American Society for Microbiology

South Central Branch Meeting

November 1 - 2, 2019

University of Mississippi - Oxford, MS

Department of Biology

AMERICAN SOCIETY FOR MICROBIOLOGY

THE UNIVERSITY of MISSISSIPPI

ASM Branches

President's Welcome

Dear Colleagues,

It is my pleasure to welcome you to Oxford, Mississippi for the 2019 Annual Meeting of the South Central Branch of the American Society for Microbiology. The Biology Department of the University of Mississippi has graciously supported hosting this year's meeting. I would like to thank the organizer of this year's meeting, Dr. Wayne Gray from the UM Department of Biology, for his efforts in developing this year's program. I am very grateful for the time and effort that 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. Joel Kostka from the Schools of Biology and Earth & Atmospheric Sciences at the Georgia Institute of Technology. Dr. Kostka 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 which include the following topic areas: Eukaryotic Microbiology, Bacteriology, Immunology, Virology, and Applied and Environmental Microbiology. In addition, we have a special education session presented by Dr. Bill Wischusen of LSU and the Charles Randall Lecture presented by Dr. Jonas King of Mississippi State University.

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,

Dr. Jon S. Blevins, *ASM South Central Branch President*

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2018-19 Officers
South Central Branch ASM

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Meeting Agenda

Friday, November 1, 2019

10 am – 12:30 pm	**Ole Miss Campus Walking Tours** Meet in Ballroom Concourse		
10 am – 2 pm	**Registration** Inn at Ole Miss Ballroom Concourse		
12:00-1:00 pm	Lunch on Your Own- Visit the Ole Miss Student Union		
1:00- 2:30 pm	**Eukaryotic- Appl. & Environ.** Selected Oral Presentations Ballroom B	**Immunology** Selected Oral Presentations Ballroom C	**Bacteriology** Selected Oral Presentations Ballroom D
2:30- 3:00 pm	**Refreshments and Poster Set-Up** Ballroom A and B		
3:00- 4:15 pm		**Virology** Selected Oral Presentations Ballroom C	**Applied & Environmental** Selected Oral Presentations Ballroom D
4:30- 5:20 pm	**Keynote Lecture- Dr. Joel Kostka, Ga. Tech** *The Role of Microbes in the Response to the Deepwater Horizon Oil Spill in the Gulf of Mexico* Ballrooms C,D		
5:20- 7:20 pm	**Poster Session & Reception** Ballrooms A,B		
7:20 pm	**Dinner on Your Own- Visit the Oxford Square**		

Saturday, November 2, 2019

7:00 - 8:00 am	**Light Breakfast and Coffee - Visit the Posters** Ballroom A, B	
8:00 - 10:15 am	**Pathogenesis -Immunology- Virology** Selected Oral Presentations Ballroom C	**Bacteriolology- Environmental** Selected Oral Presentations Ballroom D
10:30 - 11:30 am	**Education Session- Dr. William Wischusen- LSU Baton Rouge** *Active Learning in a Large Classroom, Can You Really Engage Students?* Ballrooms C,D	
11:30 am - 12:15 pm	**Charles C. Randall Lecture – Dr. Jonas King, Mississippi State University** *Host-microbe interactions in a diversity of arthropod systems* Ballrooms C,D	
12:30 - 1:30 pm	**Business Meeting and Announcement of Awards** Lunch Provided Ballroom A	

Keynote Speaker

Dr. Joel Kostka
Professor and Associate Chair
Schools of Biology and Earth and Atmospheric Sciences
Georgia Institute of Technology

We are pleased to have Dr. Joel Kostka as our American Society for Microbiology Distinguished Lecturer at this year's meeting.

"A Moveable Feast: The Role of Microbes in the Response to the Deepwater Horizon Oil Spill in the Gulf of Mexico"

This presentation will describe the ecosystem services provided by the Gulf of Mexico to the U.S., emphasizing the vastness of this "small" ocean basin and its huge ecological diversity. The science of the Deepwater Horizon oil spill and response to the disaster, with photos and video describing the scale of the disaster, will be described. The second half of the talk will discuss a case study of our ongoing research on how microbes impact the fate of oil contamination on beaches of the Gulf coast. We have observed a bloom of successive microbial populations that degrade oil, isolated new hydrocarbon-degrading bacteria, and described major impacts of oil on the microbial nitrogen cycle.

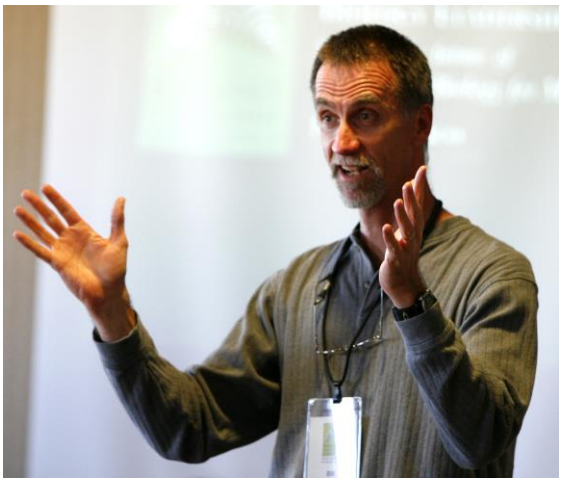
BIOGRAPHICAL SKETCH

Dr. Kostka is internationally recognized for his research in environmental microbiology which focuses on characterizing the role of microorganisms in ecosystem functioning, especially in the context of bioremediation and climate change. He is extensively published with over 110 peer-reviewed publications. He has served on numerous national or international review panels and expert committees on energy, bioremediation, and environmental microbiology. Dr. Kostka was honored as a Georgia Power Professor of Excellence, and he is currently a co-PI of the C-IMAGE3 consortium funded by the Gulf of Mexico Research Initiative to study the environmental consequences of petroleum hydrocarbon release on living marine resources and ecosystem health. In 2011, he coauthored the report "Microbes and Oil Spills: Frequently Asked Questions" published by the American Academy of Microbiology. In 2016, he participated in the ASM-AGU Colloquium on "Interactions Between Climate Change & Microbial Ecosystems." From 2009-2013, Dr. Kostka was Chair of ASM's Division N, Microbial Ecology, and he served as editor of Applied and Environmental Microbiology from 2011-2017.

Please visit Dr. Kistka's website for more info about his work: http://www.joelkostka.net

Special Educational Session

Dr. William Wischusen
Associate Professor
Biology Department
LSU Baton Rouge



"Active Learning in a Large Classroom, Can You Really Engage Students?"

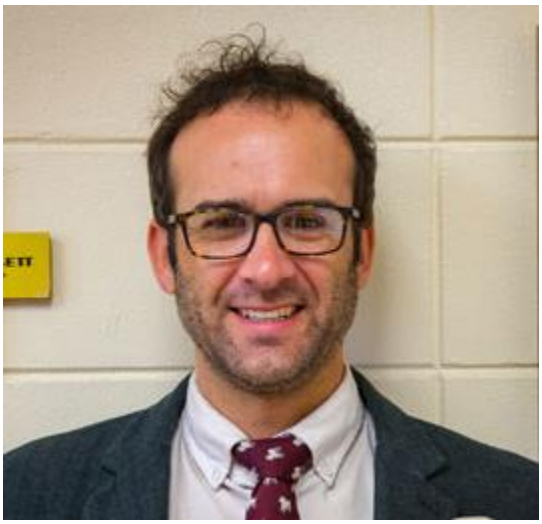
Dr. Wischusen is the Ron and Dr. Mary Neal Geaux Teach Distinguished Professor and an Associate Professor in the Department of Biological Sciences at Louisiana State University. He has degrees from Clark University (BA), The University of Alabama (MS), and Cornell University (Ph. D.) Prior to being on the faculty at LSU he was on the faculty at the University of Nevada, Las Vegas.

Dr. Wischusen's research is two-fold between vertebrate ecology and science education. He is particularly interested in understanding the animal habitat interactions of mammals and birds.

Bill has earned many awards for his teaching including the LSU Tiger Athletic Foundation Undergraduate Teaching Award, the LSU BP Award for Outstanding Undergraduate Teaching, The LSU President's Award for Excellence in Undergraduate Teaching, and the LSU Torchbearer Award for Leadership in IT advancement. Additionally he has been named a National Academies Education Fellow in the Life Sciences in 2004 and a Scientific Teaching Mentor, 2012-2019. For the past three years he has been the head of the LSU Learning and Teaching Collaborative and served as one of the co-leads for the LSU-Center for the Integration of Research, Teaching, and Learning (CIRTL) program.

Charles C. Randall Lectureship

Dr. Jonas King
Assistant Professor
Departments of Biochemistry, Molecular Biology, Entomology, and Plant Pathology
Mississippi State University



"Host-microbe interactions in a diversity of arthropod systems"

Dr. King received his Bachelor of Arts degree with a major in Biology from the University of Mississippi in 2002. In 2007, he received a Master of Science degree at the University of Mississippi working on molecular systematics and entomology with Dr. Paul Lago. He was awarded his Ph.D. in 2012 working with Dr. Julian Hillyer on host-parasite interactions and insect physiology. His continued his work on host-parasaie interactions during postdoctoral training at Fort Detrick and Johns Hopkins University.

Dr. King's current research interest at Mississippi State University focuses on insect-host interactions, insect microbiomes, and development of novel diagnostics for insect vectored diseases.

This 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 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.

Selected Oral Presentations

Session 1- Eukaryotic and Applied and Environmental Microbiology

Friday, November 1st, 1:00-2:30 p.m., The Inn at Ole Miss, Ballroom B

Chairs: Dr. Erik Hom and Dr. Peter Zee, *The University of Mississippi*

1:00 pm **Comparative dual-transcriptomics of myco- and photobionts in model "Franken-Lichens"**
Michael Clear, *The University of Mississippi*

1:15 pm **The role of collecting chlorophyll, A-1, and A0 in energy and electron transfer in the photosystem I of Synechocystis sp. PCC 6803**
Lujun Luo, *University of Louisiana at Lafayette*

1:30 pm **Transcriptomic analysis of *Listeria monocytogenes* in response to bile under aerobic and anaerobic conditions**
Damayanti Chakravarty, *The University of Southern Mississippi*

1:45 pm **How do host species and habitat influence the gut microbiomes of freshwater mussels?**
Mark McCauley, *The University of Mississippi*

2:00 pm **The antimicrobial activity and cellular targets of p-anisaldehyde and epigallocatechin gallate in the opportunistic human pathogen *Pseudomonas aeruginosa***
Yetunde Adewunmi, *The University of Southern Mississippi*

2:15 pm **Probing microbial interactions and coordinated trophic responses in biological soil crusts**
Dr. Erik Hom, *The University of Mississippi*

Session 2 – Immunology

Friday, November 1st, 1:00-2:30 p.m., The Inn at Ole Miss, Ballroom C

Chairs: Dr. Brian Doctor, *The University of Mississippi,* and **Dr. Jason Bodily**, LSU Health Sciences Center, Shreveport

1:00 pm **Mother knows best: immune-based maternal effects in response to *Mycoplasma gallisepticum* infection in eastern bluebirds (*Sialia sialis*)**
Sarah D. Amonett, *The University of Mississippi*

1:15 pm **Rotavirus NSP1 does not require active cullins to inhibit IFN-β**
Kellie N. Brown, *LSU Health Sciences Center – Shreveport*

1:30 pm **The role of marginal zone B cells in the early humoral response to *Plasmodium yoelii* Infection**
Kara O'Neal, *The University of Arkansas for Medical Sciences*

1:45 pm ***In vivo* expansion of CMV-specific anti-HIV CAR T cells following CMV superinfection**
Nathan Johnson, *Tulane National Primate Research Center*

2:00 pm **Interaction between the Human Papillomavirus oncoprotein E7 and cyclin dependent kinase 8 contributes to the suppesssion of host immune responses**
Sadie M. Rice, *LSU Health Sciences Center – Shreveport*

2:15 pm **CD8+ lymphocytes modulate Zika virus dynamics and antiviral immunity in nonhuman primates**
Blake Schouest, *Tulane National Primate Research Center*

Session 3 – Bacteriology

Friday, November 1st, 1:00-2:30 p.m., The Inn at Ole Miss, Ballroom D

Chairs: Dr. Cole Stevens and Dr. Patrick Curtis, *The University of Mississippi*

1:00 pm **Development of a highly specific ELISA for Staphylococcal toxins**
Jessica Surma, *Mississippi State University*

1:15 pm **Impact of *msaABCR* on *sarA*-associated phenotypes in diverse clinical isolates of *Staphylococcus aureus***
Joseph S. Rom, *The University of Arkansas for Medical Sciences*

1:30 pm **Investigating the role of the di-adenylate cyclase, CdaA, during *Borrelia turicatae* mammalian infection**
Clay D. Jackson-Litteken, *The University of Arkansas for Medical Sciences*

1:45 pm **Molecular and physiological logic of *msaABCR* induction by central metabolite pyruvate to regulate virulence and biofilm development**
Bibek G C, *The University of Southern Mississippi*

2:00 pm **The *Y. pestis* translational GTPase BipA promotes Pneumonic Plague pathogenesis**
Samantha D. Huckuntod, *The University of Arkansas for Medical Sciences*

2:15 pm **Regulatory role of *msaABCR* operon in persister formation against aminoglycoside stress in *Staphylococcus aureus***
Shanti Pandey, *The University of Southern Mississippi*

Session 4 – Virology

Friday, November 1st, 3:00-4:15 p.m., The Inn at Ole Miss, Ballroom C

Chairs: Dr. Jason Paris, *The University of Mississippi* and **Dr. Ritesh Tandon**, *University of Mississippi Medical Center*

3:00 pm **HCMV-induced signaling through the Epidermal Growth Factor receptor directs viral trafficking to the nucleus in primary human monocytes**
Heather L. Fulkerson, *LSU Health Sciences Center – Shreveport*

3:15 pm **MicroRNAs as predictive biomarkers of Human Papillomavirus (HPV) associated low-grade cervical dysplasia outcomes**
Ashley N. Winters, *LSU Health Sciences Center – New Orleans*

3:30 pm **Increased risk of early cytomegalovirus (CMV) infection in SIV-infected infant rhesus macaques**
Matilda Moström, *Tulane National Primate Research Center*

3:45 pm **Rotavirus NSP1 requires a functional RING domain for nuclear foci formation**
Samantha K. Murphy, *LSU Health Sciences Center – Shreveport*

4:00 pm **A 3D-spheroid cell culture system provides a sensible *in-vitro* model for HCMV infection**
Dipanwita Mitra, *University of Mississippi Medical Center*

Session 5 – Applied and Environmental Microbiology

Friday, November 1st, 3:00-4:15 p.m., The Inn at Ole Miss, Ballroom D

Chair: Dr. Peter Zee, *The University of Mississippi* and **Dr. Janet Donaldson**, *University of Southern Mississippi*

3:00 pm **The microbiome of North American crayfish carapaces differs between species and by location**
Lauren A. Lawson, *The University of Mississippi*

3:15 pm **Bacterial metabolic activity and counts near Big Creek, a tributary of the Buffalo National River, Arkansas**
Nina M. Hoffpauir, *U.S. Geological Survey, Wetland and Aquatic Research Center, Lafayette LA*

3:30 pm **Elucidating the Sloth Hair microbiome: A metagenomic comparison of Two- and Three-fingered Sloths**
Maya Kaup, *The University of Mississippi*

3:45 pm **Biodegradation of Metribuzin under various electron acceptor conditions**
Thomas J. Phillips III, *Nicholls State University*

4:00 pm **Hypersaline bacterial community response to simulated Martian conditions**
Eric A. Weingarten, *The University of Mississippi*

Session 6 – Pathogenesis, Immunology, and Virology

Saturday, November 2nd, 8:00-10:15 a.m., The Inn at Ole Miss, Ballroom C

Chairs: Dr. Jason Paris, *The University of Mississippi,* **Dr. Vicki Traina-Dorge,** *Tulane National Primate Research Center* and **Dr. William Gallaher**, *LSU Health Sciences Center – New Orleans*

8:00 am **Role of DeoR transcriptional regulators in virulence and environmental adaptation in *Listeria moncytogenes***
Dr. Reshma Ramachandran, *Mississippi State University*

8:15 am **The Plasminogen activator protease plays a dual role during primary pneumonic plague**
Dr. Srijon K. Banerjee, *University of Arkansas for Medical Sciences*

8:30 am **The degree of polymerization and sulfation patterns in heparan sulfate are critical determinants of Cytomegalovirus infectivity**
Dr. Ritesh Tandon, *University of Mississippi Medical Center*

8:45 am **Efficacy of novel recombinant Simian Varicella Virus-Simian Immunodeficiency vaccines following mucosal SIV challenge in the rhesus macaque**
Dr. Vicki Traina-Dorge, *Tulane National Primate Research Center*

9:00 am **Break**

9:15 am **The landscape of immune responses and persistent viral reservoirs in systemic and lymphoid tissues in rhesus macaques infected by a newly developed SHIV-CH848**
Dr. Widade Ziani, *Tulane National Primate Research Center*

9:30 am **Effect of stromal interferon response factor 3 on Human Papillomavirus 16 life cycle**
Dr. Gaurav Raikhy, *LSU Health Sciences Center – Shreveport*

9:45 am **HIV-1 Tat and morphine promote neuroendocrine dysfunction that may involve disruption of mitochondrial complex activity**
Dr. Jason J. Paris, *The University of Mississippi*

10:00 am **Viral membrane-interactive motifs: playing in the sequence sandbox**
Dr. William R. Gallaher, *LSU Health Sciences Center – New Orleans*

Session 7 – Bacteriology and Environmental Microbiology

Saturday, November 2nd, 8:00-10:15 a.m., The Inn at Ole Miss, Ballroom D

Chairs: Dr. Cole Stevens and Dr. Patrick Curtis, *The University of Mississippi*

8:00 am **Environment and neutral processes shape the skin microbiome of the introduced European catfish (*Silurus glanis*)**

Dr. Marlène Chiarello, Laboratory of Functional Ecology and Environment (EcoLab), University of Toulouse, Toulouse, France

8:15 am **Shared evolutionary history and the weakening of ecological priority effects**
Dr. Peter Zee, *The University of Mississippi*

8:30 am **Interaction between *Streptococcus pneumoniae* and *Staphylococcus aureus* generates $^{\bullet}$OH radicals that rapidly kills *Staphylococcus aureus* strains**
Dr. Jorge E. Vidal, *University of Mississippi Medical Center*

8:45 am **Myxobacterial response to the phytohormone methyljasmonate supports plant recruitment of micropredators within rhizobiomes**
Dr. Cole Stevens, *The University of Mississippi*

9:00 am **Break**

9:15 am ***Mycobacterium tuberculosis* Rv1075c and LipE are important for intracellular growth and *in vivo* infection**
Dr. Ying Kong, *University of Tennessee Health Science Center*

9:30 am **Analysis of *Brevundimonas subvibrioides* developmental signaling systems reveals inconsistencies between phenotypes and c-di-GMP levels**
Dr. Patrick Curtis, *The University of Mississippi*

9:45 am **The effects of diabetic urinary microenvironment on the fitness and virulence of *Streptococcus agalactiae***
Preeti John, *University of Louisiana at Lafayette*

10:00 am **Patterns in the diversity of a Mississippi River microbiome across space**
Dr. Cliff Ochs, *The University of Mississippi*

Abstracts of Selected Oral Presentations

Session 1 Talks: Eukaryotic, Applied, and Environmental Microbiology

S.1. Comparative Dual-Transcriptomics of Myco- and Photobionts in Model "Franken-Lichens"
Michael Clear and Erik Hom, *The University of Mississippi*

Phylogenetic analyses have suggested that algal ancestors to plants were pre-adapted for interactions with mycorrhizal fungi. Synthetic approaches have also revealed an apparent innate capacity for fungi to interact with photoautotrophs under certain environmental conditions. Past work identified 'sticking' between the model green alga *Chlamydomonas reinhardtii* and two model Ascomycetes as well as endophytic Ascomycetes. However, little is known about what drives the formation of these tight physical associations. Here, we provide a first look into the dual-transcriptome of *Neurospora crassa* and *Aspergillus nidulans* when in co-culture with *C. reinhardtii* and investigate common differentially expressed genes between the mycobionts and the photobiont when co-cultured with different fungi. In particular, we examine genes related to lipid synthesis and transfer, carbon and nitrogen metabolism, and cell wall remodeling processes in both partners in relation to the formation of symbiotic fungal-algal tissues.

S.2. The role of collecting chlorophyll, A_{-1}, and A_0 in energy and electron transfer in the photosystem I of *Synechocystis* sp. PCC 6803
[1]Lujun Luo, [1]Andrei Chistoserdov, [2]Sergei Savikhin, [3]John H. Golbeck, [1]Wu Xu, *[1]University of Louisiana, Lafayette LA 70504, [2]Purdue University IN 47907, [3]The Pennsylvania State University, PA 16802*

Background**:** Photosystem I (PS I) is one of two chlorophyll-containing, light-driven oxidoreductases in the photosynthetic membrane. The 2.5-Å crystallographic structure of trimeric PSI (Jordon P. et al. (2001) *Nature* 411:909-917) revealed that the collecting chlorophylls (eC-39/eC-40) among 90 antenna chlorophylls are the closest to the electron transfer chain. A_{-1} and A_0 accept electrons from the P700 reaction center and reduces the electron acceptor A_1, a phylloquinone molecule. There is a histidine residue either from PsaA (H730) and PsaB (H709) core protein forms a ligand bond with each of the chlorophylls that have been assigned as collecting chlorophylls. For A_0, there is a tyrosyl residue from the PsaA (Y692) and PsaB (Y667) core proteins forms a hydrogen bond. And there is one water molecule is held by the asparagine residue from the PsaA (N600) and PsaB (N582) and the magnesium of A_{-1}. To examine the function of all these chlorophylls, we replaced these residues with different residues, which turned out PsaA-H730L, all substitutions (N was replaced by M,Q,L,H) of A_{-1}, PsaA-Y692F and PsaA-Y692F/PsaB-Y667F, PsaA-M684H/PsaB-M659H did affect their growth rate and reduced their low-temperature 77K fluorescence intensity and oxygen evolution.
Methods**:** A PCR-based method was used to generate the mutant recombinant DNAs using pIBC and pBC templates. The wild type, almost all mutant and RWT (recovered wild type) strains were cultured in the BG-11 medium with or without 5 mM glucose under 40 µE m^{-2} s^{-1} light. Growth rates of cell cultures was monitored by measuring the absorbance at 730 nm. When the cultures reached approximately 0.8 A730/mL, which were harvested for the low-temperature (77K) fluorescence emission spectra according to the previously published method. Oxygen evolution from the whole cells, and the Photosystem I complexes were purified by sucrose-gradient ultracentrifuge also were obtained as previously published method. The room-temperature absorption spectra got from using an Agilent 8453 UV-visible spectrophotometer and were normalized at 680nm. Ultrafast spectroscopy and ($P700^+$-P700) spectra were executed as in detail elsewhere.
Results: For the collecting chlorophylls, replacement of PsaA-H730 with Leu increased the doubling time, and the chlorophylls content, 77K fluorescence intensity of photosystem I and its oxygen evolution reduced about 10%. For all the mutations about A_{-1}, especially for the replacement of PsaB-N582 with His and Met, the doubling time increased over 3 times, and significantly affected PS I function. We also found that the all double mutant strains PsaA-N600M/PsaB-N582M, PsaA-N600Q/PsaB-N582Q, PsaA-N600L/PsaB-N582L, and PsaA-N600H/PsaB-N582H couldn't grow without glucose. For the A_0, PsaB-Tyr667 with Phe, did not affect the assembly and accumulation of PS I, but its substitution with Ala reduced cellular levels of PS I. The doubling mutation of PsaA-Y692F/PsaB-Y667F and PsaA-M684H/PsaB-M659H, chlorophylls content, 77K fluorescence intensity and its oxygen evolution were significantly reduced. These PS I mutants are being characterized by spectroscopic measurements. The 77K fluorescence intensity of PsaB-W664F reduced about 15%.
Conclusion: The A_{-1} mutants in A branch grow slower in the absence of glucose. A_{-1} double mutants cannot grow without glucose. A_0 ligand mutants either from PsaA or PsaB side can grow without glucose. However, the double mutant (PsaA-M684H/PsaB-M659H) lost its photoautotrophic growth ability. PsaB-W664F cannot grow without glucose.

S.3. Transcriptomic analysis of *Listeria monocytogenes* in response to bile under aerobic and anaerobic conditions.
Damayanti Chakravarty[1], Gyan Sahukhal[1], Mark Arwick[2], Morgan Wright[3], and Janet R. Donaldson[1*]**;** *[1] Cell and Molecular Biology, The University of Southern Mississippi, Hattiesburg, MS. [2]Institute for Genomics, Biocomputing and Biotechnology,*

Mississippi State University, Mississippi State, MS. [3]Department of Biological Sciences, Mississippi State University, Mississippi State, MS.

Background: *Listeria monocytogenes* is a dangerous food borne bacterium that is the third leading cause of death from food borne pathogens in the USA. Typically acquired through consumption of contaminated ready to eat products, such as cantaloupes and ice cream, *L. monocytogenes* must be able to sense and respond to bile encountered within the small intestine. *Listeria monocytogenes* possesses response mechanisms that allow it to survive the bactericidal effect of bile. Though these mechanisms have been identified, it is not known how this response occurs under physiologically relevant anaerobic conditions. Our hypothesis was genes involving pathogenesis are differentially regulated under conditions mimicking different parts of the gastrointestinal tract.
Methods: Our investigation involved analyzing the transcriptome of *L. monocytogenes* following exposure to bile under aerobic or anaerobic conditions at a pH of either 7.5 or 5, mimicking different parts of the GI tract. After obtaining the raw RNA sequencing reads, data was analyzed using several softwares packaged into a bioinformatics pipeline. At the end of pipeline, differentially expressed genes were obtained and genes greater than $\pm$ 3 fold were selected for further study.
Results: Genes responsible for adhesion and intracellular survival were upregulated under anaerobic conditions. Genes responsible for two component system were also upregulated, which could indicate a potential novel stress recognition and response system. Interestingly, the *pdeD* gene, which codes for a known oxygen sensor and regulator of cyclic-di-GMP concentrations, was also upregulated at certain mimicking conditions. Expression of pdeD gene also increased under anaerobic conditions when measured in vitro. A *pdeD* mutant was constructed and was found to be sensitive to bile, as well as to intracellular growth. Concentration of c-di-GMP increased when the pdeD mutant was exposed to anaerobic environment compared to aerobic.
Conclusion: These data indicate that c-di-GMP may be regulated in response to oxygen availability. This needs to be further analyzed in future directions.

S.4. How do host species and habitat influence the gut microbiomes of freshwater mussels?

