as stress signals for the initiation of sporulation and early transcription of sporulation-related genes, which results in the an increase in the production of enterotoxin and delay of spore outgrowth. The effect on enterotoxin production is strain- specific.

P.77. Role of Human Papillomavirus E7 phosphorylation in Cellular Gene Expression

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Background: Although human papillomavirus (HPV) can cause a variety of cancers, HPV infections can last for decades without causing any serious health problems. The phosphorylation level of E7 of high-risk HPV types is higher than that of low-risk HPV types and CKII-mediated E7 phosphorylation may play critical roles in cell cycle control, induction of DNA synthesis, enhancement of oncogenic functions of E7, and development of HPV-associated neoplasia. In this study, we examined the role of E7 phosphorylation in the regulation of cellular gene using phosphorylation-defective HPV16 cell lines (SSAA and SSDD mutants).
Methods: Uninfected human foreskin keratinocytes (HFKs) and HFK cells containing either wild type or E7 hypophosphorylated and phosphomemetic HPV mutants, SSAA and SSDD respectively, were used in undifferentiated conditions as monolayer cultures with mouse fibroblasts. These monolayer cultures were used for RNA isolation from stroma and subsequently microarray analysis was performed. The microarray results were validated through RT-qPCR. Further, effect of phosphorylation-defective mutants on expression and phosphorylation of E7 was carried out by transfecting retroviral vectors having either wild type E7 or SSAA mutant in U2OS cells. The phos-Tag gel and Western blot performed for E7 expression levels.
Results: We examined gene expression pattern in the monolayer cultures containing uninfected HFKs, wild type HPV or E7, SSAA and SSDD, mutants. Microarray results showed the differences mainly in interferon stimulated genes (ISGs) and cytokines and inflammatory response genes. ISGs identified through microarray data analysis were IFI44L, IFIT1, Mx1, OAS2, PARP9, STAT1 and XAF1 along with many others. Other significant genes showing differences were CCNA1, IGFBP2, interleukins (IL6, IL-8) and MMPs. We confirmed expression of these genes with qPCR. The phos-Tag gels showed that though expression of phosphorylated E7 in E7 mutants is reduced but not completely abolished.
Conclusion: The ISGs have been shown to have antiviral activity and role of E7 mutants in modulating keratinocytes have not been well understood. Serine sites at position 31 and 31 in E7 protein was observed to play an important role in suppression of ISGs in the HPV containing keratinocytes. The phos-Tag gels showed that though expression of phosphorylated E7 in E7 mutants is reduced but not completely abolished. Our results also suggested the presence of phosphorylation site(s) other than at positions 31 and 32.

P.78. Incorporation of Fatty Acids into the Cellular Membrane of *Salmonella* after Exposure to Bile

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Background: *Salmonella* is a gram negative, facultative anaerobic food borne pathogen and is the leading cause of deaths related to foodborne illnesses. In order to establish an infection successfully, *Salmonella* must be able to survive in the presence of various stressors that it encounters namely changes in pH, oxygen availability, osmolarity and bile. Research from our lab has shown that exposure to bile causes a shift in fatty acid composition in the cell membrane of another enteric, *Listeria monocytogenes.* Thus, this led to the hypothesis that *Salmonella* incorporates fatty acids exogenously from bile into its membrane and thereby protects itself against bile induced damage.
Methods: To determine what effect bile has on the fatty acids in *Salmonella* and if this shift has a direct link to bile resistance, fatty acid profile of four strains of *Salmonella* (S. *Heidelberg, S. typhimurium, S. typhi*, and *S. enteritidis*) were examined using fatty acid methyl esters (FAME) analysis under aerobic conditions with exposure to 0% bile and 0.3% bile. To determine if these fatty acids had a "protective" role, cultures were pre-treated with a lipid mix containing varying concentrations of lipid mix (Sigma L0288) and then exposed to 0% and 5% bile for 1 hour, and the viability was assessed using plate counts.
Results: Under aerobic conditions, there was a change in the fatty acid profile in all four strains. The four fatty acids that consistently increased were the unsaturated fatty acids oleic acid and linoleic acid and the saturated fatty acid stearic acid. The saturated fatty acid palmitic acid increased in all three strains except *S.enteriditis*. Preliminary data from the survival analysis showed that the lipid mix had no impact on survival after bile exposure for any of the strains under aerobic conditions.
Conclusion: This study shows that the fatty acid profile of *Salmonella* changed after exposure to bile. Though our results are preliminary, these changes in the fatty acid profile did not correlate with changes in survival of *Salmonella.* Further work is needed to determine how this impacts the ability of *Salmonella* to cause salmonellosis.