Mark McCauley, Marlène Chiarello, Carla L. Atkinson, Colin R. Jackson, *The University of Mississippi, Oxford, Mississippi, The University of Alabama, Tuscaloosa, Alabama*

Background: Unionidae are a diverse, species rich family of freshwater mussels, yet, data available on their bacterial symbionts is sparse. We present the largest study of gut bacterial microbiomes in freshwater mussels, furthering our understanding of this ecologically important symbiosis. Although sedentary filter feeders, the bacterial composition of their gut is not constrained by the bacterial community locally present in the water column and sediment. However, the degree to which the environment and/or the host plays a role in the bacterial composition of the gut is unknown. Methods: We examined the gut microbiomes of 10 Unionid mussel species collected at various sites along the Sipsey river, Alabama, USA. Extracted DNA was amplified with Illumina MiSeq, producing 16S rRNA gene sequences. We assessed the alpha (observed species richness and Inverse Simpson index) and beta diversity (Bray-Curtis dissimilarity index) of the bacterial communities within host species that were present at multiple sites as well as between various host species present at each site.
Results: There were significant differences in the alpha and beta diversity of gut bacterial communities between mussel species, as well as variation within each species at different sites. Shell length and mass were not significantly correlated to the microbial community of any mussel species. Overall, Proteobacteria and Firmicutes were the dominant bacterial phyla, representing almost half of all bacteria present.
Conclusion: Freshwater mussels are able to maintain a bacterial microbiome that is distinct from the surrounding water column and sediment that they live in. The bacterial diversity present in this study indicates that the freshwater mussel hosts are selectively maintaining distinct bacterial communities, even between closely related mussel species. Further, the local environment in which mussels develop and mature likely plays a significant role in the establishment of certain bacterial taxa.

S.5. The antimicrobial activity and cellular targets of *p*-anisaldehyde and epigallocatechin gallate in the opportunistic human pathogen *Pseudomonas aeruginosa*

Yetunde Adewunmi[1], Sanchirmaa Namjilsuren[1], Dahlia Amato[2], Douglas Amato[2], William Walker[2], Olga Mavrodi[1], Derek Patton[2], and Dmitri Mavrodi[1], *[1]School of Biological, Environmental, and Earth Sciences, The University of Southern Mississippi, Hattiesburg, MS, [2]School of Polymer Science and Engineering, The University of Southern Mississippi, Hattiesburg, MS*

Background: Plant-derived aldehydes are constituents of essential oils (EOs) that possess broad range antimicrobial activity and kill microorganisms without promoting resistance. However, their widespread use is hampered by chemical instability and knowledge gaps in their mode of action (MOA). To circumvent these issues, we incorporated *p*-anisaldehyde from star anise into a polymer network via acetal linkages called PANDAs (Pro-Antimicrobial Networks via Degradable Acetals). The resultant antimicrobial polymer released *p*-anisaldehyde upon a change in pH and humidity and controlled growth of the multi-drug resistant pathogen *Pseudomonas aeruginosa* PAO1. Here, we generated 10,000 transposon mutants of PAO1 and screened them for hypersensitivity to *p*-anisaldehyde to identify cellular pathways targeted by PANDAs. To further improve the

antimicrobial efficacy of PANDAs against PAO1, we combined *p*-anisaldehyde with epigallocatechin gallate (EGCG), a green tea polyphenol that inhibits efflux in gram-positive and -negative bacteria.
Methods: We subjected PAO1 to transposon mutagenesis and screened mutants for hypersensitivity to *p*-anisaldehyde. Genome regions flanking the transposon insertion sites were identified by DNA sequencing. The interactions between *p*-anisaldehyde and EGCG were characterized using checkerboard method. Lastly, we used RNA-seq to profile transcriptomic responses of *P. aeruginosa* to *p*-anisaldehyde, EGCG, and their combination thereof.
Results: Our transposon mutagenesis screen yielded 27 unique mutants defective in components of RND efflux pumps, membrane transporters, porins, enzymes of the molybdenum cofactor biosynthesis complex, and hypothetical proteins. Our study revealed that EGCG acted synergistically with *p*-anisaldehyde ($\sum$FIC = 0.5) and significantly reduced its minimal inhibitory concentration by 2.5 folds. The response of PAO1 to *p*-anisaldehyde involved a total of 256 genes, some of which encoded nitrate reductase, energy metabolism enzymes, transporters, and components of efflux pumps and type III secretion machinery. The exposure to EGCG altered expression of 28 genes involved in signal transduction, antioxidant defense, and carbohydrate metabolism. Finally, the synergistic interaction between *p*-anisaldehyde and EGCG differentially affected components of efflux, membrane transport, stress response, and nitrate reductase pathways.
Conclusions: Results of this study highlight cellular pathways targeted by EO constituents and produce novel phytoaldehyde-containing polymer materials that effectively kill pathogenic microorganisms.

S.6. Probing Microbial Interactions and Coordinated Trophic Responses in Biological Soil Crusts
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Cooperative communities of eukaryotic and prokaryotic microbes that include fungi, bacteria, photosynthetic algae, and nitrogen-fixing cyanobacteria underlie the functions of biological soil crusts (BSCs) as ecosystem pioneers in low-water, extreme temperature environments. We are working to understand how BSC microbes interact—metabolically and physically—to impact BSC carbon and nitrogen cycling. Specifically, we are studying how microbes in dessicated crusts that exist primarily in a low-metabolic dormant state, become rapidly reanimated upon the addition of water. We will report on our early findings using whole-community metagenomics, metatranscriptomics, proteomics, and metabolomics to develop a holistic view of the microbial processes underlying BSC metabolic reactivation upon wetting. We are working to produce an integrated phylo-trophic map detailing the flow and exchange of carbon and nitrogen between key microbial taxa within BSCs and expect our findings to be fundamental for understanding the microbial community structure of BSCs and the emergent ecosystem functions of crusts.

Session 2 Talks: Immunology

S.7. Mother knows best: Immune-based maternal effects in response to *Mycoplasma gallisepticum* infection in eastern bluebirds (*Sialia sialis*)
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Background: Neonates lack adaptive immunity and are vulnerable to pathogens. In fact, adults carrying pathogens may transmit infections to their neonate offspring. In response to pathogens, mothers transfer passive immunity to offspring by transmitting antibodies via milk or yolk. Mothers previously or currently infected with a pathogen can transfer pathogen-specific antibodies to newborns, granting them immunological protection until they can synthesize their own. In birds, antibodies are deposited before eggshell formation within the mother's oviduct. It was recently discovered that wild eastern bluebirds (*Sialia sialis*) are common hosts of the avian pathogen *Mycoplasma gallisepticum* (MG). Adult bluebirds exposed to MG in the wild mount an adaptive immune response in the form of circulating antibodies. In this study, I hypothesized that adult female bluebirds transmit both MG and MG-specific antibodies to neonates, and that offspring who receive the antibody will experience faster growth throughout the nestling period.
Methods: Serum samples were collected in the breeding season of 2018 and 2019 and tested for MG antibodies using enzyme-linked immunosorbent assays (ELISAs). Choanal cleft (throat) swabs from entire eastern bluebird families are analyzed using PCR to obtain individual disease status and evaluate intrafamily disease transmission.
Results: ELISA results indicate that adult female bluebirds transmit MG-antibodies to their offspring, and these antibodies persist in young approximately 5 days post-hatch. PCR analysis indicates that both male and female adult bluebirds may transmit MG infection horizontally within the first 5 days post-hatch, most likely through feeding behaviors.
Conclusion: Maternal antibodies may confer immunological protection for neonates exposed to MG prior to immune activation. Familial transmission of this pathogen and protective antibodies may impact the fitness of wild eastern bluebird nestlings.

S.8. Rotavirus NSP1 does not require active cullins to inhibit IFN-β

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Background: Human rotaviruses lead to over 200,000 deaths in children worldwide each year by causing diarrhea leading to severe dehydration. The host innate immune response limits the spread of viruses by producing interferon-β (IFN-β), which stimulates the production of IFN stimulated genes (ISGs) and limits viral spread. Rotavirus encodes the nonstructural protein NSP1, which inhibits the production of IFN-β by inducing the degradation of interferon regulatory factor 3 (IRF3) and β-transducin repeat-containing protein (β-TrCP). The degradation of IRF3 and β-TrCP is dependent on the proteasome, suggesting NSP1 may induce degradation through the ubiquitin proteasome system (UPS). NSP1 has previously been shown to interact with cullin RING ubiquitin ligase (CRL) complexes. NSP1 was thought to exploit CRLs to mediate the degradation of IRF3 and β-TrCP, but we showed that NSP1 does not require Cul3 to induce degradation of IRF3 and β-TrCP. Because this result conflicts with another published study, our aim is to determine if inactivation of CRL complexes alters the ability of NSP1 to prevent IFN- β induction. Because our data support the hypothesis that NSP1 functions alone as an E3 ubiquitin ligase rather than part of a CRL complex, we also aim to identify E2 ubiquitin conjugating proteins that interact with the conserved RING domain of NSP1.
Methods: To determine if NSP1 inhibits the production of IFN-β when cullins are globally inactivated, cells were infected with rotavirus and then treated with the global neddylation inhibitor MLN4924. The NSP1-mediated degradation of IRF3 and β-TrCP in the absence of active CRL complexes was analyzed by immunoblotting, and IFN-β mRNA levels were analyzed by RT-qPCR. To determine if NSP1 associates with E2 ubiquitin conjugating proteins, Halo-tagged NSP1 proteins from SA11-4F and OSU strains of rotavirus were expressed in mammalian cells, along with Flag-tagged E2 proteins (UBE2D1, UBE2D2, UBE2D3, and UBE2N). UBE2D1, UBE2D2, UBE2D3 are involved in K48-linked ubiquitin linkages that target proteins for degradation. UBE2N is involved in K63 ubiquitin linkages that target proteins for cellular re-localization or promote signal transduction. HaloTag pull-down assays were performed followed by immunoblotting to detect transient association of NSP1 and E2 proteins.
Results: Inhibition of CRL complexes by MLN4924 did not prevent NSP1-mediated degradation of IRF3 and β-TrCP, nor did treatment alter IFN-β production during rotavirus infection. NSP1 from the SA11-4F rotavirus strain did not appear to associate with any of the E2 proteins analyzed. NSP1 from the OSU rotavirus strain appeared to associate with the UBE2D proteins that were analyzed. However, the results from these pull-down experiments are preliminary and will require further analysis.
Conclusion: This study shows that NSP1 does not require active cullin proteins to induce the degradation of IRF3 and β-TrCP. Also, cullin inactivation does not prevent NSP1 from inhibiting the production of IFN-β. These data support the hypothesis that NSP1 itself is an E3 ubiquitin ligase. Detection of an association between NSP1 and UBE2D1, UBE2D2, and UBE2D3 will provide evidence that NSP1 acts as an E3 ubiquitin ligase to poly-ubiquitinate IRF3 and β-TrCP. Detection of an association between NSP1 and UBE2N will provide evidence that NSP1 acts as an E3 ubiquitin ligase to influence signal transduction pathways during rotavirus infection. *In vitro* ubiquitination assays will be used to further test the hypothesis that NSP1 has E3 ubiquitin ligase activity. Future studies will also examine the purpose of the interaction between NSP1 and Cul3, as this interaction appears to be unrelated to NSP1-mediated protein degradation.

S.9. The Role of Marginal Zone B Cells in the Early Humoral Response to *Plasmodium yoelii* Infection

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Background: *Plasmodium* infection results in architectural changes to the spleen, including dissolution of the marginal zone, which leads to a loss of marginal zone B cells from this site. The role of marginal zone B cells during *Plasmodium* infection is unknown; however, they are positioned at a site that facilitates their contact with blood and its contents. *Plasmodium* is a blood borne pathogen making it likely that this B cell population can respond to infection. Our interest in this study was to determine the ability of marginal zone B cells to participate in the humoral response to *Plasmodium*.
Methods: Flow cytometry was utilized to show that loss of marginal zone B cells during *P. yoelii* infection is not due to apoptosis and that this population expresses B cell receptors specific to *Plasmodium* antigen. Adoptive transfer and flow cytometry were utilized to determine the fate of marginal zone B cells following *P. yoelii* infection.
Results: During *P. yoelii* infection, disruption of the marginal zone did not result in apoptosis of marginal zone B cells rather these cells were activated and differentiated into plasmablasts. Analysis of marginal zone B cells revealed that a proportion of these cells express B cell receptors specific for *Plasmodium* derived antigens, indicating they possess the ability to respond to infection in an antigen-specific manner.
Conclusion: This study suggests that marginal zone B cells have the capability to participate in extrafollicular antibody production during *Plasmodium* infection. In future studies, we will determine the capacity of marginal zone B cells to produce parasite-specific antibodies.

S.10. *In Vivo* Expansion of CMV-Specific anti-HIV CAR T cells following CMV Superinfection

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Background: Human Immunodeficiency Virus-1 (HIV-1) has killed over 35 million and infects 1.8 million new people each year. Antiretroviral therapy (ART), although effective in controlling plasma viremia and transmission, does not purge the latent or persistent reservoirs necessary to eliminate infection, and must be maintained for life. It is thus imperative to discover therapeutics that provide both lifetime suppression of viral loads and depletion of viral reservoirs. Recently, studies have demonstrated control of viral replication and decreasing viral reservoirs in 50% of rhesus vaccinated with a CMV vaccine vector. They propose that continuous immunosurveillance of SIV by T effector memory (T_{EM}) cells is maintained by the persistent CMV vectors. Utilizing CAR T cells, this strategy could be extended to control viral replication in HIV^+ patients.
Methods: To mimic immunosurveillance elicited by the CMV vector, we stimulated rhesus PBMCs with rhCMV peptide pools (IE1, IE2, and pp65) to expand CMV-specific T cells. In contrast to CD3/CD28 bead stimulation, which typically gives rise to T central memory cells (T_{CM}) differentiated from naïve cells, response to CMV restimulation induces expansion of T_{EM} cells. Utilizing this approach, we genetically modified CMV-specific T cells with retroviral vectors expressing CD4-CAR, converting them into HIV-specific effector cells. These vectors utilize a bicistronic design, expressing the CD4 extracellular domain with intracellular T cell signaling domains, as well as an maC46 fusion inhibitor to protect against viral entry. The genetically modified T cells target a critical step in the viral life cycle independent of MHC presentation, targeting heterogeneous viruses while avoiding the potential for viral escape. We hypothesize that continuous stimulation of CD4-CAR T cells through their CMV-specific TCR will not only maintain activated T_{EM} CTL capable of targeting HIV infected cells, but also circumvent the potential for T cell exhaustion that occurs when relying solely on *ex vivo* stimulation to generate therapeutic levels of T cells. Using a cohort of 4 macaques, we infused CMV-specific CD4-CAR/maC46 T cells. Upon CAR infusion, ART was terminated, allowing viral rebound and antigen to reappear for *in vivo* expansion of CAR T cells.
Results: We find CMV-specific CAR T cells exist *in vivo* primarily as T_{EM}, comprising on average 1.5% (SD ± 0.9) of $CD8^+$ T_{EM} in peripheral blood 5 months after infusion. To further boost these CAR T cells *in vivo*, we superinfected animals with rhCMV at 6 months post infusion. Three animals showed an increase in the circulating $CD8^+$ T_{EM} CD4-CAR population to an average of 2.7% (SD ± 0.6), while the fourth animal did not respond. Despite this expansion of CAR T cells, PVL remained unchanged.
Conclusion: These studies suggest that viral specific CAR T cells are capable of proliferating in response to endogenous viral specific TCR stimulation, and that boosting via the viral specific TCR is a viable strategy for enhancing CAR T cell frequency *in vivo*.

S.11. Interaction between the Human Papillomavirus Oncoprotein E7 and Cyclin Dependent Kinase 8 Contributes to the Suppesssion of Host Immune Responses

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Background: Persistent infection by human papillomaviruses (HPVs), small, double stranded DNA viruses that infect keratinocytes of the squamous epithelia, can lead to the development of cervical and other cancers. The viral oncoprotein E7 regulates host and viral gene expression by binding host transcriptional regulators, although mechanisms responsible for E7-mediated gene expression are incompletely understood. We have discovered that a mutation in the E7 open-reading frame, F57A, results in integration of the HPV16 genome into the host DNA, an event that disrupts the viral life cycle. Integration of the viral genome may be driven by interferon responses that promote the expression of anti-viral genes, called interferon-stimulated genes (ISGs). ISG expression is driven by the STAT1 transcription factor. STAT1 gains full transcriptional activity once phosphorylated on S727 by cyclin dependent kinase 8 (CDK8), a nuclear serine-threonine kinase. We hypothesize that E7 functions to suppress interferon signaling by interacting with CKD8 to block phosphorylation and activation of STAT1, thereby inhibiting expression of ISGs.
Methods: To determine ISG levels, RNA-sequencing was performed with total RNA extracted from human foreskin keratinocytes stably expressing E6/E7 or E6/E7 F57A viral proteins from retroviral vectors. Levels of fully activated STAT1 (P-S727) were determined by Western blot analysis. To determine whether E7 interacts with CDK8, immunoprecipitation was performed with E7 antibody and Western blot was performed for CDK8 (and *vice versa*) in cells containing wild type HPV16 genomes or HPV16 genomes harboring the E7 F57A mutation.
Results: RNA-sequencing analysis revealed that cells harboring E7 F57A protein have increased expression of a subset of ISGs (*BST2*, *IFIT1*, *IFI27*, *IFI44L*, *IFI6*, *MX2*, and *XAF1*) that are regulated by STAT1. Levels of activated STAT1 are significantly higher in E6/E7 F57A cells, as compared to E6/E7 cells. Immunoprecipitation studies demonstrated that wild type E7 interacts with CDK8, whereas the E7 F57A mutation greatly diminishes this interaction.
Conclusion: The E7 F57A mutation is harmful to the HPV16 life cycle as it causes the integration of the viral genome and increased levels of activated STAT1, which promotes the expression of many immune-related genes. We have identified host kinase CDK8 as a novel interacting partner of E7 and this interaction depends on the 57 amino acid residue of E7. Our data suggest that E7 may interact with CDK8 to block CDK8-mediated phosphorylation and activation of STAT1. Together, the ability of E7 to interact with CDK8 may be a novel mechanism by which E7 regulates host gene expression.

S.12. CD8+ lymphocytes modulate Zika virus dynamics and antiviral immunity in nonhuman primates
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Background: CD8+ lymphocytes are critically important in the protection and control of viral infections, but the role of these cells in Zika virus (ZIKV) infection remains incompletely explored in a model similar to humans immunologically. Given the recent development of the macaque model of ZIKV infection, we asked whether CD8 cells are an important correlate of protection in ZIKV infection of nonhuman primates.
Methods: In cohorts of rhesus and cynomolgus macaques, we interrogated the function of CD8+ lymphocytes in acute ZIKV infection by way of their depletion. Applying an integrative approach, we tracked viral RNA in fluids and tissues, conducted transcriptomic analysis of immune responses in blood, developed a high dimensional flow cytometry panel to phenotype innate and adaptive immune cells during infection, quantified neutralizing antibody titers, and used H&E staining to screen for neural pathology.
Results: CD8 depletion delayed serum viral loads and dysregulated patterns of innate immune cell homing and monocyte-driven transcriptional responses in the blood, which informed the development of mechanistic coculture assays. CD8-depleted macaques also showed evidence of compensatory Th1 and humoral immune responses. The absence of CD8+ lymphocytes appeared to increase viral burdens in lymphatic tissues, semen, and cerebrospinal fluid, and neural lesions were evident in both CD8-depleted rhesus macaques.
Conclusions: These findings suggest that CD8+ lymphocytes comprise an important component of immunity to ZIKV and point to an underappreciated signaling axis within the innate immune system that may be important for orchestrating host antiviral signaling, conclusions with implications for immune-based interventions such as vaccine development.

Session 3 Talks: Bacteriology

S.13. Development of a Highly Specific ELISA for Staphylococcal Toxins
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Background**:** *Staphylococcus aureus* is a Gram-positive opportunistic pathogen that causes mild skin infections to severe invasive diseases such as necrotizing pneumonia and diabetic foot ulcer. The virulence of *S. aureus* is largely attributed to the array of secreted toxins such as staphylococcal cytotoxins and superantigens. Identification of specific virulence factors expressed at the infection site is important to understand which virulence factors are contributing to diseases. Quantification of virulence factors at the protein level using western blot or ELISA is ideal. However, *S. aureus* produces Protein A which binds to the Fc region of mouse and rabbit immunoglobulin commonly used in western blot and ELISA. This creates false positives in an ELISA and reduces the accuracy when testing for specific toxin concentrations.
Methods**:** Since chicken IgY does not bind to protein A and cellular Fc receptor, we hypothesized using chicken IgY antibodies for the detection of Staphylococcal toxins will prevent the non-specific binding in ELISA. We expressed and purified histidine-tagged leukotoxin D using immobilized metal affinity chromatography. These toxins used to immunize chickens to produce IgY antibodies specific to the toxin (Aves laboratories). These antibodies were then conjugated with alkaline phosphatase (Abcam). These antibodies were used for western blot and sandwich enzyme-linked immunosorbent assay (ELISA) to detect LukD from *S. aureus* culture supernate and diabetic and non-diabetic mice tissues infected with *S. aureus*.
Results**:** From western blot analysis of *S. aureus* culture supernate or mouse tissue spiked with LukD, chicken IgY conjugated with alkaline phosphatase (IgY-AP) generated a single specific reaction to the LukD from. By contrast, rabbit IgG generated several non-specific reactions in addition to the LukD protein. These results demonstrated that IgY-AP specifically detect LukD without non-specific reactions to other *S. aureus* proteins or host cellular proteins. The sandwich ELISA using IgY-AP specifically detected as low as 10 ng of LukD. From tissue samples from diabetic mice infected with *S. aureus*, western blot assay failed to detect LukD due to the excessive amount of host proteins. By contrast, the sandwich ELISA assay was able to detect as low as 5 ng of LukD based on the standard curves generated using known amounts of LukD.
Conclusion**:** The sandwich ELISA method established in this study specifically detect staphylococcal virulence factors directly from the tissues infected with *S. aureus*. Quantification of specific virulence factors at the protein level will significantly advance our understanding of *S. aureus* pathogenesis. We will further generate chicken IgY antibodies to the remaining *Staphylococcus aureus* toxins.

S.14. Impact of *msaABCR* on *sarA*-associated phenotypes in diverse clinical isolates of *Staphylococcus aureus*

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Background**:** The staphylococcal accessory regulator (*sarA*) plays an important role in *Staphylococcus aureus* infections including osteomyelitis, and the *msaABCR* (*msa*) operon has been implicated as an important factor that modulates expression of *sarA*. In this study, we investigated the contribution of *msa* to *sarA*-associated phenotypes in the *S. aureus* clinical isolates LAC and UAMS-1.
Methods**:** Biofilm and virulence phenotypes previously observed in *sarA* mutants were assessed in *msa* mutants that had been generated in both the USA300 MRSA strain LAC and the USA200 MSSA strain UAMS-1. Concomitant mutation of *S. aureus* protease (*aur*, *sspAB*, *scpA*, and *splA-F*) or nuclease (*nuc1*) was made in regulatory mutants or wild-type strains to further investigate the mechanistic basis by which *sarA* and *msa* impact biofilm and toxin production.
Results**:** Mutation of *msa* was shown to result in reduced production of SarA, a reduced capacity to form a biofilm, and an increase in the production of extracellular proteases and nucleases, and that it does so in both strains. We also confirmed that the increased production of proteases and nucleases contributes to the biofilm-deficient phenotype of both LAC and UAMS-1 *msa* mutants. However, the results in each strain were not equivalent, with mutation of *msa* having a greater impact on protease production in LAC than in UAMS-1. Mutation of *sarA* also had a greater impact on all of these phenotypes by comparison to mutation of *msa.* This was also true as assessed by the relative virulence of LAC and UAMS-1 *sarA* and *msa* mutants in a murine osteomyelitis model. Indeed, mutation of *msa* had no impact on the virulence of UAMS-1 in this model.
Conclusion**:** Our findings support a regulatory model in which *msa* is upstream of *sarA*, but also suggest that, at least in osteomyelitis, it would be therapeutically preferable to target *sarA* rather than *msa* to achieve the desired clinical result, particularly in the context of diverse clinical isolates of *S. aureus*.

S.15. Investigating the role of the di-adenylate cyclase, CdaA, during Borrelia turicatae mammalian infection

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Background: Tick-borne relapsing fever (TBRF), a vector-transmitted bacterial disease, is globally distributed and is among the most common bacterial infections in some African countries. Despite the clear public health concern TBRF represents, the signaling pathways required for virulence of the causative Borrelia spirochetes are unknown. Cyclic di-AMP (c-di-AMP), synthesized by di-adenylate cyclases (DACs), is a dinucleotide second messenger predominantly found in Gram-positive organisms that is required for virulence and several critical physiological processes in many bacteria. Borrelia spirochetes are unique Gram-negative bacteria that encode a single DAC, CdaA, but the role of CdaA in the pathogenesis TBRF spirochetes remains unknown.
Methods: To investigate the function of cyclic di-AMP and CdaA in TBRF spirochetes, cdaA was inactivated in Borrelia turicatae using allelic exchange mutagenesis. Because c-di-AMP plays a critical role in osmoregulation in other bacteria, we assessed the capacity of cdaA mutants to resist osmotic stresses in vitro. Additionally, given the essential nature of c-di-AMP for general viability in other bacterial systems, whole genome sequencing (WGS) was used to identify possible suppressor mutations that confer survival when deleting cdaA. Finally, the ability of cdaA mutants to infect and cause relapsing fever was determined utilizing a murine infection model.
Results: cdaA mutants demonstrated significant growth defects in response to osmotic stresses. Interestingly, restoration of cdaA expression by genetic complementation failed to rescue the observed growth phenotypes. WGS of cdaA mutants subsequently revealed point mutations in genes putatively involved in osmoregulation, cell envelope metabolism, and transcriptional regulation. Murine infection experiments demonstrated that cdaA mutants are unable to establish infection, and, in contrast to the above results, genetic complementation successfully reversed this phenotype.
Conclusion: cdaA mutants exhibit increased sensitivity to osmotic stresses, likely due to the occurrence of suppressor mutations required for deletion of cdaA. Mutations identified suggest a link between c-di-AMP, osmoregulation, and cell envelope metabolism in B. turicatae. The inability of cdaA mutants to establish infection and the ability to reverse this phenotype upon genetic complementation imply that CdaA plays a critical role during infection, and this essential function is independent of the defect in osmotic stress resistance seen in the cdaA mutants. Future experiments will identify functions of c-di-AMP that render the small molecule indispensable for mammalian infection.

S.16. Molecular and Physiological Logic of *msaABCR* Induction by Central Metabolite Pyruvate to Regulate Virulence and Biofilm Development.

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Background**:** Key problem with *Staphylococcus aureus* as a pathogen is the acquisition of antibiotic resistance and their ability to form robust biofilm. They are also equipped with robust mechanism against several host responses under different host nutrient environments. Pyruvate is a critical metabolite that drives ATP production through central metabolism which is also

shown as an important signal molecule. Recent studies have shown that pyruvate alter staphylococcal metabolic flux and exoproteins expression. Furthermore, pyruvate is thought to be the inducer molecule of *CidR* which induces *CidABC* expression during biofilm development. In this study, we showed that pyruvate induces *msaABCR* expression which in turn represses *cidABC* operon during biofilm development and alter virulence. However, molecular and physiological logic of *msaABCR* Induction by pyruvate to regulate biofilm development and virulence factors is still unknown.
Methods**:** We measured expression of *msaABCR* operon in presence of pyruvate. To test whether intracellular pyruvate affects *S. aureus msaABCR* mutant's growth, metabolism and exoprotein expression, we measured growth, acetate generation and exoprotein profile of *msaABCR* and *msaABCR*/ proteases mutants in TSB supplemented with 1% pyruvate.
Results**:** Pyruvate induced expression of *msaABCR* operon. Growth of *msaABCR* and *msaABCR*/ proteases mutants is increased significantly in presence of pyruvate. These mutants also produced more acetate compared to wild type level after 6 hr. Exoprotein profile showed differential expression of exoproteins by *msaABCR* and *msaABCR/proteases* mutant in presence of pyruvate.
Conclusion**:** These results suggest that *msaABCR* expression is induced by intracellular pyruvate and represses pyruvate catabolic pathways to promote biofilm development and exoprotein production. We seek to further define molecular mechanism behind how *msaABCR* senses pyruvate to regulate staphylococcal virulence.