P.79. Expression of the Stress-Response Gene *DDR48* is Upregulated in the Yeast Morphotype by the Antifungal Drug 5-Fluorocytosine in the Pathogenic, Dimorphic, Fungus *Histoplasma capsulatum*

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Histoplasma capsulatum (Hc) is a systemic, dimorphic, fungal pathogen. *Hc* grows as a multicellular mold at environmental temperatures (25°C) whereas, upon inhalation into a human or other mammalian host (37°C), it transforms into a unicellular, pathogenic yeast. This mold-to-yeast shift is required for pathogenesis. Our research aims to elucidate the numerous stress response pathways (e.g., oxidative stress and DNA damage) that *Hc* utilizes to survive in the ever-changing environment. Specifically, we are characterizing the DNA damage-responsive protein *DDR48*, an *Hc* homolog sharing sequence similarity to *C. albicans DDR48p*. Previously in our lab an allelic replacement deletion-mutant was generated (*ddr48*Δ) to elucidate the function of Hc*DDR48*. Interestingly, *DDR48* is constitutively expressed in the mold-phase whereas it is only expressed at basal levels in the yeast-phase. However, *DDR48* expression can be modulated in the yeast-phase under stressful conditions. Upon analysis, we found that *DDR48* is required for resistance to numerous cellular stressors such as oxidative stress, DNA damage, heat shock, and antifungal drugs. This study focuses on the antifungal drug 5-Fluorocytosine (5-FC), an inhibitor of fungal DNA and RNA synthesis. We found that in the yeast-phase of *Hc*, the addition of either 50 µg/ml or 100 µg/ml of 5-FC up-regulates *DDR48* expression by 4-fold using qRT-PCR. Interestingly, there is no significant difference in *DDR48* expression in the mold-phase after 5-FC addition. Research is ongoing to elucidate other antifungals whose addition modulates *DDR48* expression.

P.80. Antibiotic Resistant Bacteria in Aquatic and Terrestrial Life Stages of Dragonflies

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Background: Antibiotics entering into the aquatic environment select for antibiotic resistant bacteria (ARB) in the microbiomes of aquatic organisms. It is possible that juvenile insects in contaminated aquatic environments could pass ARB on to terrestrial life stages during metamorphosis. In this study, aquatic juveniles (nymphs) and terrestrial adult dragonflies were tested for their ability to harbor ARB. The possibility of antibiotic exposure stimulating the proliferation of ARB in dragonfly nymphs was also assessed.
Methods: Nymph and adult dragonflies were collected from the University of Mississippi Field Station, Oxford, Mississippi and the Wolf River Greenway in Memphis, Tennessee. ARB were enumerated through culture on TSA media amended with the antibiotics Amoxicillin, Cefazolin, Kanamycin, Tetracycline and Vancomycin. Additional nymphs were collected from the University of Mississippi Field Station and incubated for 10 d in aquatic microcosms containing amoxicillin at 1x, 3x, and 5x minimum inhibitory concentrations. Following incubation, amoxicillin-resistant bacteria were enumerated in nymphs using TSA amended with amoxicillin.
Results: Dragonfly nymphs and adults caught and immediately used for culture methods contained bacteria resistant to multiple antibiotics. All dragonfly nymphs grown in amoxicillin-contaminated microcosms grew bacteria resistant to amoxicillin. The concentration of antibiotic in each microcosm had a significant effect on amoxicillin resistant bacterial growth (P=0.009), with number of resistant colony forming units increasing with higher concentrations of antibiotics.
Conclusion: Both juvenile and adult dragonflies harbored antibiotic resistant bacteria and exposure of juvenile nymphs to amoxicillin increased the numbers of bacteria resistant to that antibiotic. This demonstrates that aquatic insects can harbor ARB, suggesting that they may serve as a reservoir and potential source of ARB transmission.