S.17. The *Y. pestis* Translational GTPase BipA Promotes Pneumonic Plague Pathogenesis
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Background**:** *Yersinia pestis*, the causative agent of plague, is a highly virulent pathogen. *Y. pestis* encodes multiple virulence factors. For example, the Ysc type 3 secretion system is essential for pneumonic plague pathogenesis. While regulatory mechanisms of many of these virulence factors are known, there may be others that have yet to be characterized. The translational GTPase, BipA has been shown to regulate virulence factors in other Gram-negative bacteria such as *P. aeruginosa*, *E. coli*, and *S. enterica* serovar Typhi. Furthermore, BipA is transcriptionally upregulated in a murine model of pneumonic plague. While BipA has been characterized in these bacteria, the role of BipA in *Y. pestis* virulence and pneumonic plague pathogenesis has yet to be investigated. Here, we show that BipA promotes *Y. pestis* virulence in a murine model of pneumonic plague. We show that CO92 *ΔbipA Y. pestis* attenuation is not caused by a growth defect *in vitro.* Rather, we show that CO92 *ΔbipA Y. pestis* exhibits greater susceptibility to neutrophil-mediated killing.
Methods**:** Female C57BL/6 mice (n=10 per group) were infected with lethal doses (10^3 colony forming units) of CO92, CO92 *ΔbipA,* CO92 *ΔbipA::bipA* and survival was recorded. Furthermore, bacterial burdens of infected mice (n=10 per group) were measured at 24 (lung) and 48 (lung and spleen) hours post infection (hpi). Growth curves were constructed by inoculating CO92 *Δpgm* and CO92 *Δpgm ΔbipA* cultures in BHI broth and measuring the optical density (OD_{620}) every two hours for 16 hours. Additionally, human cell lines THP-1 (macrophage differentiated) and A549 (alveolar epithelial type II), murine macrophage cell lines (J774.A1 and MH-S), and human primary neutrophils were inoculated with CO92 *Δpgm*, CO92 *Δpgm ΔbipA*, or CO92 *Δpgm ΔbipA*::*bipA Y. pestis* using a multiplicity of infection of 1 bacterial cell to 1 mammalian cell. Bacteria were serially diluted and plated to enumerate surviving bacteria to determine bacterial killing.
Results**:** Deletion of BipA resulted in reduced bacterial burdens and increased survival of infected mice. Further, we show deletion of BipA results in higher susceptibility to neutrophil-mediated killing *in vitro* and *in vivo*.
Conclusions**:** These results identify BipA as a novel *Y. pestis* virulence factor that protects against neutrophil-mediated killing and promotes *Y. pestis* virulence in a murine model of pneumonic plague.

S.18. Regulatory role of *msaABCR* operon in persister formation against aminoglycoside stress in *Staphylococcus aureus*
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Background**:** A bacterial sub-population that shows extreme antibiotic tolerance is known as 'persister cells'. Increasing evidences suggest the association of *Staphylococcus aureus* persisters with chronic and recurrent infections. Despite this clinical relevance, there are currently no viable means for eradicating persisters. In this study, we examine the regulatory role of *msaABCR* operon in persister formation against the antibiotic stress. Methods**:** We measured the frequency of persister formation in wild-type USA300 LAC, *msaABCR* mutant and its complementation strain in presence of gentamycin. We also measured the drug uptake using gentamycin-Texas red conjugation method, and membrane potential by flow cytometry. Furthermore, we also measured the TCA cycle activity and intracellular ATP concentration in *msaABCR* mutant and wild-type strain.
Results**:** We found *msaABCR* mutant was defective in persisters in the presence of bactericidal antibiotic gentamycin. The flow-cytometry showed increased gentamycin uptake and membrane potential in *msaABCR* mutant as compared to wild-type USA300 LAC cells. Similarly, qRT-PCR showed upregulation of TCA cycle genes in *msaABCR* mutant, and subsequent higher intracellular ATP concentration as compared to the wild-type strain.
Conclusion**:** This study suggests that the *msaABCR* operon positively regulates persister formation against aminoglycoside stress dependent on the energy state of the cells including their membrane potential. This study

highlights the importance of *msaABCR* operon as a drug target for eradicating *S. aureus* persisters and overcome recalcitrant infections.

Session 4 Talks: Virology

S.19. HCMV-Induced Signaling through the Epidermal Growth Factor Receptor Directs Viral Trafficking to the Nucleus in Primary Human Monocytes

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Background: Human cytomegalovirus (HCMV) is a β-herpesvirus that infects 60-90% of the world's population and is the leading cause of congenital viral infections. HCMV pathogenesis depends upon the infection of monocytes, as this is the key cell type mediating hematogenous dissemination of the virus to secondary sites of infection such as the bone marrow compartment – the site of lifelong viral persistence. To infect monocytes, HCMV must bind and enter the cell, traffic to the host cell nucleus, translocate viral DNA into the host cell nucleus, and initiate viral replication. We are interested in elucidating how the post-entry events of HCMV infection in monocytes are regulated. We have previously demonstrated that receptor-ligand interactions between the virus and monocyte initiate signaling cascades through the c-Src kinase and the epidermal growth factor receptor (EGFR) kinase. These signaling cascades are required for efficient entry of HCMV into monocytes. Recent work has documented that c-Src signaling is required early during infection to avoid viral sorting into late endosomes and subsequent degradation. To expand our understanding of how HCMV-induced signaling regulates post-entry events in monocytes, we began investigating the role of the other major signaling axis through the EGFR kinase. In this study, we show that temporally regulated signaling through the EGFR kinase is required to promote proper viral trafficking and timing of nuclear translocation of viral DNA into the host cell nucleus.
Methods: The temporal regulation of EGFR kinase by HCMV was examined via western blot of phosphorylated EGFR over a time course. To assess the functionality of HCMV-induced EGFR signaling, in cell westerns were performed to detect signaling molecules downstream of EGFR (AKT and STAT1). To determine how EGFR kinase activity affects viral post-entry events, the specific EGFR kinase inhibitor AG1478 was utilized to block EGFR kinase activity over a time course. The effect of EGFR kinase inhibition on viral trafficking was assessed via immunofluorescence microscopy, while the effect on nuclear translocation was analyzed via cellular fractionation to isolate nuclei and quantitative PCR to determine how much viral DNA was present in the host cell nucleus upon EGFR kinase inhibition.
Results: Upon HCMV infection, EGFR is phosphorylated at early and late time points during viral post-entry events. The EGFR kinase activity induced by HCMV appears to be functional by proxy of downstream signaling molecules AKT and STAT1. 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.
Conclusions: These data highlight how the initial activation of the EGFR kinase via viral binding is necessary for efficient entry, but is not sufficient to promote the subsequent trafficking events. These new data suggest that HCMV temporally regulates EGFR kinase activity to promote the correct localization of the virus during post-entry trafficking events as well as the proper timing of nuclear translocation in primary human monocytes, both of which are required to establish productive infection in a host and promote lifelong viral persistence.

S.20. MicroRNAs as Predictive Biomarkers of Human Papillomavirus (HPV) Associated Low-grade Cervical Dysplasia Outcomes

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Background: Many women get diagnosed with HPV-associated low-grade cervical dysplasia each year. The majority of women who are diagnosed with low grade cervical dysplasia will exhibit restoration to a healthy cervix. Even though most cases resolve, there are a few that progress on to a high-grade cervical dysplasia which puts them at higher risk of developing cancer. There is a need for a better prognostic test to predict whether a women will clear the dysplasia, or will progress onto a high grade dysplasia. We evaluated microRNAs as biomarkers of progression or clearance of low-grade dysplasia.
Methods: We performed a retrospective study looking for women who had a low grade dysplasia diagnosis and either resolved their dysplasia without any intervention (controls, n=29) or advanced onto high grade dysplasia (cases, n=22). Following identification of both cases and controls, we obtained their corresponding low grade dysplastic biopsy samples and extracted the total RNA. Following extraction, we used microarray technology to probe for all known human miRNAs (LC Sciences, Houston, TX). Differential microRNA expression between cases and controls was then calculated.
Results: We identified twenty-nine miRNAs that were significantly differentially expressed between the cases and controls (p value <.01). Among these differentially expressed miRNAs identified were miR-638, a known tumor suppressor miRNA, and miR-1260a, a miRNA that has been shown to promote migration and invasion. In women who advanced from low grade to high

grade dysplasia, miR-638 was significantly downregulated, while miR-1260a was significantly upregulated. These results are consistent with pathology associated with cervical dysplasia progression.
Conclusion: a clinical test examining miRNAs of the lesion following a low grade cervical dysplasia diagnosis may provide evidence of outcomes. Specifically, miR-638 and miR-1260a expression may provide insight into whether women will advance onto high grade dysplasia or resolve the dysplasia. Further, miRNAs may have a functional role in pathology related to cervical dysplasia progression.

S.21. Increased risk of early cytomegalovirus (CMV) infection in SIV-infected infant rhesus macaques

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Background: Although AIDS-related mortality due to emergence of cytomegalovirus (CMV) as an opportunistic infection has greatly declined since the advent of potent antiretroviral treatment (ART), CMV co-infection continues to be a significant contributor to **co**-morbidities in HIV-infected individuals on ART. CMV co-infection is also a poor prognostic factor in pediatric HIV infection, which is highly prevalent in Western and Central Africa. While maternal HIV infection increases the risk of congenital CMV infection, less is known about the risk of postnatal CMV acquisition and its effect on pediatric HIV infection in the absence or presence of ART.
Method: We investigated the risk of postnatal CMV acquisition in a cohort of neonatal and infant SIV-infected rhesus macaques that were separated from their mothers within 7 days of birth (mean 2 days; range 1-7 days) and SIV-infected soon thereafter (n=9 neonates) or at 5 months of age (n=6 infants).
Results: While maternal CMV antibodies detected at birth waned to undetectable levels, three of 9 SIV-infected neonates on ART and one of 6 SIV-infected infants on ART progressed to AIDS within four months of infection and had detectable plasma CMV DNA in the absence of CMV seroconversion near endpoint. Overall, postnatal CMV infection detected in 27% of SIV-infected macaques (4 of 15) contrasted with 0% detection in a cohort of 59 SIV-negative rhesus macaques separated from their mothers within 72 hours of birth and followed for more than one year (P-Value 0.0012; Fisher's Exact test). In a 15-month longitudinal follow-up of three SIV-negative infants paired with their CMV-seropositive mothers, plasma CMV DNA followed by seroconversion was detected in only one infant after 9 months despite daily exposure to maternal CMV shed in urine, saliva and breast milk.
Conclusion: These data suggest that HIV-infected infants are at increased risk of earlier acquisition of CMV infection and accelerated AIDS progression.

S.22. Rotavirus NSP1 requires a functional RING domain for nuclear foci formation

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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 by promoting proteasomal degradation of IFN regulatory factors (IRF3, IRF5, IRF7) and β-transducin repeat-containing protein (β-TrCP), which is required for NF-κB activation. NSP1 proteins from different rotavirus strains can be described by their ability to degrade IRFs, β-TrCP or both. NSP1 binds to its substrates at the C terminus and requires an N-terminal RING domain to promote their proteasomal degradation. This project uses NSP1 from two commonly studied rotavirus strains: SA11-4F and OSU. SA11-4F NSP1 induces degradation of IRFs, whereas OSU NSP1 induces degradation of β-TrCP. Previous studies suggest that NSP1 is a cytoplasmic protein but studies by our lab indicated SA11-4F weakly localizes to the nucleus and OSU NSP1 forms foci in the nucleus. Studies by our lab also showed that SA11-4F reduces the number of promyelocytic (PML) bodies and OSU alters the morphology of PML bodies. PML bodies are involved in a number of processes in the nucleus, such as IFN induction and DNA replication. Therefore, we hypothesize NSP1 antagonizes PML bodies in the nucleus to promote viral replication.
Methods: To determine if the C terminus is required for NSP1 nuclear localization, MA-104 cells were transfected with plasmid encoding tagged C-terminally truncated forms of SA11-4F and OSU NSP1 then immunostained to detect NSP1 and lamin A/C. Additionally, to determine if the RING domain is involved in NSP1 nuclear foci formation, MA-104 cells were transfected with plasmid encoding N-terminal mutant forms of OSU NSP1 then immunostained for NSP1 and lamin A/C. To determine if PML bodies are required for NSP1 nuclear foci formation, PML knockdown HaCaT cells were infected with SA11-4F or OSU then immunostained to detect NSP1 and PML. To determine the impact of PML bodies on viral replication and spread, PML knockdown HaCaT cells were infected with SA11-4F or OSU at high multiplicity of infection (MOI) and viral titers were measured by plaque assay.
Results: Truncated SA11-4F NSP1 weakly localized to the nucleus and truncated OSU NSP1 still formed foci in the nucleus. When key residues in the RING domain of OSU NSP1 were mutated, OSU NSP1 no longer formed foci in the nucleus. When PML bodies were absent from the nucleus, OSU NSP1 no longer formed nuclear foci. Finally, PML knockdown did not affect viral titer at high MOI.

Conclusion: Nuclear localization of NSP1 is independent of IRF and β-TrCP degradation. We also showed that NSP1 foci formation requires an intact RING domain and the presence of PML bodies, which suggests NSP1-mediated ubiquitination of PML bodies may be involved in foci formation. Finally, no effect on viral replication was observed in the absence of PML bodies, but it is important to note that NSP1 is dispensable for viral replication in cells susceptible to rotavirus infection. Future studies will measure IFN-β levels and viral spread in infected PML knockdown HaCaT cells. Because PML bodies play an important role in IFN induction, our findings suggest NSP1 may inhibit IFN induction through a mechanism in addition to proteasomal degradation of IRFs and β-TrCP.

S.23. A 3D-spheroid cell culture system provides a sensible *in-vitro* model for HCMV infection

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Background: Human cytomegalovirus (HCMV) has traditionally been studied in the laboratory in a monolayer of fibroblast cell culture. It is unlikely that these two-dimensional (2D) cell cultures represent a true *in-vivo* environment. To understand the host-pathogen interaction of HCMV in a more natural cell culture setting, we are utilizing an established 3D-spheroid cell culture system that is likely to provide a sensible *in-vitro* model. Human adipose-derived stromal/stem cells were recently shown to replicate HCMV clinical as well as lab strains to high titers.
Methods: Tissue culture polystyrene plates/surfaces were coated with an ELP (elastin-like polypeptide) - PEI (polyethyleneimine) conjugate to induce 3D spheroid organization of cultured adipose cells. The 3D-spheroid adipocytes were infected with HCMV and observed under confocal microscope at 5 days post infection (d.p.i) to compare the localization of viral protein pp150 in 3D vs. 2D adipocytes. Quantitative PCR analysis was performed on harvested 3D and 2D adipocytes at 5 d.p.i to calculate the number of of viral genomes.
Results: The 3D-spheroid adipocytes were successfully infected with HCMV as indicated by the expression of late viral protein, pp150 (GFP tagged). The normal late viral protein expression and localization suggests that HCMV replication progresses to a late stage in 3D cells. Results from quantitative PCR analysis shows the presence of significant amount of viral genomes in 3D spheroid adipocytes.
Conclusion: Based on our preliminary data we can conclude that 3D-spheroid cell culture system represents a viable model to study HCMV replication. Our long-term goal in this project is to determine whether 3D spheroid cell culture model better represents the *in-vivo* replication of HCMV compared to the traditional 2D monolayer cell culture system.

Session 5 Talks: Applied and Environmental Microbiology

S.24. The Microbiome of North American Crayfish Carapaces Differs between Species and by Location

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Background: North America is home to the greatest biodiversity of crayfish in the world, but little is known of the microbiota that inhabit them. This study investigated the carapace microbiome of eight species of crayfish collected across five counties in two states. Sediment and water samples were collected from the same sites to determine whether the crayfish microbiome reflected that of the surrounding environment. Both species and geographic location had a significant effect on the composition of the crayfish microbiome. Further, it was found that the crayfish microbiome differed significantly from either the surrounding water or sediment.
Methods: We collected 54 individual crayfish from eight sites in Mississippi and South Carolina, representing eight different crayfish species: *Cambarellus shufeldtii*, *Procambarus clarkia*, *Procambarus troglodytes*, *Cambarus latimanus*, *Procambarus raneyi*, Faxonius etnieri, Procambarus vioscai, *Procambarus raneyi*, and *Procambarus ablusus.* Crayfish were frozen and transported back to the lab where they were scrubbed and bacterial DNA extracted from the resulting suspension. The V4 region of the 16S rRNA gene was amplified and sequenced using the Illumina MiSeq platform. Bacterial DNA was extracted and sequenced from sediment and water samples by the same method. Samples that yielded <5,000 sequences were removed from the data set. Bray-Curtis dissimilarity and ordination methods along with analysis of molecular variance (AMOVA) were used to assess differences between the microbiota of all samples.
Results: Species was a significant factor in carapace microbiome composition (AMOVA, $p<0.001$), as was sample location (AMOVA, $p<0.001$). Sediment and water samples clustered together on both ordinations and were distant from the crayfish samples. *C. latimanus* ($p=0.005$) and P. troglodytes ($p=0.009$) had significantly greater species richness than *P. ablusus* which had the lowest species richness observed. Crayfish, sediment, and water samples were all composed of the same major phyla, but samples varied in subphyla of proteobacteria. Crayfish samples were composed of mostly Alphaproteobacteria and Gammaproteobacteria. Sediment samples consisted of mostly Deltaproteobacteria and Alphaproteobacteria, and water samples contained mostly Alphaproteobacteria and Betaproteobacteria.
Conclusion: This study is one of the first to describe the cutaneous microbiome of crayfish. Species was the most influential factor in the composition of the carapace bacterial community, though location was also significant. At two of three sites with

co-occurring crayfish species, species remained a significant factor, implying selection for a specific bacterial community by the host species.

S.25. Bacterial Metabolic Activity and Counts Near Big Creek, a Tributary of the Buffalo National River, Arkansas
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Background: The Buffalo River in northern Arkansas is a scenic location attracting nature enthusiasts for various recreational opportunities. The Buffalo River was recognized as a National River and became part of the National Park Service (NPS) in 1972. The Buffalo River watershed includes mostly private properties with only 11% of the watershed on park grounds. A nineteen-month joint study was initiated by U.S. Geological Survey and NPS in 2017 to investigate patterns of nutrients, periphyton, and bacterial characteristics at sampling sites along the Buffalo River and a tributary, Big Creek. A confined animal feeding operation (CAFO) is located alongside Big Creek. Animal waste, feed, microbes, antibiotics, pharmaceuticals, and growth hormones may enter the river from the CAFO, as well as from other parts of the watershed via nonpoint surface runoff and groundwater infiltrations. The purpose of this laboratory study was to investigate whether differences in bacterial metabolic activity and growth occur among sites along the Buffalo River, and to relate water quality characteristics to those potential differences.
Methods: Water samples were obtained from one site on Big Creek, as well as two upstream reference sites and three downstream test sites along Buffalo River. Samples were collected monthly beginning in May 2017 and shipped on ice overnight to the laboratory for processing the next day. After two rounds of aseptic filtering to remove algae and to concentrate the bacterial cells, the filtrate was divided and used to measure bacterial metabolic activity, live and culturable bacterial counts, and total fixed bacterial counts. Bacteria were stained with 5(6)-carboxyfluorescein diacetate (CFDA) and propidium iodide (PI) to measure esterase levels to determine the metabolic activity by flow cytometry. Bacteria were plated on Luria agar and colony forming units (CFU) were enumerated following a 48-hour incubation at 37°C to obtain culturable bacterial counts. For total fixed bacterial counts, the preserved cells were stained with SYTO BC for flow cytometry with a known concentration of microsphere beads.
Results: Significant differences in metabolic activity among sites were observed for 94% (16 of 17) of the analyzed months. The CFU were significantly different among sites for 73% (11 of 15) of the analyzed months. Total fixed bacterial counts were higher than culturable bacterial counts but displayed similar significant differences among sites. The bacterial cells at Big Creek displayed the lowest metabolic activity for 75% (12 of 16) and the highest bacterial counts for 45% (5 of 11) of the months with significant differences among sites.
Conclusion: Overall, the bacterial metabolic activity for all sites was lower in the colder winter months, and highest bacterial counts were generally associated with local rain events. For three months out of the total months analyzed, the Big Creek site showed the lowest metabolic activity and the highest bacterial counts, both of which were significantly different from the other sites. Contrary to the hypothesis, the bacteria at Big Creek had the lowest metabolic activity for most of the months analyzed. This laboratory study indicated that the microbial ecology at Big Creek differed from the other five sites on the Buffalo National River, with microbes at all sites being influenced by compounds throughout the watershed. The observed inverse relationship between the metabolic activity and bacterial counts at the Big Creek site generated an additional microbiological study to examine bacterial exposure to compounds previously identified near the CAFO site.

S.26. Elucidating the Sloth Hair Microbiome: A Metagenomic Comparison of Two- and Three-fingered Sloths
Maya Kaup, Erik Hom, *The University of Mississippi, Oxford, Mississippi*

Sloths are an unusual example of a mobile ecosystem, containing a plethora of symbionts living and growing in their fur as they climb through the canopies of tropical forests. Within this fur ecosystem is a complex hair microbiome, of which microbial species have only been identified based on morphology and 18S sequencing, and bacterial species have not been surveyed. In an effort to clarify the hair microbiome of two species of sloths, we have taken a whole-community metagenomic approach. We are comparing the hair microbiome of the brown-throated three-fingered sloth and Hoffmann's two-fingered sloth sampled within one season in Manuel Antonio, Costa Rica, to determine the differences in the microbiome of these two convergently evolved species. We also sampled both the head and shoulder to determine if the location sampled on the sloth influences the community composition of the sloth hair microbiome. We will show preliminary results exploring how whole-community metagenomic techniques might allow us to understand the microbial diversity of the sloth hair microbiome.

S.27. Biodegradation of Metribuzin Under Various Electron Acceptor Conditions
Thomas J. Phillips III, Raj Boopathy, *Nicholls State University, Thibodaux, Louisiana*

Background: Metribuzin (4-amino-6-tert-butyl-3-(methylithio)-1,2,4-triazin-5(4H)-one) is a photosystem II inhibiting herbicide currently being studied as a substitute for atrazine in sugarcane farms. Metribuzin is a triazinone class herbicide and known endocrine toxin with the same method of toxicity as atrazine though the threshold is approximately 1% that of atrazine.

Methods: Biodegradation of metribuzin by bacteria from the soil of the USDA Sugarcane Research Unit in Houma, LA, bacteria from anaerobic digester sludge collected from the Thiboduax Sewage Treatment Plant and *Rhodococcus rhodochrous* will be studied under aerobic and anaerobic conditions which will include fermentative, nitrate reducing, sulfate reducing, and mixed reducing environments. Once a condition under which metribuzin is degraded is found, the bacteria from that triplicate will be streaked for isolation and identified using BioLog. The concentration of metribuzin will be analyzed by HPLC, while the byproducts of degradation will be evaluated by GC/MS.

S.28. Hypersaline Bacterial Community Response to Simulated Martian Conditions
Eric A. Weingarten, Peter C. Zee, Colin R. Jackson, *University of Mississippi, University, Mississippi*

Background: Salt pans are a class of ephemeral wetland characterized by alternating periods of inundation, rising salinity, and desiccation. This hydrogeologic regime is similar to that proposed for recurring slope lineae (RSL) on Mars which are hypothesized to be melted groundwater aquifers. Any extant microbe in these deposits would have to endure long periods of desiccation in addition to extremes of salinity, temperature, and radiation. We obtained soil cores from salt pans on the Gulf coast of Mississippi in both the inundated and desiccated state. Soil samples were mixed with a Martian regolith simulant to model the composition of Mars soil in RSL. Mixtures underwent a timeseries of temperature and moisture conditions similar to the Martian equator. Bacterial survivorship was monitored with plate count and community composition was determined by 16S rRNA gene sequencing.
Methods: 30 cm deep soil cores were collected from a salt pan at Grand Bay National Estuarine Research Reserve, Mississippi, in June and October 2018, prior to and following extreme tidal events. Soil was added to an equal mass of Mars regolith simulant amended with either no added salt, 10 wt% NaCl, 2% $Ca(ClO_4)_2$, 2% $Mg(ClO_4)_2$, 2% $NaClO_4$, or all salts combined. 20g of each soil mixture were dispensed into 100mm test tubes which were placed in anaerobic jars with O_2 replaced by CO_2. Treatments were exposed in 21d increments to: -20ºC over a desiccant, +27ºC over 100 ml water, and -80ºC over desiccant. Samples were plated and DNA was extracted at each condition change. The V4 region of the 16S rRNA gene was sequenced using Illumina MiSeq.
Results: The effects of salt type, timepoint, initial hydration status, and soil layer were all significant in determining community composition ($p<0.002$ for all, PERMANOVA). Soil layer had the strongest effect ($R^2=0.17$) followed by salt type ($R^2=0.10$), initial hydration (0.06), and timepoint (0.04). Within each of the salt treatments, there was no significant change in bacterial composition following the move from the first to the second incubation. Among the 10 most abundant bacterial taxa were Solirubrobacterales (thermophile), *Pullulanibacillus* (radiation tolerant), and *Truepera* (radiation tolerant).
Conclusions: We demonstrated that bacterial species collected from salt pans were well adapted to Mars-like conditions. The community was typified by known extremophiles and after 21d at an incubation of freezing and desiccated conditions, there was no significant decline in alpha diversity across any of the treatments, indicating the stress was not sufficient to sterilize major constituents of the community. Perchlorate tolerance, which we have demonstrated, could allow bacteria to exist in surface waters on Mars for a much longer portion of the year than would be predicted by surface temperature alone as these salts greatly depress the freezing point of water.

Session 6 Talks: Pathogenesis, Immunology, and Virology

S.29. Role of DeoR transcriptional regulators in virulence and environmental adaptation in *Listeria monocytogenes*
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Background: *Listeria monocytogenes* is a ubiquitous opportunistic foodborne pathogen capable of survival in various adverse environmental conditions. The complex regulatory mechanisms responsible for its metabolic adaptations in stressful environments are not completely deciphered. Members of the DeoR-family transcriptional regulators are present in a variety of bacterial organisms, and are known to play regulatory roles in sugar metabolism. We found seven members of the DeoR-family regulators in the genome of *L. monocytogenes* strain F2365. In this study, we have characterized the role of DeoR-family regulators in virulence and metabolic adaptation in *L. monocytogenes.*
Methods: We constructed seven mutant strains with in-frame deletions, F2365Δ*iolR*, F2365Δ*deorF,* F2365Δ*glpR2*, F2365Δ*deorC*, F2365Δ*lsrR*, F2365Δ*gntR*, and F2365Δ*fruR*, in *L. monocytogenes* F2365 strain. To determine the role in virulence, oral challenge in mice model as well as plaque assay in murine L2 fibroblast cell lines were conducted. Further, to identify their role in metabolic adaptation, growth under different environmental conditions were performed.
Results: Following oral inoculation in mice, F2365Δ*glpR2*, F2365Δ*deorC*, F2365Δ*lsrR*, and F2365Δ*gntR* exhibited a significant reduction in bacterial burden in liver and spleen compared to parent F2365 strain. Further, F2365Δ*fruR* strain was observed to be defective for cell-to-cell spread in monolayers of infected mouse L2 fibroblast. For growth in Minimal Medium supplemented with 50mM Glucose, F2365Δ*iolR*, F2365Δ*deorF,* F2365Δ*glpR2*, F2365Δ*deorC*, and F2365Δ*gntR* showed altered growth patterns compared to the parent strain. Also, for growth in acidic and saline conditions, F2365Δ*glpR2*, F2365Δ*deorC*, and

F2365Δ*lsrR* exhibited reduced growth.
Conclusion: This study shows that members of the DeoR-family regulators play significant role in *L. monocytogenes* virulence, cell-to-cell spread, sugar metabolism, and survival in adverse environmental conditions. Thus, understanding the mechanisms allowing *L. monocytogenes* survival within host cells and stressful food environments will assist in the development of intervention strategies to control *Listeria* in food products and foodborne illnesses.

S.30. The Plasminogen activator protease plays a dual role during primary pneumonic plague
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Background**:** *Yersinia pestis* causes primary pneumonic plague, a rapidly progressing and deadly form of pneumonia. A hallmark of the disease is a biphasic progression marked by an early pre-inflammatory phase where the pathogen suppresses host immune response and proliferates, and a later pro-inflammatory phase defined by a cytokine storm and heavy influx of neutrophils into the lungs leading to pulmonary damage. The virulence of *Y. pestis* is largely attributed to the delivery of *Yersinia* outer proteins (Yops) into the cytosol of target cells via the Ysc type 3 secretion system (T3SS) and a handful of virulence factors including the Plasminogen activating protease (Pla). Pla, a surface protease of the Omptin family, facilitates dissemination of the bacterium from the inoculation site after transmission via a flea vector, likely due to its ability to activate destruction of fibrin clots. In contrast, during pneumonic plague Pla plays no role in dissemination, but is important for early bacterial survival and proliferation in the lung. Bacteria lacking Pla show attenuated growth as early as 12 hpi, suggesting that Pla contributes to the initial events that occur immediately after inoculation. While the proteolytic function of Pla is essential during the later stages of pneumonic plague, its role in the early events that establish infection is not known.
Methods**:** We use an *ex vivo* human tissue infection platform and a standard mouse model of pneumonic plague for the analysis of early events during pulmonary infection with *Y. pestis*. Flow cytometric analysis of bronchoalveolar lavage and lung tissue, bacterial CFU enumeration and killing assays with primary human neutrophils were used to delineate the role of Pla.
Results**:** Our results demonstrate that *Y. pestis* utilizes Pla, in a T3S dependent manner, to suppress early neutrophil influx into the airways of the lungs after infection. The ability to check neutrophil infiltration and facilitate T3S is dependent on the adhesive properties of Pla and not its protease activity. Strikingly, our results also show that the proteolytic activity of Pla is essential for bacterial growth during the early pre-inflammatory phase and Pla employs its proteolytic function to protect *Y. pestis* against neutrophil mediated killing in the lungs. These results reveal crucial roles for an omptin-family protein Pla, as an adhesin and a protease, during the pre-inflammatory phase thereby demonstrating that this “immunologically silent” early phase of pneumonic plague is likely more dynamic than previously appreciated.
Conclusion**:** Our findings suggest that the fate of *Y. pestis* infection of the lung is decided extremely early during infection, and presence of Pla as an adhesin and protease, tilts the balance in favour of the pathogen.

S.31. The Degree of Polymerization and Sulfation Patterns in Heparan Sulfate are Critical Determinants of Cytomegalovirus Infectivity
Mohammad H. Hasan[1], Rinkuben Parmar[1], Quntao Liang[2,6], Hong Qiu[3], Vaibhav Tiwari[4], Joshua Sharp[2], Lianchun Wang[5] and Ritesh Tandon[1], *[1]Department of Microbiology and Immunology, University of Mississippi Medical Center, 2500 North State Street, Jackson, MS 39216, USA, [2]Department of Biomolecular Sciences, School of Pharmacy, University of Mississippi, Oxford, MS 38677, USA, [3]Complex Carbohydrate Research Center, University of Georgia, Athens, GA 30602, [4]Department of Microbiology and Immunology, Midwestern University, Downers Grove, IL, 60515, USA, [5]Department of Molecular Pharmacology and Physiology, University of South Florida, Tampa, FL 33612, USA, [6]College of Biological Science and Engineering, University of Fuzhou, Fujian, 350108, China.*

Background: Herpesviruses attach to host cells by initially interacting with cell surface heparan sulfate (HS) proteoglycans followed by engagement with protein receptors that culminates in in virus-host membrane fusion and virus entry. Interfering with HS-herpesvirus interactions has long been known to result in significant reduction in virus infectivity indicating that HS play important roles in initiating virus entry. In this study, we provide a series of evidence to prove that specific sulfations as well as the degree of polymerization (dp) of HS govern human cytomegalovirus (CMV) binding and infection.
Methods: We purified CMV extracellular virions and analyzed their binding with various glycosaminoglycans on a glycoarray. Second, HS derivatives with different degree of polymerization (dp) and sulfation levels were studied for their impact on CMV infectivity. Third, cell lines deficient in different glucosaminyl sulfotransferases were studied for the impact on CMV replication. Finally, cells pretreated with a peptide specific to sulfated-HS were studied for CMV replication.
Results: First, purified CMV extracellular virions preferentially bind to sulfated longer chain HS on a glycoarray compared to a variety of unsulfated glycosaminoglycans including unsulfated shorter chain HS. Second, the fraction of glycosaminoglycans (GAG) displaying higher dp and sulfation has a larger impact on CMV infectivity and titers compared to other fractions. Third, cell lines deficient in glucosaminyl 3-O-sulfotransferases (3-O-ST-1, 3-O-ST-4 and 3-O-ST-1/4) produce significantly reduced CMV titers compared to wild-type cells. Finally, cells pretreated with a peptide specific to sulfated-HS produce significantly reduced virus titers compared to the control peptide treated cells.
Conclusion: Taken together, these results highlight the significance of HS chain length and sulfation patterns in CMV attachment and infectivity.

S.32. Efficacy of Novel Recombinant Simian Varicella Virus-Simian Immunodeficiency Vaccines Following Mucosal SIV Challenge in the Rhesus Macaque

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Background: The development of an effective AIDS vaccine remains one of the highest priorities in HIV-research. The live attenuated varicella-zoster virus (VZV) Oka vaccine, known to be safe and effective for prevention of chickenpox/zoster, has the potential as a recombinant vaccine against other pathogens, including human immunodeficiency virus. Using the simian varicella virus (SVV) counterpart, this study tested the hypothesis that recombinant SVV expressing simian immunodeficiency virus (SIV) gag and env genes (rSVV-SIVgag and rSVV-SIVenv) will induce mucosal immune responses against SIV in the rhesus and provide protection against simian AIDS.
Methods: Fifteen female rhesus macaques were divided into two groups: Group 1 (Vaccine) n=8 and Group 2 (Control) n=7. Group 1 Vaccine macaques received subcutaneous and intranasal immunizations with rSVV-SIV*gag* and rSVV-SIV*env* at day 0, 8, 24, 43 and 55 weeks and were boosted with 100ug SIVenv and SIVgag proteins in Adjuplex® adjuvant at 24, 42 and 55 weeks. Group 2 Control macaques were similarly immunized, however, with a recombinant SVV expressing the unrelated respiratory syncytial virus (RSV) G-gene, rSVV-RSVg, and boosted with a PBS protein mock in Adjuplex® adjuvant. After the immunization regime, all 15 animals were challenged with repeated weekly mucosal intravaginal instillations of pathogenic SIV until all 7 control animals were infected. SIV viral loads were monitored with Real Time qPCR.
Results: The vaccine and protein boosts were well tolerated in all animals. These immunizations produced strong humoral and cellular immune responses against both SIVgag and SIVenv in the Gp.1 Vaccine animals. Strong levels of SIV neutralizing antibodies were also produced. Thirteen repeated weekly intravaginal SIVmac251 CX-1 (6.19.03) challenges resulted in infection of all 7 (100%) Gp.2 Control animals, while only 5 of 8 (62.5%) Gp.1 Vaccine animals became infected. These Gp.1 infected animals showed significantly reduced peak viral loads (p=0.04) with peak mean viral loads reduced two logs from that of the Control animals. The remaining 3 (37.5%) Gp.1 Vaccine animals remained uninfected and fully protected against the repeated SIV challenge.
Conclusions: Our results demonstrate recombinant varicella/SIV vaccines with protein boosts strongly stimulated humoral, cellular and neutralizing immune responses against SIVmac251 antigens. Following SIV mucosal challenge in the rhesus macaque, the vaccines also provided protection against infection to 3 of 8 Vaccine animals, and of those remaining 5 of 8 Vaccine animals infected, resulted in a significant reduction of peak SIV viral loads. These results suggest the strong potential of these novel rSVV vaccines for protection against mucosal transmitted SIV.

S.33. The landscape of immune responses and persistent viral reservoirs in systemic and lymphoid tissues in rhesus macaques infected by a newly developed SHIV-CH848

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Background**:** The chimeric simian/human immunodeficiency viruses (SHIVs), carrying HIV envelope glycoproteins from transmitted founder (T/F) viruses, are valuable as a challenge viruses in nonhuman primate models for validating HIV-1 vaccines, assessing viral reservoirs and latency, and developing functional "cure" strategies in HIV infection. However, few SHIV strains have been shown to establish long-term and persistent viral infection and maintain viral reservoirs in tissues recapitulating human HIV infection. This study comprehensively investigated immunological changes, viral replication and reservoir size in deep tissues of SHIV-CH848-infected rhesus macaques on antiretroviral therapy (cART).
Material & Method**:** A total of 10 rhesus macaques (*Macaca mulatta*, RMs) were intravenously (*i.v.)* inoculated with SHIV-CH848, containing *vpu-env* (gp140) sequence from T/F HIV-1 subtype C strain CH848. At 5 months post SHIV infection, 5 animals received cART (PMPA/FTC/DTG) for 6 months, and remaining 5 animals as untreated controls. Blood, lymph node and rectal biopsies were collected and plasma viral load, cell-associated viral RNA/DNA, and host immune responses were assessed.
Results**:** Similar to HIV in humans and SIVmac in macaques, SHIV-CH848 inoculation resulted in rapid viral replication, reaching peak viremia at 14dpi and maintained detectable levels afterwards. SHIV-CH848 primary infection significantly depleted peripheral and mucosal CD4+ T cells, which represent the major viral reservoirs in both systemic and lymphoid tissues of SHIV-CH848-infected animals, compared with non-CD4+ T cells. Also, SHIV-CH848 infection induced PD-1 expressing CD8+ T cell dysfunction and exhaustion. Despite gradual recovery of CD4+ T cells after cART, the levels of integrated viral DNA in tissues were stable throughout 6-months of cART, and rapid viral rebound was observed after ART interruption.
Conclusions**:** These findings suggest that the new SHIV strain, SHIV-CH848, is able to maintain persistent viral replication in macaques, and establish long-term viral reservoirs/latency in tissues, serving as a valuable model for HIV research.

S.34. Effect of Stromal Interferon Response Factor 3 on Human Papillomavirus 16 Life Cycle
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Background**:** human papillomaviruses (HPVs) infect squamous epithelia and cause several important cancers, but HPV infections can last for decades without causing any serious health problems. Understanding the mechanism behind HPV persistence in infected tissues may help us manage papillomaviruses-related infections better. Fibroblasts in the stromal microenvironment provide growth regulatory and innate immune signals that are required for persistent HPV infection in the epithelium. In the present studies, we examined the role of stromal interferon (IFN)-related factors, on the HPV16 life cycle.
Methods**:** Organotypic raft cultures were created using human foreskin keratinocytes (HFKs) containing wild type HPV16 along with human foreskin fibroblasts (HFFs). Major factors of IFN induction pathway were knocked down in stromal fibroblasts using lentiviral shRNA transduction to investigate effect on viral gene expression in keratinocytes. After two weeks rafts were harvested followed by RNA isolation from stroma and subsequently RNAseq analysis was performed. RT-qPCR and immunohistochemistry (IHC) were used for confirming the RNAseq results.
Results**:** Knockdown of the STAT1, the IFN receptor, and the interferon response factors (IRFs) 3 and 7 showed stimulated virus replication in the epithelium. This stimulation was associated with better epithelial differentiation. RNAseq of raft cultures containing IRF3 knockdown revealed the pathways that are significantly different from the non-target control were related to epithelial to mesenchymal transition (EMT). We confirmed effects on keratinocytes markers and epithelial EMT-transcription factors FLN, KRT6B, LOR, SNAIL1, TWIST1 and ZEB2 using RT-qPCR. IHC also confirmed the effect of IRF3 knockdown on late keratinocyte markers expression in epithelia.
Conclusion**:** These studies show that the IFN pathway in stromal fibroblasts has paracrine effect not only in regulation of HPV16 late gene expression in keratinocytes but also can influence keratinocyte differentiation. IRF3, an important transcription factor related to type I IFN production is positively associated with EMT .

S.35. HIV-1 Tat and morphine promote neuroendocrine dysfunction that may involve disruption of mitochondrial complex activity
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Patients infected with human immunodeficiency virus type 1 (HIV-1) are at risk for neuroendocrine dysfunction. The incidence is increased among those with a history of opioid abuse; but, neuroendocrine interactions are complex and the targets involved are unclear. In mice, we have observed that expression of the HIV-1 regulatory protein, trans-activator of transcription (Tat), interacts with morphine to dysregulate formation of neurosteroids in the brain, which are dependent on mitochondria for *de novo* synthesis. Add-back of the neuroprotective steroid, allopregnanolone (AlloP), ameliorates Tat/morphine-mediated behavioral pathology in mice and neurotoxicity in cell culture. Given that Tat is mitotoxic, we hypothesized that HIV-1 Tat and/or morphine would promote mitochondrial dysfunction that could be rescued by physiological concentrations of AlloP. HIV-1 Tat was conditionally-induced (or not) by doxycycline in Tat-transgenic mice (Tat□) and their control counterparts (Tat□). Tat□ and Tat□ brain cells were digitonin-permeabilized, energized from mitochondrial complex I via malate/pyruvate or complex II via succinate, and assessed for oxygen consumption rate (OCR) via an Oxytherm Clark-type electrode. Tat expression significantly reduced basal OCR and attenuated succinate-driven increases. No changes were observed following antimycin A or ascorbate/TMPD. Mitoplasts were prepared from C57BL/6HNsd mouse brains and were assessed following direct exposure to HIV-1 Tat (100 nM), morphine (500 nM), and/or AlloP (10 nM). When energized from complex I, either morphine or AlloP increased basal OCR. Tat significantly decreased OCR and this was not further influenced by morphine; however, AlloP significantly reversed this effect. When energized from complex II, no effects of Tat or morphine were observed, but OCR was increased by AlloP. Treatments were recapitulated in digitonin-permeabilized human neuroblastoma cells (SH-SY5Y) with similar results; albeit, morphine and Tat were found to interact to reduce OCR when cells were energized from complex II. Formation of reactive oxygen species were likewise increased (~10%) by Tat in SH-SY5Y cells or mouse primary mixed glia and these effects were attenuated by AlloP (0.1-100 nM) in a concentration-dependent manner. Interestingly, morphine interacted in glia to reduce AlloP's protective efficacy. Thus, Tat may disrupt mitochondrial complexes I and II, with cell-type selective morphine interactions at complex II. Mitochondrial disruption seems likely involved in related neuroendocrine dysfunction and administration of a neurosteroid, such as allopregnanolone, may ameliorate deficits.

S.36. Viral Membrane-Interactive Motifs: Playing in the Sequence Sandbox
William R. Gallaher, *LSU Health, New Orleans, Louisiana*

Both enveloped and naked viruses must interact with cell membranes to enter cells, organize replicative complexes, alter cell physiology, effect viral membrane biogenesis and egress of progeny virus. The best characterized are enveloped viruses of human disease. Analysis of viral entry and identification of viral fusion/entry motifs has been ongoing since 1980. However, nearly 40 years later, membrane-interactive motifs are still being discovered by playing in the sandbox that is the vast viral sequence database.

There are four recognized classes of Fusion/Entry Proteins: Class I – Paired antiparallel alpha helices, N-terminal fusion peptide or fusion loop. Flu, HIV, Ebola; Class II – beta barrel structures, internal fusion loop. Alphaviruses; Class III – Both helical and beta barrel, internal fusion loop. Rhabdovirus, Herpesvirus, Baculovirus; Class IV – FAST (fusion active small transmembrane proteins). Reovirus.

These have seven different membrane-interactive motifs: Signal peptide, in all glycoproteins; Fusion/Entry peptide, either N-terminal or internal fusion loop; Juxtamembrane aromatic-rich peptide, in many viral fusion glycoproteins of all classes; Membrane-spanning peptide, the transmembrane anchor; Viroporin/toxin peptide, structural or non-structural; CRAC motif peptide, cholesterol reactive amino acid consensus; and immunosuppressive peptide, common among Class I fusion/entry proteins.

The purpose of this presentation is to compare and contrast these sequence motifs, their mechanisms of action, and how to survey the viral sequence database for those motifs yet to be discovered.

Session 7 Talks: Bacteriology and Environmental Microbiology

S.37. Environment and neutral processes shape the skin microbiome of the introduced European catfish (*Silurus glanis*)

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Background: Fish skin microbiome is highly diverse and may participate to crucial functions for its host. Previous studies performed on cultivated and/or of economically important species, suggested it may vary according to both environmental and host-associated factors, while the relative importance of both types of factors remained understudied, and comparisons with microbiomes of wild and phylogenetically contrasted species are also lacking.
Methods: We characterized for the first time the skin microbiome of the European catfish, an introduced species native from Easter Europe, sampled in different rivers of France. We compared the effect of host-associated factors and environmental variation on the structure and the diversity of its microbiome.
Results: Catfish skin microbiome composition was distinct to that of other freshwater fish species previously studied with high abundances of *Gammaproteobacteria* and *Bacteroidetes*. We found no effect of catfish individual genotype and body size on the structure of its associated skin microbiome. Local habitat was the best predictor of catfish skin microbiome, together with neutral models of microbiome assembly.
Conclusion: Our findings contradicts previous patterns identified for other freshwater fish species, predominantly performed on cultivated fish populations and suggests that at a local scale, catfish microbiome may be finely regulated depending on environmental conditions. This raises the need of further assessing (i) the effects of fish functional traits on the skin microbiome of invasive and native fish species and (ii) possible roles of skin microbiome variability for host acclimation facing environmental variations.

S.38. Shared evolutionary history and the weakening of ecological priority effects

Peter C. Zee & Tadashi Fukami, *The University of Mississippi*

Background: The order and timing of species arrivals can influence species abundances in the resulting ecological communities. These ecological priority effects have been documented in a range of taxa, including microbial species. However, the factors determining the extent to which priority effects affect community assembly remain unclear. In this study, we set out to experimentally test this relationship between prior shared evolutionary history among species and the strength of priority effects.
Methods: Using laboratory populations of the bacterium *Pseudomonas fluorescens*, we examined whether shared evolutionary history affected the strength of priority effects. Through a combination of experimental evolution and ecological community assembly experiments in the lab, we quantified how prior shared evolutionary history affected subsequent patterns of community assembly.
Results: Consistent with this hypothesis, we found that priority effects tended to be weaker in sympatrically evolved pairs of immigrating populations than in allopatrically evolved pairs. Furthermore, priority effects were weaker under higher phenotypic complementarity. However, the strength of these patterns was contingent on the amount of time the pairs shared prior evolutionary history.
Conclusion: Together, our results suggest that the evolutionary history of organismal traits may dictate the strength of priority effects and, consequently, the extent of historical contingency in the assembly of ecological communities.

S.39. Interaction between *Streptococcus pneumoniae* and *Staphylococcus aureus* generates •OH radicals that rapidly kills *Staphylococcus aureus* strains

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Background: *Streptococcus pneumoniae* (Spn) rapidly kills *Staphylococcus aureus* (Sau) by producing membrane-permeable hydrogen peroxide (H_2O_2). The mechanism by which Spn-produced H_2O_2 mediates Sau killing was investigated.
Methods and Results: An *in vitro* model that mimicked Spn-Sau contact during colonization of the nasopharynx demonstrated that Sau killing required outcompeting densities of Spn. Compared to the wildtype strain, isogenic Spn∆*lctO,* and Spn∆*spxB,* both deficient in production of H_2O_2, required an increased density to kill Sau. While residual H_2O_2 activity produced by single mutants was sufficient to eradicate Sau, a double Spn∆*spxB*/∆*lctO* mutant was unable to kill. A collection of 20 diverse MRSA and MSSA strains showed linear sensitivity (R^2=0.95) for Spn killing, but the same strains had different susceptibilities when challenged against pure H_2O_2 (5 mM). There was no association between Sau clonal complex and sensitivity to either Spn or H_2O_2. To kill Sau, Spn produced ~180 µM of H_2O_2 within 4 h of incubation, while killing-defective Spn∆*spxB* and Spn∆*spxB*/*lctO* mutants produced undetectable levels. Remarkably, a sublethal dose (1 mM) of pure H_2O_2 when incubated with Spn∆*spxB* eradicated diverse Sau strains suggesting that Spn bacteria may facilitate conversion of H_2O_2 to a hydroxyl radical (•OH). Accordingly, Sau killing was completely blocked by incubating with scavengers of •OH radicals, Me_2SO, thiourea, or sodium salicylate. The •OH was detected in the supernatant of Spn by spin trapping and electron paramagnetic resonance.
Conclusions: Spn produces H_2O_2 which is rapidly converted to a more potent oxidant, the hydroxyl radicals, to rapidly intoxicate Sau strains.

S.40. Myxobacterial response to the phytohormone methyljasmonate supports plant recruitment of micropredators within rhizobiomes

Barbara I. Adaikpoh, Shukria Akbar, Hanan Albataineh, Sandeep K. Misra, Joshua S. Sharp, and D. Cole Stevens, *Department of BioMolecular Sciences, University of Mississippi, University, MS, USA*

Background: Chemical exchanges between plants and microbes within rhizobiomes are critical to the development of community structure. Volatile root exudates such as the phytohormone methyljasmonate contribute to various plant stress responses and have been implicated to play a role in the maintenance of microbial communities. Bacteriovorous myxobacteria are competent predators of plant pathogens and are generally considered beneficial to rhizobiomes. While plant recruitment of myxobacteria to stave off pathogens has been suggested, no involved chemical signaling processes are known.
Methods: Herein we employ untargeted mass spectrometry-based profiling and RNA sequencing to determine how methyljasmonate (MeJA) exposure impacts soil-associated myxobacteria.
Results: From a panel of four myxobacteria, we observe a robust response from the plant-associated myxobacterium *Archangium* sp. strain Cb G35 with 10 □M MeJA inducing a ≥4-fold change in transcription for 56 genes and impacting the production of at least 300 metabolites. We also observe that MeJA induces *A.* sp. motility supporting plant recruitment of micropredators via root exudates.
Conclusion: From these data, we conclude that the predatory myxobacteria respond to the plant phytohormone MeJA and that plant recruitment of micropredators within soils may only involve a subset of myxobacteria.

S.41. *Mycobacterium tuberculosis* Rv1075c and LipE are important for intracellular growth and *in vivo* infection

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Background*: Mycobacterium tuberculosis* (Mtb) relies on fatty acids and cholesterol as important nutrients during infection. Mtb lipid metabolism pathways serve as a source of carbon and energy for Mtb and influence the pathology of Mtb by modifying the quantity of primary metabolite pools. We have characterized two Mtb putative lipases Rv1075c and Rv3775 (*lipE)* and determined their impacts on Mtb intracellular growth and in vivo infection.
Methods: We overexpressed and purified rRv1075c and rLipE proteins, and characterized their lipase / esterase activities and transcriptional expression levels under stress conditions. We also investigated their impacts on Mtb intracellular survival using a macrophage cell line and human PBMC derived macrophages and their impacts on *in vivo* infection using a mouse model.
Results: rRv1075c prefers short-chain esters, with the highest activity on acetate. Its esterase activity was the highest at 45 °C and pH=9. Site-direct mutagenesis revealed that its active triad are Ser^{80}, Asp^{244}, and His^{247}. We further determined that

rRv1075c hydrolyzed triacetin and tributyrin, and it was mainly distributed in cell wall and membrane. Its expression was induced at pH 4.5, mimicking the acidic phagosome of macrophages. Mutation of Rv1075c led to reduced bacterial growth in THP-1 cells and human peripheral blood mononuclear cell-derived macrophages, and attenuated M. tuberculosis infection in mice. LipE prefers medium-chain ester substrates, with the highest activity on hexanoate. Its activity is the highest at 40 °C and pH=9. rLipE hydrolyzes trioctanoate. Besides, LipE also has a β-lactamase activity. The residues Ser^{97}, Lys^{100}, Gly^{342}, and His^{363} are essential for both lipase and β-lactamase activities of LipE. Expressions of *lipE* gene was induced under stressed conditions mimicking Mtb's intracellular niche. Gene-disrupting mutation of LipE led to significantly reduced bacterial growth inside THP-1 cells and human peripheral blood mononuclear cell derived macrophages, and attenuated Mtb infection in mice. Conclusion: Rv1075c and LipE are esterases and are important for Mtb intracellular growth and *in vivo* infection. Further investigation of their functions in Mtb lipid metabolism pathways will expand our knowledge of how Mtb utilizes host esters during *in vivo* infection.

S.42. Analysis of *Brevundimonas subvibrioides* developmental signaling systems reveals inconsistencies between phenotypes and c-di-GMP levels

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The DivJ-DivK-PleC signaling system of *Caulobacter crescentus* is a signaling network that regulates polar development and the cell cycle. This system is conserved in related bacteria, including the sister genus *Brevundimonas*. Previous studies had shown unexpected phenotypic differences between the *C. crescentus divK* mutant and the analogous mutant of *Brevundimonas subvibrioides*, but further characterization was not performed. Here, phenotypic assays analyzing motility, adhesion, and pilus production (the latter characterized by a newly discovered bacteriophage) revealed that *divJ* and *pleC* mutants have mostly similar phenotypes as their *C. crescentus* homologs, but *divK* mutants maintain largely opposite phenotypes than expected. Suppressor mutations of the *B. subvibrioides divK* motility defect were involved in cyclic-di-GMP (c-di-GMP) signaling, including the diguanylate cyclase *dgcB*, and *cleD* which is hypothesized to affect flagellar function in a c-di-GMP dependent fashion. However, the screen did not identify the diguanylate cyclase *pleD.* Disruption of *pleD* in *B. subvibrioides* caused no change in *divK* or *pleC* phenotypes, but did reduce adhesion and increase motility of the *divJ* strain. Analysis of c-di-GMP levels in these strains revealed incongruities between c-di-GMP levels and displayed phenotypes with a notable result that suppressor mutations altered phenotypes but had little impact on c-di-GMP levels in the *divK* background. Conversely, when c-di-GMP levels were artificially manipulated, alterations of c-di-GMP levels in the *divK* strain had minimal impact on phenotypes. These results suggest that DivK performs a critical function in the integration of c-di-GMP signaling into the *B. subvibrioides* cell cycle.