P.81. *msaABCR* Operon is Involved in Staphylococcal Implant-Associated Osteomyelitis

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Background: *Staphylococcus aureus* is a major cause of both health care associated and community-associated infections. *S. aureus* is a primary agent of chronic bone infection also known as osteomyelitis. Treatment of osteomyelitis is very complicated and may require surgical debridement followed by prolonged antibiotic courses. Osteomyelitis patients often experience serious life threatening complications like septicemia, thrombosis and pathological fractures thus leading to high mortality and morbidity. In this study, we have investigated the role of the *msaABCR* operon in pathogenesis of osteomyelitis caused by *S. aureus*.
Methods: In this study, we used the modified chronic osteomyelitis infection model using SD rats. Medical implants (K-wire pin) were coated with *S. aureus* biofilm (wild type, *msaABCR* deletion mutant and the *msaABCR* complement), and surgically transplanted transcortically through the metaphysis in the tibia. The infected tibias were harvested after 4, 8 and 15 days and were analyzed by microbiological, Microcomputed tomography (MicroCT), and histology.
Results: MicroCT images analysis of infected tibia bone revealed that the wild type *S. aureus* strain colonized bone tissue and triggered significant bone damage of the infected tibia, whereas the *msaABCR* deletion mutant of *S. aureus* was attenuated and unable to cause chronic osteomyelitis. Bacterial count from infected tibias, blood and other vital organs (heart, Liver, kidney, spleen, lungs) showed a significant difference in bacterial load between animals infected with the *msaABCR* mutant versus the wild type. Histological examination of the wild type infected tibia bone showed significantly increased bone resorption processes relative to the *msaABCR* deletion mutant that showed increased bone formation process comparable to negative control groups.

Conclusion: This study shows that the *msaABCR* operon plays a role in the implant-associated osteomyelitis infection cause by *S. aureus*. In particular, this model shows the importance of the *msaABCR* operon in biofilm related implant-associated chronic infections which cause significant morbidity.

P.82. Paracrine Factor Signaling Regulation by HPV16 E5 Protein

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Background: Human papillomaviruses (HPV) infect keratinocytes of squamous epithelia and cause benign hyperproliferation that in some cases may progress to malignancy. The cellular changes that promote virus persistence and progression to malignancy currently remain unclear. The stroma is an important source of growth factors that can facilitate cancer growth, as well as anti-viral cytokines such as interferons. In addition, interferons are also produced by the infected host cell. HPV actively suppresses interferon production and signaling in infected keratinocytes and stromal cells. Interferon-kappa (IFNκ), a keratinocyte-specific constitutively active interferon, as well as interferons alpha and beta are suppressed by HPV.
Methods: Keratinocytes were transfected to make cells containing the complete HPV16 genome. E5 Stop cells were generated by transfected keratinocytes with a plasmid containing the complete HPV16 genome, but with a stop codon mutation in the E5 open reading frame. Cells were treated with various inhibitors to target signaling pathways. RT-qPCR analysis was performed on RNA isolated from HPV16-containing and E5 Stop cells. Protein levels were assayed via western blotting procedures.
Results: We observed decreased levels of interferons alpha (IFNα), beta (IFNβ) and IFNκ in cells containing the complete HPV16 genome versus uninfected keratinocytes. Further, we observed increases in all three IFNs in E5 Stop cells. Interestingly, cells treated with the EGFR-specific inhibitor AG1478 showed increased levels of IFNs alpha and beta, but not kappa, exhibiting the differential regulation of IFNκ. Further analysis of signaling pathways showed activated Jun-amino terminal kinase (JNK) in E5 Stop cells.
Conclusion: We have shown that IFNα, IFNβ, and IFNκ are suppressed in HPV16-containing cells in a manner that depends upon the E5 protein. We are currently testing the hypothesis that *the regulation of IFN production in HPV16-containing cells requires the E5 protein and that JNK signaling plays a role in the production of IFNs in E5 Stop cells.*