S.43. The effects of diabetic urinary microenvironment on the fitness and virulence of *Streptococcus agalactiae*

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Background: The increased incidence and severity of urinary tract infections (UTI) in individuals with diabetes are conventionally attributed to impaired immune system, glycosuria, incomplete emptying of bladder due to autonomic neuropathy. The spectrum of UTI in diabetics ranges from asymptomatic bacteriuria and cystitis to severe complications such as pyelonephritis and urosepsis. Gram positive *Streptococcus agalactiae* (GBS) is an important urinary pathogen in non-pregnant adults with underlying conditions such as diabetes. GBS is hemolytic, coccoid pathogen that colonizes gastrointestinal and lower reproductive tracts of healthy humans as a commensal. The annual burden of GBS-UTI is estimated to be approximately 160,000 individuals in the US. Interestingly, the fitness and virulence of GBS in diabetic urinary tract has not been experimentally evaluated. We hypothesized that diabetic urinary bladder microenvironment will enhance the virulence of GBS.
Methods: We compared gene expression by qRT-PCR and virulence characteristics such as biofilm formation and host epithelial adherence among GBS cultivated in nutrient rich medium, or in human urine with or without 300mg/dl glucose (to recapitulate moderate glycosuria). Our studies used GBS serotype Ia strain A909 and serotype V strain CNCTC 10/84.
Results: We observed that moderate glycosuria enhances GBS virulence characteristics like biofilm formation, adherence to 5637 human bladder cell lines and resistance to reactive oxygen species (ROS). In addition, compared to GBS cultivated in plain urine, moderate glycosuria rapidly (in 2h) induces expression of virulence genes encoding pore-forming toxins β hemolysin/cytolysin (*cylE*), CAMP factor B (*cfb*), and surface factors involved in teichoic acid dealanylation and resistance to AMP (*mprF*, *dltA*) as adjudged by qRT-PCR. We also observed upregulation of *brpA*, a mediator of cell wall integrity and biofilm formation and of genes encoding two-component signal transduction systems in GBS cultivated in urine + glucose for 2h.
Conclusion: Our observations indicate that glycosuria may afford fitness advantage to GBS by augmenting its virulence in turn facilitating infection of the urinary tract.

S.44. Patterns in the Diversity of a Mississippi River Microbiome Across Space (Over 1300 Km Downriver) and Time (1 Year)

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

Background: The dynamics of the microbiome composition of large rivers has been poorly studied. In response to spatial and temporal variation in environmental properties we expect variation in the microbiome, but it is unclear in response to what conditions, and over what time-frame, and to what degree the microbiome may vary. In this study, we examined patterns of variation in taxonomic diversity of the Mississippi River bacterioplankton microbiome over space and time.
Methods: Measurements were made for free-living and particle-associated bacterial communities over two weeks with distance downriver ("spatial study"), and over a year at a single mainstem location ("temporal study"). For the spatial study, there were 13 sample sites, sampled in sequence, along 1,300 kilometers spanning the Upper to the Lower Mississippi. This study section included confluences with five tributaries, river cities, and extended stretches without major inputs to the Mississippi. Samples for bacterial community composition were characterized by Illumina MiSeq next generation sequencing.
Results: For the spatial study, composition and proportional abundance of dominant bacterial phyla varied little among large tributaries, or downriver in the Mississippi except for a substantial but transient anthropogenically-induced bump at Memphis. At a finer scale of taxonomic resolution (OTUs), however, there were notable patterns in downriver variation in community alpha diversity (richness within a site) and beta diversity (variation in composition among sites). Relatively large shifts in beta diversity occurred following major tributary confluences, while in long stretches between these points diversity typically varied more gradually. For the temporal study, measurements were made at a site on the Lower Mississippi on a daily, weekly, and monthly basis. In contrast to the spatial study, we found substantial variation over time in composition and proportional abundance of dominant bacterial across a range of taxonomic resolution from phyla to OTUs. The pattern of change resembled an arc, with divergence (dissimilarity) in community composition compared to the initial community increasing gradually over the first six months, and then decreasing gradually over the following six months of the study.
Conclusion: We conclude that within a site variation in the Mississippi River microbiome occurs in response to factors related to seasonal change, but that within a season there is downstream variation related largely to the influence of major tributaries.

Poster Session Abstracts

Applied and Environmental Microbiology Posters

P.1. Survival of *Salmonella* spp. and microbial indicators on catfish fillets subjected to slush-ice chilling

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Background: Slush-ice chilling has been applied to catfish fillets by processors for several years. However, little is known about the effect of this system on fillets' safety and quality. The objective of this study was to determine *Salmonella* spp. behavior and microbial indicators on catfish fillets subjected to slush-ice chilling and refrigerated storage.
Methods: Catfish fillets were inoculated with a cocktail of *Salmonella* strains and treated with a mixture of slush ice and brine. Samples were held in a cooler at 2°C for up to 12 days to enumerate *Salmonella* spp. Additionally, fillets taken before-water chill, after-water chill, and after 24-h slush-ice, were stored in a cooler at 2°C for up to 12 days to analyze psychotrophic plate (PPC), total coliforms (TCC), and generic *E. coli* counts.
Results: *Salmonella* counts were reduced (P≤0.05) between 0.55 - 0.83 log CFU/g by slush ice treatments (0% - 4.5% salt). *Salmonella* reduction was similar (P>0.05) among all treatments after 12 d of storage. Psychotrophs, coliforms, and *E. coli* counts were similar (P>0.05) among fillets collected before and after 24 h in slush ice.
Conclusion: Under the assessed conditions, slush-ice treatments had minimal impact on *Salmonella* spp., and the growth of microbial indicators was inhibited with 24-h treatment. However, slush-ice chilling did not have a residual effect during refrigerated storage.

P.2. Response to methyljasmonate exposure by *Archangium* sp. suggests niche roles within rhizobiome

Barbara Adaikpoh, D. Cole Stevens, *The University of Mississippi, Oxford, Mississippi*

Background: Plants have been known to curate and maintain distinct rhizobiomes, some of which are utilized to combat pathogenic microorganisms as well as herbivorous insects. While the exact recruitment mechanisms of these beneficial microbes are not yet well understood, it has been shown that certain volatile organic compounds including methyljasmonate are associated with plant defense responses. Myxobacteria are competent predators of plant pathogens and have been observed to activate predatory characteristics when exposed to exogenous quorum signals produced by prey bacteria. We hypothesize that the plant-associated myxobacteria, *Archangium* sp. Cb G35 senses and responds to methyljasmonate (MeJA) in a manner that promotes symbiosis with the associated plant.
Methods: Untargeted mass spectrometry-based profiling of the MS/MS datasets obtained from extracts of *Archangium* sp. Cb G35 grown in culture conditions exposed to ecologically equivalent titers of MeJA and simultaneous RNA sequencing was utilized to observe changes in metabolome and transcriptome respectively.
Results: Our observations showed generation of new specialized metabolites belonging to various molecular families and an interesting absence of certain native metabolites depicting a switch on/off in biosynthesis. Furthermore, this switch on/off phenomena was similarly observed in the RNA sequencing data where, with above a 4-fold change ($p<0.05$), 15 genes were over-expressed while 41 genes were under-expressed in the exposed cultures. Among these over-expressed genes were the NodB-like xylan/chitin deacetylase and bifunctional hydroxymethyl pyrimidine kinase which have been associated with plant-microbe symbiosis. The metabolic profile of exposed cultures when compared to unexposed showed impact of supplementation on at least 9 metabolites and significant modifications to MeJA.
Conclusion: This study gives a glimpse into plant recruitment of beneficial myxobacteria and the potential for inducing generation of new specialized metabolites in bacteria.

P.3. Presence of Sulfonamide Resistant Bacteria in a Rural Sewage Treatment Plant in Southeast Louisiana

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Background: The presence of antibiotic resistance is a growing global concern which has affected Southeast Louisiana in recent years. Improper usage and improper disposal of antibiotics by consumers, hospitals, industry, and sewage treatment facilities have enhanced the emergence of antibiotic resistance in the waterways of southeast Louisiana with reports existing of human contact with antibiotic resistant bacteria through interaction with the waterways. Southeast Louisiana culture revolves around the waterways being used both recreationally and commercially and the impact of antibiotic resistance cannot be understated. Throughout previous years, the Environmental Biotechnology lab at Nicholls State University has studied the increasing presence of antibiotic resistance in the waterways of Southeast Louisiana and has correlated some of this increase in resistance to processes of local sewage treatment facilities.
Methods: In this study, samples were collected from a sewage treatment facility in Thibodaux, Louisiana and two bacteria resistant to sulfonamide antibiotics, one able to grow in the presence of Sulfamethoxazole and one able to grow in the presence of Trimethoprim, were isolated. These bacteria were then subjected to various concentrations of antibiotics in the

presence of glucose. Bacterial growth and carbon and nitrogen metabolism of the bacteria were monitored.
Results: The results of this study showed growth in both cultures in all concentrations of antibiotics including the highest concentration tested at 1000 mg/L of the sulfonamides indicating antibiotic resistance.
Conclusion: Research is currently being conducted to determine whether these bacteria can use sulfonamides as the sole source of carbon and whether these bacteria contain sulfonamide resistance genes, sul1, sul2, sul3, and sulA.

P.4. Predation Triggers Persistent Populations of *Pseudomonas putida* that Elude Predatory *Cystobacter ferrugineus* and Develop Resistance upon Exposure to Antibiotics

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Phenotypic resistance to antibiotics attributed to persister cells ubiquitous to both commensal and non-commensal bacterial populations has been linked to increased incidents of antibiotic therapy failure. While factors that influence persistence within host-pathogen environments have been identified, information concerning triggers for persistence removed from host-pathogen environments remains limited. Considering myxobacteria utilize antimicrobial metabolites during predation and antibiotic exposure triggers persistence, we sought to investigate the role of persistence during predator-prey interactions. Utilizing an ecologically relevant predator-prey pairing of *Cystobacter ferrugineus* and *Pseudomonas putida*, we infrequently observe populations of *P. putida* capable of surviving during predation. These *P. putida* survivors were determined to include persister cells demonstrating diminished growth when exposed to antibiotics. Surviving populations of *P. putida* generated by predator exposure were able to rapidly develop antibiotic resistance and endure subsequent predation. Ultimately, we conclude that persistence triggered by predation increases to prey survivability and could contribute to proliferation of antimicrobial resistance within non-commensal environments.

P.5. Lipid metabolism in *Acinetobacter baylyi ADP1*: Optimizing lipid production through genetic modification of lipid biosynthesis pathways

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Background: *Acinetobacter baylyi* ADP1 has a natural ability to produce triacylglycerols (TAGs), precursors for biodiesel manufacturing. To compensate for low production yields, it is crucial to examine and adjust key enzymatic steps in lipid metabolism pathway. Our goal is to genetically engineer *A. baylyi* to enhance production and accumulation of TAG by inactivating genes of competitive/degradation pathways and overexpressing enzyme catalyzing major steps in the synthesis pathway.
Methods: Genes targeted for mutagenesis were knocked out using the *cre-loxP* site specific recombination system. For expression analysis, genes were cloned into the pGEM-T vector and enzyme activities were measured. The lipid composition of all strains was monitored by thin layer chromatography.
Results: Initially, two putative lipase genes (*lip1* and *lip2*) associated with TAG degradation and a putative acyl-CoA dehydrogenase (*fadE*) gene involved in β-oxidation of fatty acids were consecutively inactivated. The genome of *A. baylyi* ADP1 harbors multiple copies (10-11) of the *fadE* gene. For the next step, a second putative acyl-CoA dehydrogenase (*fadE*) and the gene for phosphate acetyl transferase (*pta*), the product of which is involved in acetate production/utilization were knocked out. Inactivation of the *fadE* gene copy (ACIAD3191) resulted in loss of growth on oleic acid while the mutation in the *pta* gene led to reduced growth on acetate as well as reduced acidification of culture medium. Each of these mutations was combined with the lipase and the first *fadE* mutations. Phosphatidic acid phosphatase (PAP) mediates the synthesis of diglyceride, a precursor for TAG. Three putative *A. baylyi* PAP genes were expressed and evaluated for their potential activities and roles in TAG production.
Conclusion: Mutations in the genes: *lip*, *fadE* and *pta* resulted in increased accumulation of TAG. Two of the overexpressed phosphatases show enhanced PAP activity. This study underlines the ability of *A. baylyi ADP1* to be used a model organism for studying metabolic engineering of the TAG biosynthesis pathway.

P.6. Quantifying Species-Specific Microbial Defense Traits in Response to Predation

Tyler Bacon, *The University of Mississippi, Oxford, Mississippi*

Background: Predator-prey relationships are among the most important and widespread ecological interactions across all diverse ecosystems, including microbial communities. *Myxococcus xanthus* is a social bacterium that acts as a predator to other microbes yet is subject to predation by larger organisms. Understanding its strategies for resisting predation are relatively unknown. In the laboratory, using a variety of defined *M. xanthus* mutants, the importance of potential defense traits can be measured in response to predation. Furthermore, determining whether the response is species-specific can be tested
Methods: In this study, two nematode species, *Pristionchus pacificus* and *Caenorhabditis elegans*, were used as predators and allowed to interact with laboratory mutant strains of *M. xanthus* within a simulated environment. Quantification of the population growth of different *M. xanthus* strains reflected their relative resistance to nematode predation
Results: Preliminary experiments resulted in a variety of defense responses to predation from the laboratory mutant strains of M. *xanthus*. This suggests that select mutant strains resisted predation from the nematodes more successfully than others. In addition, *M. xanthus* exhibited different resistant responses to both *C. elegans* and *P. pacificus.* Further experiments are planned to be conducted to evaluate the interactions between predator identity and bacterial genotypes to determine the genetic basis for *M. xanthus* resistance to predation.

Conclusion: The continuing work of identifying significant traits in response to species-specific predation using an experimental approach will ultimately contribute to the development of a microbial system for testing the evolution of complex multispecies communities.

P.7. The Influence of Time and Xenobiotics on Bacteria Typically Found in Confined Animal Feeding Operations

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Background: The primary objective of this laboratory study was to complement results of an interdisciplinary study pertaining to the potential influence of a confined animal feeding operation (CAFO) on water quality of the Buffalo National River (BNR), Arkansas. Bacteria from river water at a site near a CAFO exhibited lowest metabolic activity among the six sites studied over 19 months. Confined animal feeding operations can be a source of animal excrement, feed, microbes, antibiotics, pharmaceuticals, and growth hormones. In this laboratory study, three bacterial species were exposed to five xenobiotic compounds previously identified in BNR's watershed, and the metabolic activity was measured to investigate microbial responses that may be occurring in the BNR. The hypothesis was that bacteria that may be found in watersheds near CAFOs would be differentially influenced by individual xenobiotics.
Methods: *Escherichia coli* (stock strain)*, Streptococcus suis* (avirulent strain derived from pig)*,* and *S. dysgalactiae* (virulent strain derived from silver carp, *Hypophthalmichthys molitrix)* were cultured, and the exponential phase bacteria were incubated with the xenobiotics*.* Two concentrations of atrazine (herbicide), butylparaben (preservative in food, pharmaceuticals, and cosmetics), 17 α-ethynylestradiol (EE_2, synthetic estrogen), trenbolone (anabolic steroid), and tylosin (macrolide antibiotic that inhibits protein synthesis; clinically used to treat porcine infections caused by *S. suis*) were used. The high concentrations were set according to the U.S. EPA environmental thresholds, whereas the low concentrations were half that level in the exposure water. Treatment groups included each xenobiotic at two concentrations with three bacterial species incubated for two time periods. Incubations were for 2.5 and 21 hours using an orbital shaker in the dark at 35°C. Cells were then stained with propidium iodine and 5(6)-carboxyfluorescein diacetate (CFDA) for esterase activity, indicative of metabolic activity, then analyzed by flow cytometry. Numbers of both the PI-stained (inactive) and CFDA (active) cells were counted up to 300,000 events per sample run in triplicate, and proportions of metabolic inactivity were calculated.
Results: Metabolic activity among bacterial species was significantly different at 21 hours, but not at 2.5 hours; *S. suis* showed the highest level of metabolic inhibition ($P<0.0001$) over all treatments except atrazine at 21 hours. For *S. dysgalactiae* and *E. coli* over all treatments, metabolic activity was significantly lower at 2.5 hours than at 21 hours ($P<0.0001$*;* $P<0.0001$). Among xenobiotics, atrazine at both concentrations inhibited *S. dysgalactiae* and *E. coli* metabolic activity at the highest level for both time periods ($P<0.0001$*,* $P<0.0001$). Trenbolone caused a larger decrease in metabolic activity after 21 hours than after 2.5 hours for *S. suis* ($P=0.0004$). Among the xenobiotics for the 2.5 hr exposure, metabolic activity among species was shown to be significant only for EE_2 and trenbolone ($P=0.0046$; $P=0.0405$). The two different concentrations of compounds did not have a significantly different effect on metabolic activity for all three bacterial species.
Conclusion: The xenobiotics used in this study included growth hormones, an herbicide, an antibiotic, and a preservative previously found in BNR. The bacteria were representatives that can be found in watersheds in that *E.coli* is found in animal waste, *S. dysgalactiae* is a causative agent of bovine mastitis, and *S. suis* strains are swine pathogens. Long-term exposures of *E. coli* and *S. dysgalactiae* caused lower levels of metabolic inhibition by xenobiotics than did *S. suis*. Generally, the various bacterial species were differentially susceptible to exposure, with metabolic inhibition levels varying with exposure time and compound. Overall, these results showed that bacterial metabolic activity was inhibited by each of the xenobiotics tested, thus helping interpret results from the BNR field study; the microbial ecology is likely being influenced by the presence of xenobiotic compounds typically found in the BNR watershed.

P.8. Recovery and analysis of ancient DNA from Maya Dental Calculus

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Background: Analyses of human oral microbiomes can reveal substantial amounts of information about health, diet, and diseases of individuals and their communities within the archaeological record. The data will show which bacteria were present in the oral cavities of individuals who lived in the past, while providing a novel glimpse into their health and disease. In this study we utilized two subsamples of Maya populations from Belize; one from a large populated city center (Xunantunich), and one from a smaller satellite village (San Lorenzo). Examining aDNA from these distinct sites, we can parse out how societal status and diet affected oral health and disease in the Maya population.
Methods: Samples of ancient dental calculus were removed from archaeologically recovered skeletons, and processed in the specialized aDNA laboratory in Copenhagen, Denmark. The DNA was extracted, built into libraries using the BEST protocol, and metagenomically sequenced at BGI on a BGISEQ-500. Data was then processed initially using the Paleomix Pipeline. The Kraken metagenomics toolkit and MetaPhlAn2 were used to map the DNA sequences and establish the taxonomies and composition of the oral microbiome for each individual sample. Pavian, STAMP, and Rvegan were used to calculate the statistics and visualize the metagenomic results.
Results: The sequenced and collapsed aDNA had read lengths between 80-130 bp, and analyses were able to yield bacterial identification at the species level. Statistical tests show that there is significance between the bacterial compositions of the two sites, potentially indicating that there is a higher level of pathogenic bacteria at Xunantunich, where there would have been higher-caste individuals.

Conclusion: This project showed that these methods were effective for reconstructing ancient DNA oral microbiomes, and that the microbial landscape of ancient Central America is ripe for exploration. Furthermore, our data show differences in microbial relative abundance between two geographical locations, providing insights into potential transmission of pathogens in ancient populations, and new sources for exploring evolution of pathogens.

P.9. Bacterial Load in Virtual Reality Headsets

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Background: Objects undergoing constant human interaction are known to be colonized by many different strains of bacteria. Virtual Reality (VR) headsets are an increasingly popular technology that comes into close contact with users faces and facial orifices. In this study, we show that bacterial colonization of Virtual Reality headsets subject to extended use is a problem that should be addressed by increased sanitation procedures.
Methods: The study focused on VR headsets of a Computer Science laboratory at the University of Mississippi. Prior to the study, headsets were sterilized with 70% ethanol, after which users were instructed to follow their usual usage and cleaning procedures. Once per week, for six weeks, ~10 cm^2 of the VR headset forehead and nosepiece were swabbed and swabs placed in sterile saline solution. Samples were diluted to10^{-4} and plated on trypticase soy agar (TSA), mannitol salt agar (MSA), and eosin methylene blue (EMB) plates. After incubation for 48 h, colonies were counted and the number of CFU's from the original sample determined.
Results: Bacterial load increased substantially over the course of the study, coinciding with increased use of the headsets. While EMB plates were general negative, suggesting few coliforms, high numbers of yellow/gold colonies on the MSA plates suggest a high prevalence of *Staphylococcus* (and likely *Staphylococcus aureus*) present on the headsets. This suggests that regular cleaning techniques are not sufficient to sanitize the headsets for sustained use.
Conclusion: This study shows that increased usage of VR headsets positively correlates with the bacterial load. This suggests that current cleaning techniques are not sufficient and should be increased in order to properly sanitize the headsets.

P.10. Long-term Prevalence Patterns of the *Burkholderia* Bacterial Farming Symbiont in *Dictyostelium discoideum* Social Amoeba Populations

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Background: The relationship between the social amoeba *D. discoideum* and its endosymbiotic bacteria, *Burkholderia* provides a model system for studying how symbiotic relationships develop. *Burkholderia* confers "farming" to *D. discoideum*, allowing the amoeba to carry food bacteria to a new location, which is beneficial in times of food scarcity. Only three particular *Burkholderia* species, *B. agricolaris*, *B. hayleyella*, and *B. bonniea* have been demonstrated to confer farming, however, the ecological relevance of this farming symbiosis is not clear. Each of these farming symbiont species differ in laboratory measurements of their farming ability and costs of infection. In this study, we identify the prevalence and identity of *Burkholderia* infection in *D. discoideum* populations sampled across numerous locations in Virginia. We will compare our results to two previous Virginia collections done in the past 20 years, and we hypothesize that beneficial symbiont species will increase in prevalence in natural populations over time.
Methods: We collected soil samples from three locations and over ten different microhabitat conditions in Virginia. Samples were grown on plates in the lab, and amoeba fruiting bodies were collected, and DNA was extracted. PCR was performed using *Burkholderia* and *Dictyostelium* specific primers. Using Sanger sequencing, we were able to identify *Burkholderia* infection status and identity in our *D. discoideum* samples.
Results: *B. agricolaris* showed 53% increase from 2000 and a 67% increase from 2014. *B. hayleyella* was not found. *B. bonniea* showed a 0.91% decrease from 2000 and still exhibits low prevalence (it was not found in 2014). *B. phytofirmans* (7.9%), *Paraburkholderia dipogonis* (4.8%), *Paraburkholderia ginsengisoli* (1.6%), and *Paraburkholderia phenazinium* (1.6%), were also found. Their symbiotic properties are not know.
Conclusion: The increase in *B. agricolaris* suggests that this species is a beneficial symbiont of *D. discoideum.* Not finding *B. hayleyella* suggests that it is more pathogenic, which is consistent with laboratory measurements of its high fitness costs. The decrease in *B. bonniea* prevalence could indicate that it is less relevant to *D. discoideum* fitness.

P.11. Microbial Analysis of Drinking Water in Kanembwe, Rwanda

Mackenzie Hoogshagen, *University of Central Arkansas, Conway Arkansas*

Background: In many third world countries, diarrhea is one of the most common diseases that citizens face on a daily basis. Many gastrointestinal diseases are commonly caused by microorganisms that can colonize in drinking water when it is not properly treated before distribution. Our research is based in Kanembwe, Rwanda, a small rural village. In surveys conducted during previous research projects, some residents reported their children having diarrhea once a month lasting up to one week. Such frequent bouts of diarrhea can interfere with nutrient uptake, especially in children, thus further complicating malnutrition, another common health problem within the village.
Methods: We hypothesized that the drinking water in Kanembwe contains microbes that could be causing these diseases. To investigate this, we travelled to Rwanda in June 2019 where we collected samples of water from one of the village sources and tap water from three additional cities. We filtered the water through 0.2 micron filters to capture the microorganisms and then brought the filters back to the University of Central Arkansas. We then extracted the DNA and sent it to be sequenced.

Results: When we receive the sequences, we will begin analysis in order to characterize the microbial makeup of the drinking water in Kanembwe and cities throughout Rwanda.

P.12. Pneumococcal Resistance to Granule-mediated Killing by Human Neutrophils

Jay Jackson, Jessica Surma, Jackson Holland, Yoonsung Hu, Justin Thornton, *Mississippi State University, Starkville, MS*

Background: *Streptococcus pneumoniae* is a significant human pathogen and the leading cause of community-acquired pneumonia and acute otitis media. Pneumococcus can also asymptomatically colonize the mucosal surfaces of the nasopharynx. One of the primary defense mechanisms of the human immune system against pneumococcal infection involves extracellular granule-mediated killing of bacterial cells by neutrophils. While this mechanism has previously been shown to kill about half of pneumococci *in vitro*, we hypothesized that some pneumococcal strains have evolved to be more resistant to this granule-mediated killing. A cell wall-associated protein HtrA has been identified as a possible protective protease against neutrophil granules.
Methods: Twenty-three invasive strains, which were isolated from the blood or cerebrospinal fluid of patients with systemic pneumococcal disease, were exposed to granules from human neutrophils to determine sensitivity. Additionally, fourteen carriage isolates were tested. The same protocol was used to test for HtrA protection, but HtrA was supplemented alongside degranulate. An HtrA knock out mutant of T4R was created to assess the role of HtrA in the defense of granule-mediated killing.
Results: We observed varying sensitivity among invasive isolates, some being killed by over 90% to a few showing complete resistance. We found varying sensitivity among the colonization strains similar to the rate of resistance in invasive strains. An HtrA knockout mutant of T4R was killed more by degranulate than the wildtype. We have also observed substantial protection of pneumococci from degranulate by purified HtrA.
Conclusion: The similar rates of resistance among invasive and carriage isolates shows that many isolates have developed resistance to granule-mediated killing, but resistance is not specific to invasive strains. The ability of purified HtrA to protect pneumococci from degranulate and the increased susceptibility of the HtrA knockout to degranulate show that HtrA may be a mechanism of pneumococcal resistance. Developing strategies to abrogate this resistance would enhance innate immunity to this important pathogen.

P.13. Monitoring Levels of Fecal Indicator Bacteria in Water Bodies Impacted by Aerobic Sewer System Effluent

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Background: Aerobic sewer systems are used to treat wastewater in many rural areas of the United States. Many of these systems discharge effluent directly into ditches and ravines that rely on sunlight for disinfection purposes. This practice is problematic since very few studies have examined whether effluent from aerobic sewer systems is adequately disinfected by sunlight. In many cases, the disposal ditches or ravines that receive effluent from aerobic sewer systems flow directly into or near major recreational water bodies.
Methods: The environmental impacts of aerobic sewer system effluent were examined by monitoring the microbial quality of water from: 1) Ditches and ravines where aerobic sewer system effluent gets discharged and 2) The Calcasieu River, which intersects with several ditches and ravines that collect aerobic sewer system effluent. The microbial quality of ditch, ravine, and river samples was evaluated by monitoring concentrations of *Escherichia coli* and fecal coliform bacteria using viable plate count assays, which were performed with CHROMagar ECC medium.
Results: *E. coli* and fecal coliforms were present at levels of up to 1.2×10^5 cfu/100 ml and greater than 3.0×10^6 cfu/100 ml, respectively in ditches and ravines where aerobic sewer system effluent was disposed. *E. coli* and fecal coliform bacteria were typically undetectable in the Calcasieu River during periods of dry weather, but were present at levels of up to 9.5×10^3 cfu/100 ml and 8.8×10^4 cfu/100 ml, respectively following rain events.
Conclusion: These results of this work show that fecal indicator bacteria from aerobic sewer systems persist in disposal ditches and ravines and are then flushed into the Calcasieu River during rain events. These observations represent a significant public health concern since the Calcasieu River is used for a variety of recreational activities including fishing, boating, jet skiing, and swimming.

P.14. Understanding the Microbiomes of Water and Milk Kefir

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Kefir is a traditional beverage produced by inoculating and fermenting milk or sugar water with “kefir grains” (KGs), symbiotic aggregates of diverse bacteria and yeast. It is claimed to be of nutritional and probiotic value, although the molecular basis for many of these claims remain to be scientifically demonstrated. There are several studies that describe the microbial composition of liquid kefir and KGs from around the world, but there has not been a systematic study to determine what microbes might be common across these cultures. Is there a core set of microbes that define water or milk kefir? How are water kefir and milk kefir microbiomes different, and how interchangeable are KGs in making water or milk kefir? To answer these questions, we are working to isolate and identify microbes from kefir (liquid and grains) and to pursue whole community metagenomics of kefir grains, which serve as the inocula/propagules of kefir cultures. In addition, we are performing reciprocal “transplantation” experiments of grains (milk KGs cultured in sugar water and water KGs cultured in milk), to understand

essential community members that are sufficient and necessary for kefir production. In this poster, we will share results on the methods we have developed to effectively isolate DNA from polysaccharide-rich KGs, species we have isolated and identified to date, and some early results on our KG transplantation experiments.

P.15. Quantitative PCR analysis of antibiotic resistance genes neighboring a wastewater treatment plant in an urbanizing watershed

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Background: Development and spread of antimicrobial resistance (AMR) and multidrug resistance (MDR) through propagation of antibiotic resistant genes (ARGs) in various environments is a global emerging public health concern. The role of wastewater treatment plants (WWTPs) as hot spots for the dissemination of AMR and MDR has been widely pointed out by the scientific community. Therefore, it is important to monitor, understand and mitigate the occurrence and distribution of ARGs in urban waterways particularly associated with effluent discharges from WWTPs.
Methods: In this study, we collected surface water samples from sites upstream and downstream of two WWTP discharge points in an urbanizing watershed in the Bryan-College Station (BCS) area in Texas over a period of nine months. Quantitative real-time polymerase chain reaction (qPCR) method was used to measure eight ARGs – *tetA, tetW, aacA, ampC, mecA, ermA, blaTEM, and intI* in the surface water collected at each time point.
Results: The concentrations of all ARGs were substantially higher in the downstream sites compared to the upstream sites, particularly in the site immediately downstream of the WWTP effluent discharges (except *mecA*). Overall, highest concentrations of tetracycline resistance genes (*tetA*+*tetW*) were observed to be 9.9×10^5 and 7.3×10^4 copies/ 100 ml surface water in the downstream vs. the upstream sites. Class I integrons are genetic elements that are deemed to play a major role in the distribution of AMR and development of MDR among bacteria in a variety of environments. The integron integrase gene (*intI*) is the key fragment of the functional structure of Class I integrons as it is responsible for antibiotic resistance element-containing gene cassettes to be acquired, expressed and disseminated across bacterial species. Our study detected the *intI* gene cassette in high amounts at all sites and all sampling points, and were ~40X higher in the downstream sites (2.5×10^7 copies/ 100 ml surface water) compared to the upstream sites (1.2×10^6 copies/ 100 ml surface water).
Conclusions: Our findings suggest a significantly increased prevalence of ARGs downstream of the wastewater treatment plant, suggesting that the WWTP may have served as a gateway to the development and release of these ARGs into the water bodies located in its immediate vicinity, through the effluents. Data from this study will benefit establishment of improved environmental regulations and practices to help manage ARG discharges into the environment.

P.16. Presence of multi-drug resistant pathogens and antibiotic resistance genes in waterways and seafood populations of rural Southeast Louisiana, USA

Ethan Naquin, Hiba Soorya, and Raj Boopathy, *Nicholls State University, Thibodaux, Louisiana*

Background: The spread of antibiotic resistance is a growing global concern in recent years. Improper usage and disposal of antibiotics by consumers, hospitals, and industries has furthered the emergence of antibiotic resistance in the waterways of Southeast Louisiana, namely Bayou Lafourche and Bayou Terrebonne, a main source of drinking water impacting over 70,000 individuals that live along its banks. Additionally, there are existing reports of exposure to antibiotic-resistant bacteria through direct contact with seafood. In Louisiana, one out of every seventy jobs are related to the seafood industry, and this region exports roughly 1 billion pounds of seafood each year at a value of 2.4 billion USD. Although the implications of an increasing presence of antibiotic resistance in the rural environment is alarming, there are very few studies dealing with this phenomenon in other similar locations.
Methods: In this study, water samples were collected as well as samples from various species of freshwater fish and shellfish—common seafood caught in the area—and the occurrence of antibiotic-resistant bacteria was monitored. This survey of antibiotic-resistant bacteria and genes was accomplished using Kirby-Bauer Assay as well as PCR techniques for gene display.
Results: The results of this study show the presence of multi-drug resistant bacteria exhibiting resistance to all antibiotics tested. Also, various pathogens showing antibiotic resistance isolated from water samples were also similarly isolated from fish swabs. Furthermore, various bacteria containing the *sul1* and *sul2* genes, genes for sulfonamide drug resistance, were isolated. Further work will continue to search for additional antibiotic resistance genes.
Conclusion: This study shows evidence of antibiotic resistant bacteria present in both water and fish samples. It also displays how antibiotic resistant pathogens in the water are appearing on seafood and, consequently, making it easier for humans to gain exposure to them.

P.17. Identification of the Biosynthetic Gene Cluster for Dolastatin 10/Symplostatin 1/Monomethyl Auristatin D from a Black Band Disease-Associated Polyculture

Thuy M. Nguyen, Erik F. Y. Hom, *The University of Mississippi, Oxford, Mississippi*

A mixed Non-Ribosomal Peptide Synthase/Polyketide Synthase (NRPS/PKS) gene cluster probably encoding for the historically important cytostatic dolastatin 10 (Dol-10), was identified from *Roseofilum reptotaenium* polyculture, which contains a marine phycoerythrin (red) cyanobacterium - *R. reptotaenium*, and several different bacteria. This polyculture was

successfully maintained in the laboratory condition and can produce Dol-10 and its derivatives, Symplostatin 1, and monomethyl auristatin D (MMAD) by cultivation. MMAD, a synthetic analog of Dol-10, playing an important role in clinical application as a "payload" in antibody-drug conjugates (ADCs) for cancer therapy, can now be produced naturally. The metagenome of *R. reptotaenium* polyculture was separately by using genome binning software; all the contigs predicted to come from the same genome were grouped into a cluster. The dolastatin-containing contig was identified belongs to a red cyanobacterium *R. reptotaenium* cluster, which means the cyanobacterium is the organism in the polyculture produced Dol-10, not the bacteria. Applying genome binning tool to determine the dolastatin suit of compounds derived from the cyanobacteria is important because making the axenic cyanobacteria culture is more likely impossible and time consuming.

P.18. Effect of Sliver Oxide Nanoparticle on Carbon and Nitrogen Removal by Bacteria in the Gulf of Mexico

Christopher Oubre and Raj Boopathy, *Department of Biological Sciences, Nicholls State University, Thibodaux, LA*

Background: Nanoparticle use in engineering, medicine, cosmetics, personal care products, and manufacturing is becoming more common. Nanoparticles are incredibly useful because of their ability to add properties to the compound they are made of, but unfortunately have been reported to be toxic to microbes including bacteria. Because nanoparticles are becoming commonplace, it is likely that they are being disposed of improperly and will eventually end up in the coastal waters of South Louisiana due to drainage of Mississippi watershed into this coastal ecosystem. Nanoparticles in coastal waters should have an impact on the bacteria that play a key role in biogeochemical cycles such as carbon, nitrogen, and phosphorous cycles, but to our knowledge no study on this subject has been reported.
Methods: In this study, sediment samples were collected from Grand Isle and Cocodrie of coastal Louisiana and were enriched for common heterotrophic bacteria that carry out carbon and nitrogen cycles in coastal waters. These enriched bacteria were exposed to various concentrations of silver oxide nanoparticle.
Results: The results show that the nanoparticle at concentrations 10 mg/L or above were lethal to the bacteria and bacterial growth was inhibited resulting in no removal of carbon and nitrogen in the media. The lethal concentration 50 (LC_{50}) of silver oxide for the coastal bacteria was found to be 3.92 mg/L for the Grand Isle consortium and 6.01 mg/L for the Cocodrie consortium with increases in silver oxide nanoparticle concentration having increasingly adverse effects on carbon and nitrogen removal for both cultures.
Conclusion: Two coastal sites were assessed for their sediment bacteria's ability to tolerate the presence of silver oxide nanoparticles and both were found to display adverse effects on carbon and nitrogen removal in the presence of the nanoparticle. Further research is to be conducted subjecting these same cultures to the presence of zinc oxide nanoparticles.

P.19. Effect of trans-cinnamaldehyde supplementation on the antioxidant status of channel catfish following *Edwardsiella ictaluri* infection

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Background: Commercial channel catfish is an economically important agricultural commodity in the United States. Bacterial pathogens including *Edwardsiella ictaluri,* a causative agent of enteric septicemia of catfish, threaten profitable catfish production through inventory losses and emergence of antimicrobial resistance. Previously, trans-cinnamaldehyde (TC) was found to inhibit the growth of *E. ictaluri in vitro* as well as decrease the mortality in catfish fingerlings infected with *E. ictaluri***.** The objective of this study was to investigate the antioxidant activity of catfish following dietary TC supplementation in the presence and absence of *E. ictaluri* challenge.
Methods: Catfish fingerlings procured for the study were fed either basal diet, or basal diet supplemented with TC (20 mg/kg diet) for 10 days. Then, the level of five different antioxidant enzymes in the hepatic tissue were measured. Similarly, in the second experiment, catfish were fed either basal diet, or basal diet supplemented with TC for 10 days, and then were infected with *E. ictaluri*. Thereafter, the antioxidant enzyme activities were measured on 1, 6, and 14 days post-infection (DPI).
Results: Catfish fed TC supplemented diet showed a significant increase in superoxide dismutase (SOD) activity and a significant decrease in malondialdehyde (MDA) concentration in the hepatic tissue. Whereas catalase (CAT) and reduced glutathione (GSH) activities, and nitric oxide (NO) concentration were not significantly different between control and TC fed groups. Following *E. ictaluri* challenge, catfish fed TC exhibited an increased CAT activity compared to those fed basal diet on 6 DPI. Furthermore, SOD and GSH activities were upregulated in the TC fed group on 1 and 14 DPI. On the other hand, NO and MDA levels were lowered in TC group as opposed to the control group on 1, 6, and 14 DPI.
Conclusion: Our findings suggest that feeding TC could improve the antioxidant status of channel catfish via ameliorating the oxidative stress as well as enhance resistance against *E. ictaluri* infection. Further, this antioxidant effect could be the potential mechanism by which TC serves *in vivo* antimicrobial activities against *E. ictaluri*.

P.20. Host Range and Fitness Consequences of *Burkholderia* Bacterial Symbiont Infection Across the Social Amoeba Phylogeny

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Background: The bacteria *Burkholderia* have a unique symbiotic relationship with the amoeba host *Dictyostelium discoideum*. Certain *Burkholderia,* upon infecting *D. discoideum,* are known to confer a farming trait to the amoebas. While normally amoebas feed on bacteria, fully digesting them, farming is the ability to carry food bacteria through the amoeba life cycle. This

is beneficial if the amoebas are dispersed to locations lacking food, as they can grow their own. While potentially advantageous to a wide range of amoeba species, this mutualism has only been studied in *D. discoideum*. Research suggests that the *Burkholderia* farming symbionts may have co-evolved with their *D. discoideum* host and are host specific, yet the extent of that specificity is unknown. Our goal is to test this by infecting other social amoeba species in the lab. We predict that *Burkholderia* will be able to infect and confer farming better in amoeba species more closely related to *D. discoideum* as compared to the more distantly related species, and that there will be a fitness cost to the more distantly-related amoebas upon infection.
Methods: Four species of *Burkholderia* that confer farming were infected into seven different amoeba species across the the Dictyostelid phylogeny. One week after infection the presence and strength of infection were assayed using PCR with *Burkholderia*-specific primers. Amoeba species were grown for an additional generation to to test for persistent *Burkholderia* infection. The cost of infection for each amoeba species will be determined by measuring spore production with and without *Burkholderia* infection.
Results: Preliminary data suggests that species of amoeba closely-related to *D. discoideum* are able to be infected with the *Burkholderia* farming symbionts and confer farming, but there is a cost to infection. We are currently assessing infection levels and spore production across all seven social amoeba species.
Conclusion: These results will show how specific the host-symbiotic relationship is between *Burkholderia* and *D. discoideum*. This will make *D. discoideum* a more useful model for studying host-bacteria interactions, and in particular the mechanisms of symbiont infection and maintenance.

P.21. The Bacterial Microbiome of the Social Amoeba

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Background: *Dictyostelium discoideum* is a common model organism for studying bacterial-eukaryotic interactions. It was recently shown that some wild *D. discoideum* carry viable bacteria both intracellularly and within their multicellular fruiting bodies. However, the full bacterial microbiome has yet to be characterized, and it is unknown whether other social amoeba species also have bacterial microbiomes. Our objective is to characterize the natural bacterial microbiome of multiple social amoeba species, and assess the roles of host species and environment on microbiome composition.
Methods: We collected a total of 14 soil samples from nine different natural areas and brought them back to the laboratory where they were plated to allow for amoeba fruiting body formation. We collected fruiting bodies from 11 different species, then extracted DNA directly from the fruiting bodies and from their respective soil samples. DNA was sent for next generation sequencing of bacterial 16S rRNA gene amplicons. We will process sequences in the computer program QIIME2, where we will identify taxa and perform various diversity analyses
Results: We expect that 1) intraspecific microbiomes will be more similar than interspecific microbiomes, regardless of sampling location, 2) the amoeba microbiomes will be less diverse than the microbiomes of surrounding soil, and 3) both amoeba and soil microbiomes will vary with environmental factors
Conclusion: The bacterial microbiomes of social amoebae have the potential to act as simple models for studying microbiome dynamics. If intraspecific microbiomes are more similar than interspecific microbiomes it would suggest coevolution of bacteria with their specific hosts. Alternatively, if there is significant inter-individual variation within amoeba species, social amoebae microbiomes would be similar to the human gut microbiome, which may make them an even more valuable model.

P.22. The Effects of Tillage on Soil Microbial Diversity in Corn-Soybean Systems

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Background**:** Although considerable focus has been given to the chemical and physical property impacts on soil quality, proposals for sustainable land use have shown that there is a lack of much research on the assessment of “soil quality” from a soil microbiological point of view. In this study, we studied the interactions between chemical, physical and biological indicators under different management systems to establish strategies to achieve soils that effectively support sustainable agriculture.
Methods**:** We report results from a three-year study to determine the effects on changes in soil microbial diversity from tillage (two reduced intensity tillage practices and two with increased intensity) in a corn-soybean production system. Soil samples (0 to 7.5-cm depth), were collected from tillage treatments at locations in the Southern Coastal Plain (Verona, MS) and Southern Mississippi River Alluvium (Stoneville, MS) MLRA’s, were extracted for 16S rRNA genes and sequenced with NGS (Illumina Mi-Seq platform). Quality DNA libraries were assembled, and produced nearly 15,000 OTUs for each soil sample.
Results**:** The Mantel test of correlations indicated that differences among microbial community members, i.e., OTUs, were significantly influenced by tillage regime (rM = 0.15, p = 0.002). Both Bray-Curtis ordination and alpha diversity indices (Simpson) were used to track annual changes in microbial diversity wherein the first year under the lowest intensity tillage had the highest level of diversity. In the second and third year we measured significantly greater diversity in both reduced tillage treatments than in the two tillage practices with increased intensity. For both study sites, differences in tillage intensity effects on the abundance of *Proteobacteria* followed the general order of lower intensity paired with lower abundance.
Conclusion**:** Higher tillage intensity led to greater abundance of *Proteobacteria* by making substrate (crop residues) more available for these decomposers. This study indicates that soil management via tillage regime had a profound effect on both diversity and the composition of microbial communities. Further, the intensity of soil management influences the structure of soil microbiomes over relatively short periods of times, and the temporal trend is similar across diverse crop production regions. The observed structure is expected to be highly correlated with results from functional genomics analysis.

P.23. Using a molecular-genetic approach to investigate the interactions between non-legume plants and plant growth-promoting bacteria

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Background: Non-legume plants such as rice and corn can form beneficial associations with plant growth-promoting bacteria (PGPB). Several studies have shown that these PGPB promote plant growth via nitrogen-fixation and hormone synthesis. Our current understanding of the molecular aspects and signaling that occur between the plants and PGPB is limited. In this study, we are investigating these interactions at a molecular and genetic level.
Methods: We used different experimental systems to study the growth promotion effect and root colonization in rice and corn plants by PGPB such as *Azospirillum brasilense* and *Burkholderia unamae*. We will perform dual RNA-seq experiments to study the regulation of gene expression occurring in the plant roots and bacteria. We are also optimizing a protocol to generate a bacterial (*Azospirillum brasilense*) mutant library to be used for a genome-wide Tn-seq experiment.
Results: We successfully set up an experimental system where PGPB such as *Azospirillum brasilense* and *Burkholderia unamae* could colonize plant roots and promote growth in rice and corn. We are currently extracting total RNA from roots of rice and corn for the dual RNA-seq experiments. Our preliminary data from the bacterial mutagenesis experiments suggest that we were not able to obtain a highly randomized mutant library.
Conclusion: Our experimental systems are appropriate to study the interactions between non-legume plants (rice and corn) and PGPB (*Azospirillum brasilense* and *Burkholderia unamae*) at a transcriptomic level. Results from the dual RNA-seq experiments will identify the transcriptomic changes occurring in both the host plant and bacteria. We will continue optimizing the protocol to obtain a highly randomized bacterial mutagenized population to be used for a Tn-seq experiment.

P.24. The Diversity of Fungal Symbionts of Social Amoeba

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Background: Amoebae are natural predators of bacteria and fungi, feeding on them using phagocytosis. In bacteria, research suggests that amoebae can act as an “evolutionary training ground” for intracellular adaptation. Since the bacteria are put under constant selective pressure to evolve resistance to phagocytic digestion in nature, they may also be able to resist digestion by macrophage cells. Little is known, however, if this process can extend to fungi. The purpose of this study was to survey the diversity of fungal symbionts in social amoebae to understand the ecological relevance of amoebae as potential reservoirs for fungal intracellular adaption. We are also exploring the nature of these symbiotic relationships. If fungal symbionts have host specific relationships with particular amoeba species, then this may suggest a co-evolved mutualistic relationship. Given observations from natural populations, we hypothesized that fungal species are present within amoeba fruiting bodies and that the biological partnership is host specific.
Methods: We collected five amoeba species from the same location to identify their fungal symbionts. We ran a PCR using ITS fungal-specific primers and cloned individual PCR fragments. The cloning product was sent for sequencing and the NCBI BLAST database was used to identify fungal species based on sequence similarity. Phylogenies have been constructed to show the relationships between our samples and known fungal species.
Results: Preliminary sequence analysis suggests a variety of previously undetected fungal species may be symbiotic associates, and in some amoeba species the relationship could be host specific. For example, in the amoeba *P. pallidum* only one fungal species, *Cunninghamella elegans* isolate CFR-C11 was identified.
Conclusion: Analyzing the relationship between fungi and amoebae can help to piece together factors that shape the complete social amoeba microbiome and shed light on the role of amoebae in fungal intracellular adaptation. Future studies are focused on surveying the diversity of fungal symbionts of amoeba in multiple locations.

Bacteriology and Eukaryotic Microbiology Posters

P.25. The GcrA/CcrM epigenetic regulatory system has predominantly divergent regulatory targets in closely related bacteria

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CcrM is a cell-cycle regulated DNA methyltransferase present in *Caulobacter crescentus*, which methylates adenines in GANTC motifs. Chromosome methylation changes during the cell cycle in a regular fashion, and impacts gene regulation. GcrA is a global regulator whose activity is dependent on methylation within its binding site, making GcrA and CcrM an epigenetic regulatory system. Both CcrM and GcrA are highly conserved across the Alphaproteobacteria, including *Brevundimonas subvibrioides,* an Alphaproteobacterium which is closely related to *C. crescentus* and occupies a similar environmental niche. Using RNA-seq, 129 genes were misregulated in a *B. subvibrioides ccrM* mutant, which is similar in number to the 152 genes misregulated in *ccrM* mutants in *C. crescentus*. Using RNA-seq and ChIP-seq, 42 genes were directly regulated by GcrA in *B. subvibrioides*, compared to *C. crescentus*, where 204 genes are under direct regulation of GcrA. A closer look at the individual genes showed that very few of these regulatory targets were common to both organisms. Only 4 genes were common in CcrM specific regulons, 9 genes common for GcrA regulons, and only 2 genes were common to

all four regulons. The expression of a *ctrA* promoter is dependent in both GANTC methylation as well as GcrA binding in both organisms but due to the presence of multiple methylation sites in *B. subvibrioides ctrA* promoter, its expression is different compared to *C. crescentus ctrA* promoter which contains only one methylation site, though the role of these additional methylation sites is unclear. Despite the presence of GcrA binding and demonstrating misregulation in a *B. subvibrioides gcrA* mutant, the expression of *sciP* promoter seems dependent on only CtrA. These results suggest that even though the GcrA/CcrM system is conserved in Alphaproteobacteria, the targets of the regulatory system can become highly specialized in individual organisms, even over short evolutionary distances.

P.26. Analysis of the *Brevundimonas subvibrioides* developmental signaling system reveals inconsistencies with previous developmental schemes

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Caulobacter crescentus is a Gram-negative oligotrophic bacterium and a developmental model organism for Alphaproteobacteria. *C. crescentus* displays a dimorphic life style coordinated by complex signaling pathways, including the DivJ-DivK-PleC signaling system. *Brevundimonas subvibrioides* is another gram-negative oligotrophic alphaproteobacterium with a dimorphic lifestyle closely related to *C. crescentus*. It was expected that the *B. subvibrioides* cell cycle regulatory systems would be identical to *C. crescentus*; however phenotypic assays of mutants revealed surprising differences. The *divJ* mutant displayed the same phenotypic characteristics in between species, *pleC* had some differences, while *divK* had completely opposite phenotypes. Suppressor mutations of the *B. subvibrioides divK* motility defect were found in c-di-GMP related genes *dgcB* and *cleD*, but not in the diguanylate cyclase *pleD*. Disruption of *pleD* in *B. subvibrioides* had no effect on the phenotypes of *divK* or *pleC*, but did remove adhesion from *divJ* with little effect on its other phenotypes. While suppressor mutations altered the phenotypes of a *divK* mutant, they had inconsistent impacts on c-di-GMP levels. Conversely, when c-di-GMP levels were manipulated in the *divK* background minimal phenotypic impact was observed. These results call into question how c-di-GMP signaling is integrated into the *B. subvibrioides* cell cycle.

P.27. Engineering Bacteriophage Delivery of an Antimicrobial CRISPR/Cas9 System to *Streptococcus pneumonia*

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Background: *Streptococcus pneumoniae* is a gram-positive, facultative anaerobe and is the most common cause of pneumonia, meningitis, and otitis media. As antibiotic resistance becomes an increasing concern, there is demand for novel treatments for bacterial infections. One such innovative solution is phage therapy. The CRISPR and CRISPR-associated Cas9 genes are used as a genome editing system in bacteria. We hypothesize that by integrating a CRISPR/Cas9 system into the genome of a temperate pneumococcal phage, we will develop a system for phage-mediated clearance of *S. pneumoniae*.
Methods: Synthetic oligos specific to the *ply* gene (spacer sequence) were cloned into the pKS1 vector containing a promoter, leader sequence, and direct repeats to create pKS2. The pre-crRNA (promoter, leader sequence, DR, and spacer region) was amplified from pKS2 and cloned downstream of the genes for the tracr-RNA and Cas9 (originating from *Streptococcus pyogenes* SF370) to generate pKS4. Non-essential gene segments from *S. pneumoniae* temperate phage MM1 along with an erythromycin cassette for selection were cloned into pKS4 to flank the CRISPR/Cas9 system, thus resulting in creation of pKS5 and allowing for homologous recombination into the pneumococcal chromosome.
Results: We have successfully created the ply-targeted CRISPR/Cas9 system in the plasmid pKS5 for integration into the *S. pneumoniae* genome. Transformations to integrate the system are currently underway. Once complete, CRISPR phage will be induced by mitomycin C induction and tested for antibacterial activity against *S. pneumoniae*.
Conclusion: Use of phage-delivered CRISPR/Cas9 systems targeting the pneumococcal chromosome will provide a novel and safe treatment strategy against pneumococcal infection without the use of antibiotics and will avoid potential complications such as development of resistance.

P.28. Anti-Psychotic Drugs Inhibit *Coxiella burnetii* Growth in Human Macrophages

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Background: Coxiella *burnetii* is a Gram-negative, obligate intracellular bacterial pathogen commonly found among livestock. Inhalation of contaminated aerosols leads to human Q fever, a debilitating flu-like illness that manifests with symptoms ranging from high fever and fatigue to endocarditis. *C. burnetii* preferentially infects alveolar macrophages wherein the pathogen replicates in a phagolysosome-like vacuole called the parasitophorous vacuole (PV). *C. burnetii* uses a type IV secretion system (T4SS) to secrete proteins into the host cell cytoplasm where they modulate host cell processes to maintain infection. Standard treatment for Q fever is doxycycline; however, the threat of antibiotic resistance and lack of a vaccine approved for widespread use creates a need for new therapeutic strategies, such as host directed anti-microbial drugs (HDADs). Here, we screened a panel of FDA-approved drugs, known to block a variety of host processes, for their ability to antagonize *C. burnetii* intracellular replication.
Methods: *C. burnetii* expressing red fluorescent mCherry was used for this study to aid assessment of bacterial growth in macrophages and visualization by fluorescence microscopy. 726 FDA-approved compounds, from the NIH Clinical Compound (NCC) Library, were screened for the ability to reduce *C. burnetii* growth during infection of human macrophages when cells

were treated prior to infection. Anti-psychotic drugs from the NCC library were then assessed for their ability to reduce *C. burnetii* growth in human macrophages when cells were treated post-infection and during growth in axenic medium. Cell viability assays were used to assess eukaryotic cell death caused by each drug. Currently, host signaling pathways targeted by anti-psychotic drugs are being assessed using Western blot analysis.
Results**:** 91 compounds from the NCC library inhibited intracellular *C. burnetii* growth indicated by decreased mCherry expression. Multiple anti-psychotic drugs, when added 24 h post-infection, decreased mCherry fluorescence during macrophage infection; however, these drugs did not affect mCherry fluorescence in axenic medium culture. Cell viability assays indicated multiple anti-psychotic drugs were not cytotoxic.
Conclusion**:** Overall, these data suggest multiple anti-psychotic drugs inhibit intracellular growth of *C. burnetii* in macrophages. This inhibition is not due to cytotoxic effects or direct targeting of the bacteria by the drug. Decreased activation of distinct host signaling may drive inhibition of host processes by these anti-psychotic drugs. This result would suggest those host processes are necessary for *C. burnetii* replication. Identification of these drugs as HDADs suggests potential use as future Q fever treatments.