P.83. Methylene Blue Inhibits Bacterial NOD Ligand Induced Rabbit Kidney (RK13) Tubule Cell Death

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Background: Methylene blue (MB) is an essential WHO medicine used in the treatment of systemic and focal diseases. MB is currently being evaluated in a clinical trial against septic shock. The specific aim was to assess the therapeutic potential of MB against septic shock/acute renal failure by evaluating the anti-apoptotic activity of MB against NOD2-induced acute renal cell death.
Methods: MDP (NOD2 ligand) and C12-iE-DAP (NOD1 ligand) were applied to rabbit kidney (RK_{13}) cell cultures (MDP was previously shown induced RK13 cell apoptosis) that were incubated for 1 h at 37ºC in media containing different concentrations of Löffler's MB solution diluted in culture media. The cells were viewed microscopically for apoptotic cells (rounded cells with blebbing cytoplasm and fragmented nuclei) and were harvested (4-6 h post incubation) when 30-50% cell death occurred in the NOD ligand treated cells. The cell lysates were processed for caspase-3 activity, DNA was extracted and ethidium bromide staining polymeric DNA bands visualized post gel electrophoresis. The relative DNA band density was determined using imagej software.
Results: MB inhibited RK_{13} cell death, caspase activation, and DNA ladder formation induced by NOD ligands in a dose dependent manner (1:40-1:640 dilution). More specifically, MB diluted 1:80 to 1:640 inhibited microscopically assessed cell death, caspase-3 and -6 activity by 40-90%, and apoptotic DNA levels (endonuclease) by up to 100%. The 50% inhibitory dose (ID_{50}) for all parameters was obtained with a 0.5% MB solution. MB treatment at 3 h post challenge was less effective in inhibiting NOD2-induced RK_{13} cell death (apoptotic DNA).
Conclusion: MB treatment exhibits anti-apoptotic activity against NOD ligand-induced caspase-mediated apoptosis of rabbit renal tubule epithelial cells. Thus, the results suggest that the anti-apoptotic activity of MB may play a significant role in the treatment of acute renal failure during severe sepsis.

P.84. Potential of Mycobacteriophage as Candidates for Phage Therapy - Identification of Phage that Infect Nontuberculous Mycobacteria Pathogens

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Background: Nontuberculous mycobacterial (NTM) infections cause a wide range of debilitating diseases and are increasing in prevalence in the US and worldwide. With increased resistance to antibiotics by NTM pathogens, phage therapy is gaining attention as an alternative for the treatment of such infections. Over 7,000 bacteriophages that infect the non-pathogenic species *Mycobacterium smegmatis* have been isolated. Host-range tests have identified specific subclusters of phage that can infect multiple *M. smegmatis* strains and a non-pathogenic strain of *M. tuberculosis*, indicating that they may exhibit broad host range and can possibly infect NTM pathogens.

Methods: Spot tests and direction infection of three pathogenic Mycobacterium species by Mycobacteriophages isolated from *M. smegmatis* were performed to assess the broad-host range infectivity of these phage isolates against the NTM of interest. Efficiency of plating (e.o.p.) was calculated in comparison with *M. smegmatis* infection rates.
Results: Members of the A3 subcluster have multiple phage that are potentially broad host range. In addition, these phage share a specific variant of a gene (GP5) encoding a putative minor tail protein. We have tested the ability of a large series of A3 phage to infect three pathogenic Mycobacterium species (*M. abscessus*, *M. chelonae*, and *M. fortuitum*) to determine if A3 broad host range extended to NTM species and if the infectivity correlates with the gene variant. The A3 phage Rockstar and Vix display significant broad host-range infectivity for one or more of the NTM hosts. In addition, as part of the SEA-PHAGES broad host-range project, we have extended our study to thirty non-A3 phage isolates. The Mycobacteriophage Alma (A9), BPs (G1), Cain (K6), Jeon (W), Larva (K5), Muddy (singleton), PegLeg (M1), SherlockHolmes (K3), Trixie (A2), Wintermute (K4), and ZoeJ (K2) have successfully infected one or more of these NTM hosts as well. We are currently investigating the existence of related minor tail proteins in these isolates which may correlate with their broad host-range infectivity.
Conclusions: Our results demonstrate the broad host-range infectivity of a large number of mycobacteriophages isolated from the non-pathogenic *M. smegmatis* host. The ability of these phages to infect pathogenic strains of NTM provides compelling evidence for their future development as novel phage therapy candidates for NTM infections.

P.85. The Human Cytomegalovirus Tropism Regulator UL148 Induces ER stress During Infection

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Background: Enveloped viruses require the secretory pathway of host cells for processing and proper folding of viral glycoproteins. Eukaryotic cells express three different ER stress sensors, IRE1, ATF6, and PERK, that detect misfolded proteins and activate the unfolded protein response (UPR). During the UPR basal mRNA translation is inhibited while genes involved in ER associated degradation (ERAD), glycoprotein folding, and programmed cell death are upregulated. HCMV triggers the UPR, but the virus also modifies ER stress signaling pathways, for instance by degrading the ER stress sensor IRE1. Our laboratory identified that a viral endoplasmic reticulum (ER)-resident glycoprotein, UL148, strongly influences human cytomegalovirus (HCMV) tropism for epithelial cells, likely by stabilizing the expression of glycoprotein O (gO), a viral glycoprotein absolutely required for infectivity of cell-free HCMV virions. We have found that UL148 interacts with SEL1L, a subunit of the E3 ubiquitin ligase Hrd1 that plays key roles in ERAD. We also find that UL148 triggers ER stress: Ectopic expression of UL148 triggers the UPR, and leads to the induction of unusual ER structures that resemble ER quality control (ERQC) compartments. Furthermore, we find that prominent ERQC compartments occur during HCMV infection, and that their formation depends on UL148. Here, we address the influence of UL148 on ER stress in the context of the HCMV infected cell, and investigate whether ER stress is required for the effects of UL148 on viral glycoprotein expression.
Methods: Human foreskin fibroblast (HFF) cells were infected with wild-type (WT) HCMV strain TB40/E (TB_WT) carrying a S-tag at the C-terminus of gO, or a *UL148*-null mutant version of the same virus, TB_148$_{STOP}$. Cells were lysed at various time points following infection, subjected to BCA assay to allow for normalization of protein content across samples, and analyzed by Western blotting. In siRNA experiments, HFF were reverse-transfected with siRNA against IRE1, ATF6, PERK, ATF4 or a non-targeting control (NTC) siRNA. To facilitate detection of gO, TB_WT and TB_148$_{STOP}$ derivatives that express an S-tag at the C-terminus of gO were used to infect cells (MOI=2) at 30 h post siRNA transfection. At 96 h post infection, lysates were harvested for western blot analysis.
Results: In cells infected with WT HCMV, upregulated expression of ATF4 occurred by at 48 hpi, roughly coinciding with the onset of UL148 expression. In contrast, *UL148*-null infected cells did not accumulate ATF4 at levels comparable to WT infections until 96 hpi. These results suggest that UL148 induces ATF4 expression during HCMV infection. Treatments with siRNA against ATF4 or ATF6 led to enhanced levels of gO in both WT and *UL148*-null infection settings. IRE1 siRNA however, led to enhanced levels of gO during infection with WT, but not *UL148*-null virus. Remarkably, ATF6 siRNA treated cells showed signs of markedly enhanced viral replication relative to the non-targeting control siRNA or to any of the other siRNA conditions: the observed cytopathic effect was more severe, viral late proteins were expressed at more robust levels, and higher titers of virus were released to infected cell supernatants. This observation, while preliminary, suggests that ATF6 may restrict HCMV replication. Taken together, our results suggest that the HCMV ER resident protein UL148 triggers ER stress to modulate the expression of gO, and imply that viral interplay with ER stress signaling pathways exert a powerful influence on HCMV cell tropism and infectivity.