P.29. Metagenomics of Preterm Infant Skin

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Background: Although the skin microbiota of preterm infants includes potential pathogens, its development and role in infection is poorly understood. This is due, in part, to the technical challenges presented by sampling their skin and a lack of resolution for bacterial species and strains utilizing 16S rRNA sequencing.
Methods: To begin to characterize the preterm infant skin microbiota at the species and strain level, we performed a prospective, longitudinal metagenomic study. For two preterm infants in the neonatal intensive care unit, five skin sites and fecal material were sampled biweekly for two months. Samples were treated with propidium monoazide to eliminate extracellular DNA and whole genome sequencing was done.
Results: A total of 113 microbial species were detected from 38 samples. Stool samples were the most species-rich (averages=25.7 species) although one baby's stool samples were dominated by *Citrobacter koseri*. The nares, axillary vault, periumbilical cord, and upper chest skin sites were the least species-rich (6.4-9.3 species), and the inguinal crease skin site was of intermediate species richness (15.6 species). *Staphylococcus epidermidis* was detected in the most samples (n=35) and at least two strains of *S. epidermidis* were identified based on 4,486 biallelic SNPs shared across 14 tested samples.
Conclusion: These results demonstrate that it is possible to overcome the technical challenges of sampling the microbiota of preterm infant skin, and characterize its community at the level of species and strain. This approach may be applied to enhance the diagnosis, treatment, and prevention of infections.

P.30. Identification of host cellular receptor of *Streptococcus pneumonia*

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Background**:** *Streptococcus pneumoniae* (pneumococcus) is a common colonizer of humans that is capable of causing otitis media, pneumonia, and meningitis. The currently available pneumococcal conjugate vaccines are composed of capsular polysaccharides from the serotypes causing the most invasive infections and elicit serotype-specific immunity that successfully prevents invasive disease in both children and adults. However, the overall rates of colonization have remained fairly constant and vaccine serotypes are frequently replaced with non-vaccine serotypes through genetic exchange. Therefore, it is necessary to develop non-serotype specific, protein-based vaccines for preventing both invasive and non-invasive pneumococcal disease. We hypothesized that Far-western technique could be used to identify host receptors to pneumococcal adhesins as a first step in developing a vaccine against colonization.
Methods**:** We constructed a staphylococcal expression vector expressing the pneumococcal surface protein, SP_1650 (PsaA), which is known adhesin. The recombinant protein was purified by affinity chromatography and biotinylated for use as bait protein in far-western blot. Cell lysate proteins from the human nasopharyngeal cell line, Detroit 562, were used as a prey proteins for 1D and 2D far-western analysis to identify specific ligand proteins of the host cell that can interact with PsaA. We have transfected and transduced the human kidney cell line HEK293 T/17 with Lentivirus expressing annexin A2 (AnxA2). Additionally, we performed adhesion assays Detroit 562, HEK293 T/17 control cells, and AnxA2-transduced HEK293 T/17 cells to determine if AnxA2 expression increases adherence of pneumococci expressing PsaA versus isogenic mutants lacking PsaA.
Results**:** We could successfully express other five of pneumococcal proteins (SP_1003, SP_1241, SP_1650, SP_1860, and SP_2239) in staphylococcal expression system. From far-western and mass spectrometry analysis we identified that PsaA interacts with human annexin A2 (AnxA2) from Detroit 562 cells. The AnxA2-transduced HEK 293 T/17 cells were confirmed to overexpress AnxA2 by western blot probed with AnxA2 antibody.
Conclusion**:** This study shows that AnxA2 serves as a host cellular receptor for pneumococcal PsaA. Thus, adhesion assay will be able to determine the adherence ability of pneumococcus in presence of AnxA2 and vice versa. we have preliminary data that 20% of pneumococcus could adhere to Detroit 562 cell versus 5% of pneumococcus adhere to HEK 293 T/17 cell. This study will develop a platform for identifying important pneumococcal adhesins, their host receptors, and allow for development of vaccines aimed at blocking colonization.

P.31. Novel Compounds with Efficacy against *Acinetobacter baumannii*

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Background: Several bacteria (the "ESKAPE" pathogens) are considered a high threat to human health due to nosocomial infections and antibiotic resistance, including *Acinetobacter baumannii*. Among the strategies recommended by CDC to combat this increased prevalence of antibiotic resistant bacteria is the development of new antibiotics. Our lab has produced a variety of new compounds based on pyrazole, many with potent antibacterial activity. In this study we report the results of antimicrobial testing of several of the newly synthesized compounds in this series.
Methods: Following organic synthesis, compounds were screened for antimicrobial activity using minimum inhibitory concentration testing (MIC) against multiple strains of *Staphylococcus aureus,* two other Gram positive bacteria, and *A. baumannii* 19606. In this study, compounds with low MICs against *A. baumannii* were studied further. These compounds were tested for their ability to generate Reactive Oxygen Species (ROS) using the indicator 2',7'-dichlorofluorescein diacetate (DCFH-DA). Additionally, damage to *A. baumannii* cells was determined using the propidium iodide/Syto-9 ratio.
Results: Over three dozen compounds were tested with more than half showing significant activity (MICs of 6.25 µg/mL or less) against either *Staphylococcus* spp. or *A. baumannii*. Four compounds with MICs against *A. baumannii* ranging from 6.25 down to 1.56 µg/mL were selected for further testing. All four compounds, when tested at their MICs, elevated the levels of ROS as compared to untreated bacteria. Compound #25 significantly increased the propidium iodide to Syto-9 ratio at 2x its MIC, indicating either a specific impairment of membrane function or a bactericidal effect.
Conclusion: Specific modifications to pyrazole-based molecules continue to result in the creation of compounds with potent antimicrobial activity. Numerous avenues of research are underway to learn more about the efficacy of these compounds using *in vivo* systems as well to discover more clues as to their mechanism of action.

P.32. LukAB and HlgCB Cytolytic Toxin Production and Activity during *Staphylococcus aureus* Infection of Human Macrophage-Like Cells

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Background**:** *Staphylococcus aureus* is an opportunistic pathogen and commensal bacterium of the upper respiratory tract. Approximately 500,000 individuals in the U.S. are infected with *S. aureus* annually, with ~ 50,000 succumbing to infection-related complications. Due to extensive antibiotic use, multi-drug resistant *S. aureus* strains have emerged. With no vaccine against *S. aureus*, and antibiotics becoming a limited treatment option, therapeutics have focused on a diverse panel of *S. aureus* virulence factors. *S. aureus* produces over 40 exotoxins, including the human-specific cytolysins Panton-Valentine Leukocidin (PVL) and α-toxin (Hla), and varying toxin repertoires between *S. aureus* strains contributes to difficulty in treating infection. During infection of human alveolar macrophages (hAMs), LAC, a methicillin-resistant strain, is significantly more cytotoxic than UAMS-1, a patient-acquired methicillin-sensitive strain. LAC does not produce PVL during hAM infection, and Hla, while produced, is inactive, suggesting other virulence factors are responsible for LAC cytotoxicity. Here, we investigated human-specific LukAB and HlgCB toxin activity during *S. aureus* infection of human macrophage-like THP-1 cells.
Methods**:** THP-1 cells were infected with LAC or UAMS-1 for 24 h. Western blot analysis was used to probe THP-1 lysates and supernatants for the presence of LukAB and HlgCB subunits. Recombinant LukAB and HlgCB were used in cell viability assays that measured cell death caused by each toxin. LAC and UAMS-1 grown in eukaryotic media were compared to bacteria cultured in prokaryotic media using Western blot analysis to assess LukAB and HlgCB production.
Results**:** Supernatants from LAC-infected THP-1 cells contained LukA, LukB, HlgC, and HlgB, while toxin subunits were differentially produced by UAMS-1. CCK8 assays showed a dose-dependent increase in THP-1 cell death when treated with HlgCB, indicating both subunits of the HlgCB complex are required for cytotoxicity. *S. aureus* cultured in eukaryotic or prokaryotic media containing pyruvate produced LukAB and HlgCB, while subunit production differed in media lacking pyruvate.
Conclusion**:** These results suggest that components, such as pyruvate, that alter *S. aureus* metabolism contribute to cytotoxicity through increased production of distinct *S. aureus* toxins. Overall, these findings suggest increased LAC cytotoxicity may be the result of differential LukAB and HlgCB production.

P.33. Investigating the Novel Protein-Protein Interaction between *Gl*eIF4A RNA Helicase and *Gl*eIF3i of the Preinitiation Complex in Primitive Eukaryote *Giardia lamblia*

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Background: The parasite *Giardia lamblia* is a pear-shaped, unicellular eukaryotic microorganism that is responsible for causing diarrheal disease, Giardiasis, worldwide. *Giardia* is also considered as one of the earliest branching Eukaryotes and has become a model organism for gaining insight into the evolution of various cellular processes, including eukaryotic translation initiation. Typical translation initiation in eukaryotes begins with the recruitment of the preinitiation complex (PIC) to the 5' cap by the trimeric complex eIF4F. The protein complex eIF4F is composed of the cap binding protein eIF4E, a DEAD-box RNA helicase eIF4A, and the scaffolding protein eIF4G connecting the complex to the subunits of the PIC. The primary differences between *Giardia* translation initiation and other mammalian cells are centered around *Giardia's* lack of 3 key translation initiation factors: eIF4B, eIF4H, and eIF4G. Both eIF4B and eIF4H are thought to stimulate the RNA helicase eIF4A's affinity for RNA, increase its activity, and prevent reannealing of unwound ssRNA. And eIF4G is the aforementioned

scaffolding protein. Therefore, in the absence of eIF4G, it is not clear as to how the PIC and RNA helicase are recruited to the 5' end of mRNA in *Giardia.*
Methods: A novel protein-protein interaction was discovered between *Gl*eIF4A and the subunit *Gl*eIF3i, indicating a possible way for *Gl*eIF4A to be recruited to the PIC. Analysis of both the *Gl*eIF4A protein structure and the ZDOCK model of potential docking conformations between *Gl*eIF4A and *Gl*eIF3i was performed to identify amino acid residues within eIF4A that could be important for binding.
Results: The ZDOCK modeling yielded 10 residues where *Gl*eIF4A and *Gl*eIF3i are potentially interacting. This result lead to the synthesis of several mutant *Gl*eIF4A constructs via site-directed mutagenesis, and protein-protein interactions are currently being tested within a yeast-2-hybrid system. Additionally, the helicase activity of eIF4A is also being explored through the application of an NADH-coupled ATPase assay that monitors the rate at which ATP is being hydrolyzed by the *Gl*eIF4A in an in-vitro system.
Conclusion: The primitive translational machinery that exists within *Giardia lamblia* and the unique mechanisms surrounding its recruitment and regulation are being explored. Additional research is also being conducted to determine the potential for *Gl*eIF3i as a stimulator of *Gl*eIF4A. The protein structure of *Gl*eIF4A and other eIF4A homologs contain two domains separated by an amino acid linker sequence. eIF4a is considered a minimal helicase since it has very little N-terminal extensions and no C-terminal extensions with which to interact with ATP, RNA, or other proteins. Consequently, eIF4A homologs are highly reliant on other translation initiation factors, specifically eIF4B and eIF4H, to bring the two domains together. As previously mentioned, *Giardia* does not have these initiation factors, and it is hypothesized that eIF3i is filling that role.

P.34. Analysis of amino acids residues involved GleIF4E2 interactions with GleIF2beta in *Giardia lamblia*

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Background: *Giardia lamblia* is a parasite that infects various number of mammals, including humans. The main mode of transmission is via water or food infections, and some symptoms include diarrhea, greasy stools that tend to float, and dehydration. eIF4G, eIF4B, and eIF4H are common translation initiation factors identified in most eukaryotic organisms, however, *Giardia* is different because it lacks these 3 key initiation translation factors. Translation initiation factor eIF4G is known to interact with RNA helicase eIF4A and cap-binding protein eIF4E. This leads to recruitment of the preinitiation complex to the 5' cap and subsequent translation of mRNA. Translation initiation factor eIF4G binds to the PolyA binding protein 1 thus the messenger RNA's poly A tail. Translation initiation factor eIF4H stimulate the initiation of protein synthesis at the level of mRNA unwinding. *Giardia* like other Eukaryotes do have translation initiation factor eIF4E2, but not EIF4G. The mechanism of ribosome recruitment to mRNA in the absence of eIF4G is more clearly understood in *Giardia*.
Method: A recent novel interactions was discovered between GleIF4E2 and GleIF2beta. ZDOCK computer modeling of GelIF4E2-GleIF2beta was used to identify various residue being yeast two hybrid of the GelIF4E2 that are involved in binding to GelIF2beta.
Results: ZDOCK program has identified several residues that are involved in protein-protein interaction. Site-directed mutagenesis was performed in order to test their effect on protein-protein interactions.
Conclusion: The residues identified by ZDOCK are conserved in eukaryotic initiation factors eIF4E that are involved in binding to eIF4G. The mutants that disrupt the interactions will be further tested in *Giardia*.

P.35. Prevalence of *Burkholderia* Bacterial Symbionts in Natural Populations of Social Amoebae

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Background: *Burkholderia* bacteria have a myriad of different lifestyles, many of which are host-associated and range from pathogenic to mutualistic. The mechanisms that underlie these host/symbiont interactions, however, are unclear. Three different species of *Burkholderia, B. agricolaris, B. hayleyella, and B. bonniea,* have been shown to be symbiotically associated with the social amoeba *Dictyostelium discoideum.* Usually, bacteria are phagocytized and digested by the amoeba, but the presence of *Burkholderia* gives them the ability to carry undigested bacteria to a new location to use as a food source, which is referred to as "farming". Farming has only been identified in *D. discoideum*, conferred by these particular *Burkholderia* species. In this study, we aim to identify the prevalence and identity of *Burkholderia* in natural populations of social amoebae to see if the same *Burkholderia* species are also symbionts to other social amoeba species.
Methods: We collected soil samples from 18 locations throughout Arkansas and neighboring states. Samples were plated and fruiting bodies of 10 different amoeba species were collected for DNA extraction, PCR, and sequencing. Amoeba-specific primers were used to confirm amoeba species identity. *Burkholderia, Chlamydiae,* and *Amoebophilis* specific primers were used to identify the presence of particular bacterial genera and were sequenced for additional identification of species.
Results: Preliminary data shows an infection rate of over 75% throughout all locations that were screened for *Burkholderia. Burkholderia* was found to infect multiple species of social amoebae. In these other species we have found the farming symbiont *B. agricolaris*, as well as other *Burkholderia* species: *B. phenazium*, *B. arboris*, and *B. caledonica*.
Conclusion: The widespread prevalence of *Burkholderia* suggests that these bacteria play an important role in the ecology of many social amoebae. Further research into this topic can reveal how *Burkholderia* is able to effectively form this relationship and what the effects are of these other *Burkholderia* species are on their amoeba hosts.

P.36. The role of ABC and BCCT-type transporters of *Pseudomonas synxantha* 2-79 in the response to water stress and colonization of plant rhizosphere

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Background: Drought is a major form of abiotic stress that imposes adaptive constraints on both the host plants and microorganisms associated with them. Much attention has been given to uncovering the mechanisms of drought tolerance in plants, but the effect of water limitation on rhizosphere microbial communities remains poorly understood. There is a growing evidence that plants actively select and shape the rhizosphere microbiome that can help them adapt to life in arid soils, but details of this process remain poorly understood. In this study, we characterized the contribution of ABC- and BCCT-type transporters for quaternary ammonium compounds (QACs) to the ability of the biocontrol strain *Pseudomonas synxantha* 2-79 to handle water stress and colonize plant rhizosphere.
Methods: We analyzed the genome sequence of 2-79 and identified genes involved in the uptake and metabolism of QACs choline (CHO), glycine betaine (GB), sarcosine (SAR), and carnitine (CAR). We used RNA-seq and ice nucleation reporter assays to measure changes in the expression of QAC metabolism genes in response to root exudates of *Brachypodium distachyon*. We then constructed isogenic mutant derivatives of 2-79 and evaluated these strains for the capability to catabolize individual QACs and utilize these metabolites for osmoprotection. Finally, we compared the ability of the WT strain and its transporter gene mutant to colonize the rhizosphere of *B. distachyon* under drought stress.
Results: The transcriptomic studies demonstrated that root exudates perturb multiple genes associated with the uptake and metabolism of QACs. Under water-replete conditions, 2-79 used the CbcWV transporter to take up CHO, which was then catabolized (along with GB and SAR) as a source of C and N. In contrast, under water stress, CHO, GB, and CAR were transported by BetT1, BetT2, and OpuC and used by 2-79 for osmoprotection. Upon deletion of the five predicted QAC transporter genes, 2-79 retained its ability to grow on GB under water-replete condition but lost its ability to utilize all tested QACs under water stress. The gene reporter assay further supported these findings by indicating that these transporter genes are differentially regulated under water stress. When inoculated individually, the WT 2-79 and its QAC transporter mutant did not differ in their ability to colonize the rhizosphere of drought stressed *Brachypodium*. However, when the strains were co-inoculated into the plant, the WT parent outcompeted the mutant after 5 weeks of drought stress.
Conclusions: Our results revealed that rhizosphere pseudomonads carry extensive and redundant sets of transporters that function in the uptake of plant-derived QACs. Depending on the environmental conditions, these QACs serve as nutrients or osmoprotectants and contribute to the adaptation of microorganisms to the rhizosphere lifestyle.

P.37. Regulation of PCD by *msaABCR* operon during Staphylococcal biofilm development

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Background: The potential for *Staphylococcus aureus* to cause chronic infection due to the formation of biofilms has become a growing health concern. Past studies have shown that the major constituents for the biofilm extracellular matrix are released by programmed cell death (PCD) of a subpopulation of the *S. aureus* biofilm. PCD is known to be potentiated in *S. aureus* by *cidABC*-mediated acetate and reactive oxygen species (ROS) generation. In addition, deletion of *msaABCR* operon has been shown to cause a decrease in biofilm thickness and an increase of cell death relative to the wild-type. The mechanism underlying this unregulated cell death in the *msaABCR* mutant biofilms, however, is still largely unknown. We hypothesized that the *msaABCR* operon may regulate programmed cell death processes during biofilm development in *S. aureus.*
Methods: To further elucidate the mechanism for programmed cell death used by *msaABCR* operon, we grew LAC USA300 and UAMS-1 *msaABCR* mutant strains in TSB supplemented with 35mM glucose in order to simulate the microenvironment of the *S. aureus* biofilm. Under these conditions, we measured stationary phase survival as well as glucose consumption and acetate generation rates of these strains. In addition, we quantified the expression of genes involved in PCD in the *msaABCR* mutant.
Results: The *msaABCR* mutant exhibited an increased rate of cell death in stationary growth phase compared to the wild-type strain under excess glucose condition. In addition, the *msaABCR* mutant consumed glucose and generated acetate at higher rates than wild-type. We also observed an up-regulation of *cidR* regulon in the *msaABCR* mutant.
Conclusion: These results suggested an increased weak acid-dependent cell death in *msaABCR* mutant via the *cidABC* pathway. Therefore, for future experiments, we seek to determine whether the msaB protein directly or indirectly regulates the *cidR* regulon using ChIP-seq analysis. To further verify *cidABC*-mediated cell death in *msaABCR* mutant, we also plan to measure biomass volume, cell viability, and cell death within *msaABCR/cidABC* mutant biofilms using confocal laser scanning microscopy (CLSM).

P.38. A Survey of Sloth Hair Algae Using Culturing Techniques

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Sloths possess a coarse outer layer of fur that hosts a complex microbiome with various species of algae, fungi, and bacteria. Although sloths are popular in pop culture and social media, they are under-studied scientifically. Sloth hair algae has been primarily studied via 18S sequencing and microscopy-based morphological identification. To our knowledge, the photobiont diversity of sloths has not been well surveyed nor cultured for more careful investigation. We collected samples of hair from

the backs and heads of sloths from Costa Rica in collaboration with The Sloth Institute. Hairs were placed in liquid enrichment tubes and on agar plates of a custom culture medium developed in our lab for optimal cultivation of diverse species of algae and cyanobacteria. Cultured samples were repeatedly sub-cultured and transferred in an effort to isolate species and we now have a diverse library of cultured sloth algae/cyanobacteria that can be used to elucidate photobiont diversity associated with sloths and the ecosystem benefits of these algae to the sloth, as well as for bioprospecting discovery efforts.

P.39. Genomic Prediction of Vancomycin Nonsusceptibility in *Staphylococcus epidermidis*

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Background: *Staphylococcus epidermidis* is a common opportunistic and multidrug-resistant pathogen. Vancomycin is widely used for treating *S. epidermidis* infections, but the detection of certain nonsusceptible phenotypes is challenging. The development of genetic tests to detect vancomycin nonsusceptibility has been hindered by the polygenic basis of the phenotype in staphylococci. We hypothesized that a genetic test that accounted for the cumulative effects of many mutations might accurately predict vancomycin nonsusceptibility.
Method: We studied the genomes of 30 vancomycin-susceptible *S. epidermidis* (VSSE), 15 vancomycin-intermediate *S. epidermidis* (VISE), and 11 heterogeneous VISE (hVISE) collected from St. Jude Hospital during 2010-2016. We performed a genome-wide association study to estimate the effect size of each mutation on the phenotype. Each strain was then assigned a genomic score based on the sum of the estimated effect sizes of its alleles.
Results: Five mutations were associated with the phenotype at the genome-wide level ($P<0.05$, FDR-corrected), but a total of 2694 unique mutational patterns were observed and these represented a range of effect sizes. A linear relationship was discovered between the genomic score and the phenotype, when modeled separately for each of three strains (ST2: R^2=0.654, P=2.64E-04; ST5: R^2=0.769, P=8.24E-05; ST83: R^2=0.855, P= 2.77E-03). Although these models were based on very small sample sizes, their predictive R^2 remained close to the unadjusted R^2 (0.611, 0.637, 0.825, respectively).
Conclusion: The vancomycin nonsusceptibility of *S. epidermidis* can be reasonably well-predicted by taking into account the effects of multiple mutations on the phenotype. Future work could make use of larger sample sizes for model development and evaluation.

P.40. Transcriptional Regulation of Extracellular Proteases *Staphylococcus aureus*

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Background: *Staphylococcus aureus* produces a vast repertoire of virulence factors. Among these are extracellular proteases, which are known to promote tissue invasion, the acquisition of nutrients, and subversion of host defenes. There are 10 known of these proteases organized in 4 different operons. Maintaining a tight and adequate regulation of these proteases is extremely important for *S. aureus* virulence as mutations abolishing their production results in hypervirulence. On the other hand, mutation of *sarA*, a known repressor of extracellular proteases, results in virulence attenuation. The attenuated virulence phenotype observed in a *sarA* mutant can be restored to wild type levels by eliminating protease production. Given the importance of extracellular proteases in the outcome of *S. aureus* infections we investigated the promoters controlling the production of these proteins to define the mechanisms behind their regulation.
Methods: We used a DNA affinity chromatography-pulldown approach with each of for protease promoters (~500 bp upstream from the translational start site of the operon) coupled with mass spectrometry of the obtained eluates to identify the proteins binding to these DNA fragments. This was done using whole cell lysates from the hypervirulent USA300 strain LAC and an isogenic *sarA* mutant to determine proteins binding only in the presence/absence of SarA. Additionally, we made transcriptional reporters of these promoters with sequentially truncated versions of each of them and transduced these constructs into both LAC and the isogenic *sarA* mutant to identify the location of the binding sites of SarA.
Results: We found that SarA regulates the production of all 10 extracellular proteases by binding directly to their promoters. Additionally, we identified other regulatory proteins that bind to these promoters in an operon dependent manner. Finally, we identified the location and potential consensus sequence for the SarA binding sites in these promoters.
Conclusion: This study shows that SarA binds directly to the promoters controlling the production of all ten known extracellular proteases and that other regulatory loci are also involved in controlling their production in an operon-dependent manner.

P.41. Role of unusual promoter structure in the regulation of the pilin protein-encoding gene *pilA* in *Caulobacter crescentus*

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Background: The gram negative alpha-proteobacterium *Caulobacter crescentus* divides asymmetrically to give rise to a swarmer cell, which harbors pili and a flagellum, and a non-motile stalked cell. While most pilus structural genes are expressed earlier in the cell cycle, the *pilA* gene (encoding the sole pilin) is expressed at the time of cell division by the global regulator CtrA, which occurs later than other CtrA-dependent genes. CtrA binds to 4 sites upstream of the *pilA* transcriptional start site and activates its transcription, which is unusual as most CtrA dependent promoters have a single CtrA-binding site. It was hypothesized that this unusual architecture of the *pilA* promoter plays a role in the timing of this promoter.
Methods: To test this hypothesis, β-galactosidase reporter fusions were created where various CtrA-binding sites were deleted or mutated and tested for activity.

Results: The construct with only Site 1 (P1), which overlaps the -35 region of the promoter, showed an activity twice that of the WT promoter (P1234). Mutating just Site 2 (P123:2) also had the same effect while mutating Site 3 (P123:3) resulted in reduced activity, suggesting that Site 1 drives *pilA* expression and other sites repress expression to various degrees. When these constructs were used in synchronized swarmer cells, *pilA* transcription was found to occur about 20 minutes earlier in P1 than in the WT construct. A construct with three tandem repeats of Site 1 displayed the same repression of activity, but a similar construct of a PhoB-dependent promoter in *Escherichia coli* did not, demonstrating this is a property specific to CtrA.
Conclusion: These results suggest that CtrA is both an activator and a repressor of the same promoter and may form a completely novel regulatory mechanism.

P.42. Cobalt Chloride inhibits the growth of *Mannheimia haemolytica*

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Background: *Mannheimia haemolytica* is an opportunistic pathogen that proliferates in the nasal pharynx of cattle. It is gram negative, non-motile, and the cause of bovine pneumonic pasteurellosis. When the animal is under stress and its immune system is compromised, the bacteria is able to invade the lower respiratory tract and cause pneumonia. *M. haemolytica* is a known contributor to bovine respiratory disease, also known as shipping fever, which costs the cattle industry up to one billion dollars annually. Cobalt has been shown to improve the immune system and is also a metal that is known to inhibit bacterial growth. A study done by Paterson & MacPherson (1990) showed that cattle on a depleted Co diet had a lowered immune response and more severe bacterial infection than Co supplemented cattle. However, when too much cobalt is taken into the body, it can cause harmful effects.
Methods*:* The goal of this experiment is to find the optimal concentration of $CoCl_2$ to use to inhibit the growth of *M. haemolytica* at varying temperatures. *M. haemolytica* was grown in the presence of different concentrations of $CoCl_2$ under varying temperature and cell density conditions. A spectrophotometer was used to measure the optical density of the samples.
Results: Our results show that M. haemolytica has optimal growth in BHI media at 37 C^o, and grows less efficiently as the temperature increases., with 5 mM almost completely inhibiting growth at 37^{o}C. At higher temperatures, the effect seems preserved. When the input cell-density was lowered, the $CoCl_2$ concentration needed to stop growth was lower as well. When the input cell-density was raised, the 0.5 mM, 1 mM, and 1.5 mM all behaved similarly to the control.
Conclusion: The effect of cobalt chloride on *M. haemolytica* growth is concentration-dependent, cell density dependent, and temperature dependent.