P.86. Co-Infection Studies with *M. haemoltica* and Bovine Herpesvirus Type 1

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Background: Bovine herpesvirus (BHV-1) and *Mannheimia haemolytica* are key factors in the development of bovine respiratory disease (BRD), a poly-microbial condition that that costs the US cattle industry billions of dollars every year. While *M.haemolytica* is a commensal respiratory microbe, BHV-1 is not and initiates an acute viral infection either through spread from another infected animal or due to reactivation from latency. Typically, both the viral infection and the underlying bacterial infection are required for disease presentation. In this study, we focused on how BHV-1 and *Mannheimia haemolytica* affect each other's replication cycle when co-infecting bovine cells.

Methods: We established a series of dual infections using cultured bovine kidney cells, BoHV-1 stocks and logarithmic growing cultures of *M. haemolytica*. Co-infections occurred for approx. 24 hours. We varied *M. haemolytica* concentrations to observe changes in viral infectivity due to increasing amounts of the bacterium. BoHV-1 was quantitated using plaque assay.
Results: Our results suggest that viral infectivity decreases in the presence of increasing concentrations of *Mannheimia haemolytica*. In addition, this effect may be dependent upon the number of infecting BoHV-1 particles.
Conclusion: Replication of individual BRD microbes is affected by replication/metabolism of other's when in a co-infection scenario. We are currently working to test different viral and bacterial concentrations and times of infection, in addition to testing additional BRD pathogens.

P.87. Assessing the Use of Human Transferrin (hTF) to Improve a Mouse Model of *Neisseria gonorrhoeae* Upper Genital Tract Infections

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Background: The increasing antimicrobial resistance of *Neisseria gonorrhoeae* (Gc) raises a growing public health concern. There are approximately 800,000 new cases each year in the United States. This sexually transmittable pathogen is often asymptomatic and can ascend from the lower genital tract (LGT) to the upper genital tract (UGT) causing complications in women that include: pelvic inflammatory disease (PID), ectopic pregnancy and infertility. Ultimately, the development of new drugs for gonorrhea treatment is necessary. Usable iron sources are not abundant to Gc in host tissues, particularly in the UGT. However, Gc can use human transferrin (hTF) as an iron source. Here we characterized UGT Gc infection in hTF-treated mice. Understanding the mechanisms behind Gc colonization and ascension in a mouse model will provide further knowledge to guide future vaccine experiments.
Methods: Two groups of 17-β-estradiol female BALB/c mice were infected with Gc strain FA1090 by either transcervical or vaginal inoculation. One group was treated with hTF. Colonization load was measured via culturing of UGT (endometrium and oviducts) and LGT. ELISA assays were used to quantify antibody (total IgG) concentrations in samples of venous serum. Histopathology studies were done on UGTs on day 20 post-bacterial inoculation.
Results: Graphed data show increased colonization of UGT and LGT in mice treated with hTF, although the difference was inconsistently significant in the LGT ($p<0.05$). Swelling and fluid accumulation in the oviducts (hydrosalpinx) and endometrium of infected hTF-treated mice were observed on day 20. Analysis of ELISA results showed an increase in serum total IgG between day 10 and 20 post-infection.
Conclusion: Administration of hTF to mice increases Gc colonization in the UGT and LGT. Gross histopathology of the UGT revealed inflammation at day 20 in hTF-treated mice that were transcervically infected with Gc. Preliminary data showed evidence of a Gc-specific antibody response to infection as infection progressed. These results support the use of hTF supplementated mice as a model of Gc PID.

P.88. Arthropod-Borne Diseases Since 1996: A Review of World-Wide Outbreaks

Veronica A. Stewart, Kara L. Ashworth, and William H. Dees, *McNeese State University*

Background: Over the past twenty years the World Health Organization has reported disease outbreaks from all over the world via Disease Outbreak News (DON; www.who.int). Disease outbreaks were reviewed, and the biome in which they occurred was determined.
Methods: We reviewed arthropod-borne disease outbreaks occurring since 1996 using DON and other sources. Outbreaks were categorized by disease, continent, and biome. For each disease, a timeline was constructed to show outbreak occurrence. The overall case fatality rate for each outbreak also was included.
Results: The arthropod-borne diseases reported by DON include: dengue/dengue hemorrhagic fever, yellow fever, chikungunya, Rift Valley fever, Zika, malaria, O'nyong-nyong, Japanese encephalitis, St. Louis encephalitis, Venezuelan equine encephalitis, West Nile fever, leishmaniasis, Crimean-Congo hemorrhagic fever, relapsing fever, tularemia, typhus (louse-borne), and plague. The five most reported diseases were yellow fever, dengue/dengue hemorrhagic fever, Zika, plague, and Rift Valley fever. The biome in which four out of the five most reported diseases occurred was tropical. Zika and dengue/dengue hemorrhagic fever occurred mainly in humid tropical regions; whereas, plague and yellow fever occurred mainly in semi-arid tropical regions. Rift Valley fever occurred mainly in the desert (tropical) region.
Conclusion: The majority of disease outbreaks reported occurred in the tropical biome. *Aedes* species vectored the majority of disease pathogens reported. Plague was the only non-mosquito borne pathogen reported of significance. Understanding the environmental conditions affecting disease transmission provides further information for preventing and controlling vector-borne disease outbreaks.

P.89. Bacterial Diseases Since 1996: A Review of World-Wide Outbreaks

Veronica A. Stewart, Kara L. Ashworth, and William H. Dees, *McNeese State University*

Background: Over the past twenty years the World Health Organization has reported disease outbreaks from all over the world via Disease Outbreak News (DON; www.who.int). Disease outbreaks were reviewed, and the biome in which they occurred was determined.
Methods: We reviewed bacterial disease outbreaks occurring since 1996 using DON and other sources. Outbreaks were categorized by disease, continent, and biome. For each disease, a timeline was constructed to show outbreak occurrence. The overall case fatality rate for each outbreak also was included.

Results: The bacterial diseases reported by DON include: anthrax, cholera, leptospirosis, botulism, diphtheria, enterohemorrhagic *E. coli*, Legionnaire's disease, bacterial meningitis/meningococcal disease, typhoid fever, shigella/dysentery, pertussis, and listeriosis. The three most reported diseases were cholera, bacterial meningitis/meningococcal disease, and Legionnaire's disease. Cholera and bacterial meningitis occurred mainly in semi-arid tropical regions; whereas, Legionnaire's disease occurred mainly in humid temperate regions. Cholera and bacterial meningitis were reported ten times more often than any other bacterial disease.
Conclusion: The majority of disease outbreaks reported occurred in the tropical biome. The narrow zone of Africa, from Guinea-Bissau to Ethiopia, is predominantly a semi-arid tropical region. This zone, often called the meningitis belt, is where the majority of bacterial disease outbreaks have been reported. Understanding environmental conditions that affect disease transmission provides further information to prevent and control bacterial outbreaks in the future.

P.90. Assessment of Reovirus Nonstructural Protein Σ1s Function in Different Genetic Backgrounds

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Previous work from our laboratory revealed that reovirus nonstructural protein σ1s is facilitates efficient replication of a serotype 1 (T1) reovirus by promoting viral protein production. Here, we assessed the function of T1 and serotype 3 (T3) σ1s proteins in different viral genetic backgrounds. We compared viral replication and protein production for wild type and σ1s-deficient T1 and T3 viruses (T1L and T1L σ1s-null; T3D and T3D σ1s-null). Reoviruses have segmented genomes allowing for generation of reassortants between T1L and T3D. We tested wild type and σ1s-null reassortants where the S1 gene, which encodes attachment protein σ1 and nonstructural protein σ1s, was exchanged between the T1L and T3D genetic backgrounds. T1L/T3DS1 contains 9 segments from T1L and the S1 gene from T3D, whereas T3D/T1LS1 contains 9 genes from T3D with the S1 gene from T1L. We found that σ1s was required for efficient viral protein production for all four genetic backgrounds. Markedly less viral protein was synthesized for T1L σ1s-null, T3D σ1s-null, T3D/T1LS1 σ1s-null and T1L/T3D σ1s-null compared to the cognate wild type viruses. Replication of T1L σ1s-null, T1L/T3D σ1s-null, and T3D/T1L σ1s-null were markedly diminished compared to wild type viruses. Although T3D σ1s-null produced lower viral yields than T3D, the difference was not as large as the disparity observed in the other genetic backgrounds. Together, these results indicate that T1 and T3 σ1s proteins function to promote reovirus replication by enhances viral protein synthesis. These findings also indicate the viral genetic background can influence the requirement for σ1s.

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1984	University of Arkansas for Medical Sciences, Little Rock, AR
1985	McNeese State University, Lake Charles, LA
1986	Louisiana State University Medical Center, Shreveport, LA
1987	Southeastern Louisiana University, Hammond, LA
1988	Louisiana State University, Baton Rouge, LA
1989	Perdido Beach, Florida (Inter-Branch Meeting)
1990	University of Arkansas for Medical Sciences, Little Rock, AR
1991	University of Mississippi Medical Center, Jackson, MS
1992	Mississippi State University, University, MS
1993	Louisiana State University, Baton Rouge, LA
1994	Louisiana State University Medical Center, Shreveport, LA
1995	University of Arkansas for Medical Sciences, Little Rock, AR
1996	Tulane University School of Medicine, New Orleans, LA
1997	University of Mississippi Medical Center, Jackson, MS
1998	University of Alabama, Montgomery, AL
1999	Louisiana State University Health Science Center, New Orleans, LA
2000	University of Arkansas for Medical Sciences, Little Rock, AR
2001	Louisiana State University Veterinary School, Baton Rouge, LA
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2004	Mississippi State University, Starkville, MS
2005	University of Louisiana at Lafayette, Lafayette, LA
2006	Louisiana State University School of Veterinary Medicine, Baton Rouge, LA
2007	University of Arkansas at Little Rock, AR
2008	University of Texas, Austin, TX
2009	Nicholls State University, Thibodaux, LA
2010	The University of Southern Mississippi, Hattiesburg, MS
2011	The University of Louisiana at Monroe, Monroe, LA
2012	Mississippi State University, Starkville, MS
2013	Joint Meeting with Texas Branch at Tulane University, New Orleans, LA
2014	University of Arkansas, Fayetteville, AR
2015	The University of Southern Mississippi, Hattiesburg, MS
2016	The University of Louisiana, Lafayette, LA
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