P.43. Invasion of Human Middle Ear Epithelial Cells by Nonencapsulated *Streptococcus pneumoniae* Expressing Green Fluorescent Protein

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Streptococcus pneumoniae (pneumococcus) colonizes the human nasopharynx and causes infections such as pneumonia, conjunctivitis, and otitis media (OM). Currently licensed pneumococcal vaccines have reduced the incidence of invasive pneumococcal disease but have not effectively reduced mucosal infections such as OM. Nonencapsulated *S. pneumoniae* (NESp) have been isolated from up to 8% of OM cases. In prior experiments, a chinchilla model of OM showed that NESp MNZ41 produces a high bacterial burden and results in invasive disease. However, the mechanism by which MNZ41 invades middle ear epithelial cells (MEECs) and causes invasive disease is unknown. To determine the host-pathogen interactions between MNZ41 and MEECs, we aimed to isolate human MEECs (hMEECs) containing intracellular bacteria. We hypothesized that NESp expressing green fluorescent protein (GFP) would allow detection of hMEECs containing intracellular NESp. Thus, we transformed MNZ41 and validated the presence of the *gfp* cassette using PCR. Furthermore, GFP expression was confirmed by fluorescent microscopy and flow cytometry. The GFP-expressing MNZ41 cells were used in an adhesion and invasion assay to determine if they adhere to and invade hMEECs. Using a multiplicity of infection (MOI) of 1000, we were able to detect intracellular MNZ41 in hMEECs. Future studies will involve isolation of hMEECs with intracellular MNZ41 by cell sorting. The sorted cells will be used in dual-RNA sequencing of hMEECs and intracellular MNZ41 to reveal interactions between NESp and the host. Overall, this study will allow for a better understanding of NESp virulence mechanisms during OM.

P.44. The Combination of Fluconazole and Atorvastatin Shows *In vitro* Synergy Against Fluconazole-resistant *Candida glabrata*

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Background**:** Fluconazole-resistant *Candida glabrata* is a not uncommon cause of invasive infections in immunocompromised patients. An increase in multidrug antifungal resistance and a lack of new antifungals increases the demand for new therapeutic approaches. Statins, drugs used for lowering cholesterol, have previously been found to have antifungal activity. In this study, we investigate the *in vitro* interaction of atorvastatin and fluconazole against fluconazole-resistant *C. glabrata*.
Methods**:** Three clinically unique bloodstream isolates of fluconazole-resistant *C. glabrata* were collected from patients at Ochsner Medical Center in New Orleans, Louisiana during 2010-2011. Fluconazole and atorvastatin MICs were determined by broth microdilution. Synergy testing was performed using a checkerboard broth microdilution method. The summation fractional inhibitory concentration index ($\sum$ FIC) was calculated for each isolate and interpreted as follows: $\leq$0.5, synergy; >0.5-1, additivity; >1-4, indifference; and >4, antagonism.

Results**:** The MICs (µg/mL) for atorvastatin were: 128, 128, 64, and for fluconazole, all 3 were >256 (resistant). Two *C. glabrata* isolates showed synergy (∑FICs 0.02, 0.03) with the combination of fluconazole (0.5 µg/mL) and atorvastatin (2 µg/mL). The third isolate showed synergy (∑FIC 0.1) with fluconazole (0.5 µg/mL) and atorvastatin (8 µg/mL). Synergy was observed using fluconazole concentrations as low as 9 two-fold dilutions below the MIC and atorvastatin, using concentrations 5-6 two-fold dilutions below the MIC. No antagonism was seen.
Conclusion**:** *In vitro* synergy with the combination of fluconazole plus atorvastatin was observed in the three fluconazole-resistant *C. glabrata* isolates using subinhibitory concentrations of both agents. This suggests that a combination of fluconazole and atorvastatin may serve as a new therapeutic approach in fluconazole-resistant *C. glabrata* infections, but additional fluconazole-resistant *C. glabrata* isolates should be evaluated for synergy via checkerboard broth microdilution and other methods for comparison. Synergy may not occur in all strains of fluconazole-resistant *C. glabrata*. Also, *in vitro* synergy may or may not correlate clinically.

P.45. Classifying the Activity of SP1466 Protein in Streptococcus pneumonia

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Background: Streptococcus pneumoniae (pneumococcus) is the leading cause of community-acquired pneumonia and otitis media. It commonly colonizes the upper respiratory tract of healthy individuals, but it can lead to diseases such as pneumonia, bacteremia, or meningitis, particularly in high risk populations such as infants, immunocompromised individuals, or the elderly. Pneumococcus is only known to express a single protein cytolysin, pneumolysin. However, the gene sp1466 encodes a putative hemolysin and is part of the core genome. SP1466 has homology to type III family of hemolysins and could therefore play a role in virulence. The majority of research done on type III hemolysins has been in bacteria including Bacillus and Vibrio. The purpose of this study is to identify the potential role of SP1466 in pneumococcal colonization/pathogenesis.
Methods: We utilized a gene deletion technique employing splicing by overlap extension PCR (SOEing PCR) to create an isogenic mutant lacking sp1466 in the TIGR4 strain of S. pneumoniae. We are currently cloning and expressing SP1466 using the TOPO-pET100 E. coli expression vector. We have designed primers and successfully amplified the gene by PCR for restriction digestion and eventual ligation into pET100. Once the protein is expressed, various cell types including epithelial cells, white blood cells, red blood cells, etc. will be exposed to SP1466 to determine cytotoxicity.
Results: We have successfully created an isogenic mutant that lacks the sp1466 gene and confirmed replacement of the gene with an erythromycin resistance cassette. We have also successfully cloned sp1466 into the expression vector for protein purification by affinity chromatography.
Conclusion: Characterizing the function of SP1466 in S. pneumoniae will provide novel information about this potential virulence factor.

P.46. The *msaABCR* and TCA cycle genes form persister cells dependent on ATP content of *Staphylococcus aureus* cells

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Background**:** Persister cells are phenotypic variant of bacterial population that shows extreme antibiotic tolerance without undergoing any genetic modification. The presence of persister cells during *Staphylococcus aureus* infections contribute to treatment failures by becoming dormant under environmental stress. When antibiotic treatment is ceased, they resume growth and cause recurrent infections imposing high socio-economic burden in the public health. We found the involvement of *msaABCR* operon in persister formation in *S. aureus.* Herein, we attempt to identify the regulatory mechanism of persister formation by the *msaABCR* operon dependent on TCA cycle genes in *S. aureus* cells.
Method**:** We measured the growth pattern and minimum inhibitory concentration (MIC) of gentamycin in the mutants as compared to wild-type USA300 LAC strain. Then, we measured the persister frequency in stationary growth phase of all the strains against gentamycin stress. We also measured the intracellular ATP concentration in all the strains tested.
Result**:** Similar growth pattern and same MIC was found in the mutants as compared to wild-type strain. On mutants of TCA cycle genes, we observed increased persister formation in presence of gentamycin. The ATP content was higher in *msaABCR* mutant while it was lower in the mutants of TCA genes as compared to the wild-type strain.
Conclusion**:** Since, persister cell formation is associated with the depletion of ATP; we further plan to study whether ATP content plays a deterministic role in persister formation in the stationary growth condition. We further plan to study the role of *msaABCR* in relation to the TCA cycle activity in persister formation in *S. aureus*.

Immunology and Virology Posters

P.47. Human antibody-mediated inhibition and enhancement of *Chlamydia trachomatis* infection *in vitro*

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Background: Antibody classes vary in function and distribution. The endocervix, the primary site of *Chlamydia trachomatis* infection in women, possesses a repertoire of locally produced IgG and IgA with varying contributions from serum antibody. In contrast to other mucosal sites, IgG is the predominant isotype rather than secretory IgA. Here, we determined the functional ability of antibodies of different classes and from different locations to inhibit or enhance *C. trachomatis* inclusion formation *in vitro* in human primary-like endocervical epithelial cells and investigated whether this effect is influenced by history of infection and the infecting serovar.
Methods: IgG and IgA were purified from serum and cervicovaginal lavages (CVLs) from women currently infected with *C. trachomatis* without complications. Elementary bodies (EBs) from *C. trachomatis* serovar D were incubated with normalized concentrations of *C. trachomatis*-specific purified antibodies. These were then added to confluent endocervical epithelial cells (A2EN) and incubated for 48 hours. Inclusions and nuclei were stained, imaged by fluorescence deconvolution microscopy, then analyzed in CellProfiler to determine the capacity of antibodies to inhibit or enhance inclusion formation. *C. trachomatis* genotyping of clinical samples was performed by amplification and sequencing of the *ompA* gene, followed by alignment of sample sequences with known *ompA* sequences.
Results: CVL-derived antibodies significantly protected against *C. trachomatis* infection compared to antibodies from serum. Additionally, more samples of local IgA protected against infection compared to local IgG, and more samples of serum IgG exhibited enhanced inclusion formation compared to local IgG and both local and serum IgA. Serum IgA from patients with a history of *C. trachomatis* infection significantly inhibited inclusion formation compared to that from patients without a history of infection, and a similar trend was observed for IgA from CVLs. Antibodies isolated from patients infected with *C. trachomatis* genotypes with *ompA* sequences related to that of serovar D appeared to more efficiently inhibit serovar D inclusion formation *in vitro*, although this observation was dependent on whether or not the patient had reported a history of *C. trachomatis* infection.
Conclusion: Our results indicate that antibodies produced locally in the female genital tract, especially IgA, are capable of neutralizing EBs and inhibiting infection *in vitro*, while antibodies in serum are not protective. Additionally, our data show that a history of infection correlates with increased capacity of patient antibody to inhibit inclusion formation *in vitro* and suggests that this protection may be specific to the genotype of the infecting strain. Studies are currently underway to examine how the infecting genotype may influence the functional capacity of antibodies to inhibit or enhance infection of other genotypes *in vitro*.

P.48. Effect of E- cigarette Vapor on Lung Inflammation

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Background: The e-cigarette is a popular recreational device often used for nicotine delivery by adults as well as children in the US. Even though e-cigarettes were originally marketed as safer alternatives to conventional cigarettes, the mounting experimental evidence suggests a number of adverse health effects of vaping on the respiratory physiology and immunity. In this study, our objective was to define the e-cigarette vapor mediated changes in the lung pro-inflammatory cytokine production induced by respiratory pathogens.
Method: We prepared cigarette smoke extract (CSE), e-cigarette vapor extract with nicotine (EVE^{+NIC}) and without nicotine (EVE^{-NIC}) by passing cigarette smoke or e-cigarette vapor through culture medium. We concurrently exposed murine bone marrow derived macrophage (BMDM) and human lung epithelial cell lines (A549) with heat killed *Staphylococcus aureus* and either EVE^{+NIC}, EVE^{-NIC}, CSE or plain MEM (control). The expression of proinflammatory cytokine at different time points post-exposure was assessed by qPCR and ELISA.
Results: We observed that both CSE and EVE^{+NIC} but not EVE^{-NIC} significantly suppressed *S. aureus* induced production of IL-6 and TNF-α by BMDM as well as by A549 cells. Using western blot, we also evaluated the role of p38, JNK and ERK MAP kinases in this process.
Conclusion: Our results show that similar to previously reported results using CSE, EVE^{+NIC} exposure also suppresses the proinflammatory cytokine production by macrophages and A549 cells in response to respiratory pathogens. Exposure to EVE^{-NIC} does not affect cytokine production by these cell types in our system.

P.50. Dysregulation of SMAD3 and SMAD7 signaling and TLR2 mediated upregulation of Transforming Growth Factor-beta in Simian immunodeficiency virus infected macaques

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Background: Transforming growth factor-beta (TGF-β), a pleiotropic potent immunoregulatory cytokine exerts its biological effects via Smad cell signaling pathway and Smad independent pathways. A Smad signaling pathway is initiated with oligomerization and activation of receptors upon binding with TGF-β ligand, followed by interactions between Receptor regulated-Smad/R-Smad (Smad2, Smad3) and common partner Smad/Co-Smad (Smad4) to regulate diverse biological conditions. Smad7, being regulatory/inhibitory, suppresses the pathway by inhibiting the phosphorylation of R-Smad. TGF-β promotes chronic immunosuppression in human immunodeficiency virus (HIV)/simian immunodeficiency virus (SIV) infection directly or indirectly through its inhibitory effects on the adaptive as well as the innate immune system. Moreover, microbial translocation is an important inducing factor for persistent systematic inflammation in HIV/SIV infected human/animal even after viral suppression with highly active antiretroviral therapy suggests that a link might exist between TGF-β upregulation and microbial translocation. In the present study, we have analyzed the TFG-β level in the plasma and tissues from SIV infected macaques. We have also measured the SMAD signaling pathways to determine their kinetics during SIV infection. Since the activation of TLR is evident due to microbial translocation, we have performed an in-vitro study on the effect of different TLR agonists in regulating TGF-β production.
Methods: Indian rhesus macaques were used for this study. Animals were infected with SIVmac251 intravaginally. We had measured TGF-β concentration in the plasma and tissues by ELISA and flow cytometry respectively. We had also performed the quantitative-PCR targeting the Smad3 and Smad7 genes to study the expression profile of these genes. To study the effect of TLR on TGF-β production, peripheral blood mononuclear cells were treated with different TLR agonists viz. SAC, Poly I:C, LPS and CpG as TLR2, TLR3, TLR4, and TLR9 agonist respectively. The expression of TGF-β was determined by flow cytometry assay.
Results: Significant increase in TGF-β production was detected both in circulation as well in mucosal tissue. We had also noticed a significant upregulation of Smad3, but not Smad7, along with days post SIV infection. There was no significant difference in the SMAD3/7 expression in uninfected animals. This finding correlates with increased plasma/tissue TGF-β production. We had observed the production of TGF-β from cells treated with TLR2 agonists. However, we could not notice the significant release of TGF-β with other TLR agonists.
Conclusion: Together these results indicate that SMAD mediated pathway plays a crucial role in regulating TGF-β expression. We also believe that microbial translocation plays an important role in the production of TGF-β via TLR2 pathway. Dysregulation of the SMAD pathway and increased TLR2 mediated increased TGF-β production was thought to be a key contributor to the dysfunction of CD4+ and CD8+ T cells, intestinal epithelial apoptosis and disease progression. Understanding this mechanism will strengthen our future attempt to alleviate the production TGF-β and thus counteract its negative effect on the disease progression.

P.51. Genomic and expression analysis of a putative new gene in Bovine Herpesvirus 1

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Background: The Bovine Respiratory Disease (BRD) is a serious condition that negatively affects cattle health and it is recognized as one of the biggest economic hurdles of the cattle industry. Bovine Herpes Virus 1 (BoHV-1) is one of the several viruses that can initiate this disease. When originally sequenced in 1996, the BoHV-1 genome was found to be 140kbp with 67 open reading frames (ORFs) on both forward and reverse strands. Previous work in our lab discovered several regions in the genome that potentially produced a protein. These regions mapped to un-annotated parts of the viral genome. Twelve of these regions produced an mRNA transcript, indicating the potential for protein coding.
Methods/Results: Using RT-PCR, we analyzed the expression pattern of one putative new gene, termed ORF-M, using primers close to the suspected 5' terminus. A time course analysis of viral ORF-M expression in infected cells revealed that ORF-M is likely a late gene expressed 12-24 hours post infection. Bioinformatic analysis was conducted to compare the ORF-M sequence to other alphaherpesviruses.
Conclusion: The results allowed for a reference point to begin the study of ORF-M function. When viral mutants unable to express ORF-M are generated, we will begin to understand whether the protein produced from ORF-M changes the behavior of BoHV-1 and/or the behavior of its infection.

P.52. Nonclassical Leukocyte Immune-Type Receptors in Channel Catfish, *Ictalurus punctatus.*

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Background: The Leukocyte Immune-Type Receptor (LITR) family consists of activating and inhibitory immunoregulatory receptors and is unique to teleosts. Family members vary in their number of immunoglobulin (Ig) domains, which are phylogenetically related to both Fc receptors and to receptors encoded within the leukocyte receptor complex. Previously, it was thought that all LITRs shared similar D1 and D2 Ig-domains, however we recently identified a subset that lacked these domains. One member of this nonclassical LITR subset, LITR622, contains eight Ig-domains beginning with a unique D1 that exhibits <42% amino acid identity with classical D1 domains. The LITR622 gene also encodes a distinct signal peptide and a D3 Ig-domain specific to this subset.
Methods: Using a combination of Southern blot and genomic sequencing we predict there are 13 copies of this D3 exon in the catfish haploid genome. That a subset of CC41 mAb-reactive LITRs, expressed on catfish TS32.15 CTLs and NK cells, was upregulated in catfish during an anti-channel catfish virus (CCV) response, led us to investigate LITR622 gene expression in CCV-infected cells. Clonal G14D T cells were infected with CCV (MOI of 10) and total RNA was isolated from mock- and CCV-infected cells at 3hr, 5hr and 7hr post-infection. Primers to the D1 and D1-D3 of LITR622 were used to assess expression by RT-PCR in both CCV-infected and poly(I:C)-stimulated G14D.
Results: Detectable at 5- and 7-hours post-infection, this time course confirmed that LITR622 gene expression was dependent upon viral replication. Additionally, poly(I:C) treatment also stimulated expression of LITR622, with D1-D3 expression detectable at 24 hours post-stimulation, but not detectable in mock-treated cells.
Conclusions: Such findings may indicate that some LITR622 proteins function as stress molecules. These LITRs may be recognized by certain “more classical” LITRs and further demonstrates the complexity of the LITR gene family.

P.53. The M2 Gene is a Determinant of Reovirus Pathogenesis

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Introduction**:** Mammalian orthoreovirus (reovirus) is a non-enveloped, dsRNA virus. A key step in reovirus attachment to host cells is binding to the receptor junctional adhesion molecule-A (JAM-A). Previous studies revealed that the M2-encoded outer capsid protein µ1, which mediates 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. Here, we assessed the role of M2-encoded µ1 protein in reovirus pathogenesis.
Methods**:** We assessed survival and measured reovirus titers in organs from neonatal wild-type and JAM-A$^{-/-}$ mice infected intracranially (IC) and orally (PO) with T1L or T1L/T3DM2.
Results**:** T1L was non-lethal in wild-type and JAM-A$^{-/-}$ mice following IC or PO inoculation. All wild-type mice inoculated with T1L/T3DM2 succumbed to infection. Whereas, JAM-A$^{-/-}$ mice were resistant to T1L/T3DM2. In wild-type mice, T1L/T3DM2 reached peak titers in organs with more rapid kinetics than T1L. In JAM-A$^{-/-}$ mice, T1L/T3DM2 and T1L failed to produce viral titers in organs other than the site of inoculation. Finally, we found signs of myocarditis in hearts of mice infected with T1L/T3DM2.
Conclusion**:** Our findings indicate that the M2 gene is a determinant of reovirus virulence. Further, M2 dictates the capacity of reovirus to induce myocarditis. Our results also indicate that JAM-A is required for M2-mediated pathogenesis.

P.54. MAVS and MDA5 are Required for Control of Reovirus Infection *In Vivo*

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Background: Viral RNA is detected by cytoplasmic pathogen recognition receptors retinoic acid-inducible gene-I-like receptor (RIG-I) or melanoma differentiation-associated gene 5 (MDA5) and signal through mitochondrial antiviral-signaling protein (MAVS) to induce type-1 interferon (IFN-1) production. Mammalian orthoreovirus (reovirus) elicits IFN-1 responses through RIG-I/MDA5/MAVS signaling axis in cultured cells. However, the function of the RIG-I/MDA5/MAVS pathway during reovirus infection *in vivo* is unknown.
Methods: Survival, viral titers, and IFN-1 levels were measured in neonatal wild-type (C57BL/6), MDA5$^{-/-}$, MAVS$^{-/-}$, and IFN-1 α-receptor IFNAR1$^{-/-}$ mice following oral (PO) or intracranial (IC) infection with reovirus strain type 1 Lang (T1L).
Results: T1L infection was non-lethal in wild-type mice following PO or IC inoculation. MAVS$^{-/-}$ and MDA5$^{-/-}$ mice also were resistant to T1L PO inoculation, but IFNAR1$^{-/-}$ mice succumbed by day 12. Following IC inoculation, all IFNAR1$^{-/-}$ and MAVS$^{-/-}$ mice died by day 8. Approximately 30% of MDA5$^{-/-}$ mice succumbed to T1L. T1L produced comparable titers in wild-type, MAVS$^{-/-}$, and MDA5$^{-/-}$ animals at day 4, but at later times viral titers were higher in knockout mice than wild-type controls. MAVS$^{-/-}$ mice produced higher IFNα and IFNβ levels than wild-type or MDA5$^{-/-}$animals in response to T1L.

Conclusions: These findings indicate that IFNAR1 signaling is vital for control of reovirus infection. In contrast, the requirement for MAVS and MDA5 differs by the route of inoculation. MAVS and MDA5 are more important for controlling reovirus following IC inoculation compared to oral infection. Increased IFN-1 levels in MAVS-deficient mice indicate alternative mechanisms stimulate IFN-1 responses to reovirus. Together, these findings begin to unravel how MAVS and MDA5 contribute to innate immune responses to reovirus *in vivo.*

P.55. Effect of dietary trans-cinnamaldehyde supplementation on expression of immune related genes in channel catfish immune tissues

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Background: Commercial channel catfish (*Ictalurus punctatus*) production is an economically important aquacultural commodity in the United States. *Edwardsiella ictaluri,* a causative agent of enteric septicemia of catfish (ESC), threaten profitable catfish production through inventory losses. As a result of selection pressure, resistant microbial strains are emerging, hastening the need for alternative therapies. We previous demonstrated a plant derived antimicrobial, namely *trans*-cinnamaldehyde (TC), inhibits the growth of *E. ictaluri in-vitro* and enhances survival of catfish fingerlings following *E. ictaluri* infection. No information is available regarding effect of TC on immune related genes expression in catfish. The present study was conducted to investigate the protective effect of TC against *E. ictaluri* as well as to assess the impacts of TC on expression of immune-related genes.
Methods: After feeding TC for ten days, fish were challenged with *E. ictalurid* by immersion 10^7 cfu/ml) and liver, spleen, and anterior kidneys were collected at various timepoints. Subsequently, real-time PCR was employed to determine the expression levels of twelve immune-related genes
Results: Results showed upregulation of MHC-I and MHC-II on day 7 and CD8-α , IgM, and TNFα on day 14 in TC administrated fish. Moreover, on day 28 genes including IgM, CD8-α, CD4-2, MHC-II, IL-1β, TNFα, and IFN-γ were upregulated.
Conclusion: This finding demonstrated that dietary TC supplementation can improve immune status in catfish through initiation of innate and adaptive immune responses in catfish immunocompetent tissues. This research provides clues on use of TC as an antimicrobial alternative in aquaculture.

P.56. Viroporin Motifs Encoded by Human Enteroviruses D68 and C105

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Background: Picornaviruses are RNA viruses causing many human diseases including poliomyelitis. Classically, picornaviruses express a single mRNA, translated as a polyprotein precursor, for all viral proteins. An upstream (UP) open reading frame (ORF), which may facilitate viral entry, was discovered recently by others. We queried the untranslated 5' region (5'UTR) of enteroviruses D68 and C105, associated with acute flaccid myelitis (AFM), for other ORFs with potential membrane-destabilizing motifs.
Methods: The 5'UTR of multiple picornaviruses from Genbank were queried for ORFs using the NCBI ORFinder utility. Candidate conserved ORFs, with membrane-destabilizing properties associated with unusual concentrations of aromatic residues, were assessed by PepDraw, an online utility of the Wimley lab of Tulane University, and EMBOSS. Conservation and phylogeny were assessed by nucleotide and protein BLAST utilities from NCBI, CLUSTAL W, and TreeView 1.66.
Results: A conserved viroporin-like motif, prototype PHWRQWSRLRWRPTH in D68, was found within an ORF beginning at nt323. The multiple basic and aromatic residues are similar to Ebola Delta viroporin. The motif is highly conserved with polioviruses type 2 and 3, enteroviruses 33 and 71, and many other enterovirus strains. Two other motifs with similar properties are found in C105 and Hepatitis A viruses.
Conclusion: Many, but not all, picornaviruses associated with human diseases encode viroporin-like peptides within the 5'UTR. They are highly conserved and with high potential for membrane destabilization similar to the known viroporin/enterotoxins of other viruses like Rotavirus and Ebola.

P.57. PUMA-dependent apoptosis suppresses synthesis of inflammatory cytokines and positively modulates expression of pro-angiogenic factors

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Background**:** The p53-up-regulated modulator of apoptosis (PUMA) protein is a pro-apoptotic, BH3-only member of the BCL2 family of effector proteins responsible for promoting cell death. PUMA is required for resolution of pneumococcal pneumonia in mice, as mice deficient of PUMA exhibit greater numbers of *S. pneumoniae* CFU and higher mortality rates than observed in wild-type.
Methods**:** Wild-type and *puma-/-* mice were intranasally challenged with TIGR4 pneumococcus and sacrificed 24 hr post-

infection. Differences in cytokine levels from blood and whole lung tissue were detected by MILLIPLEX MAP Mouse Cytokine/Chemokine Magnetic Bead Panel. Lung transcriptomes from wild-type and *puma-/-* mice were prepared from total lung RNA using NEBNext Poly(A) mRNA Magnetic Isolation Module and NEBNext Ultra RNA Library Prep Kit for Illumina. Libraries were read by Illumina NovaSeq and transcript reads were referenced to *Mus musculus*.
Results**:** PUMA-deficient mice exhibited significant differences in G-CSF, GM-CSF, IFN-ϒ, IL-1α and -β, -6, -9, -10, -12 (p40 and p70), -13, and -17, IP-10, KC, MCP-1, MIP-1α and -β, MIP-2, RANTES, and TNF-α compared to wild-type mice. Lungs of *puma-/-* mice exhibited approximately 3-, 200-, and 20-fold higher levels of IL-12 (p70), IFN-ϒ, and IP-10, respectively. Loss of PUMA also resulted in expression of the pro-angiogenic genes *adam19* and *neurexin II*. Additionally, wild-type and *puma-/-* mice displayed similar patterns of colonization, but *puma-/-* mice were more susceptible to subsequent dissemination to the lungs and blood than wild-type mice.
Conclusion: Polymorphonuclear cells (PMNs) were previously demonstrated to be one of the responsible innate cell types for Puma-dependent resolution of pneumococcal pneumonia in mice. Interestingly, these data suggest that this resolution is propelled by suppressing the inflammatory response via the inhibition of IL-12 maturation and consequent IFN-ϒ release from PMNs. The increase in *adam19* and *neurexin II* also suggest a consequential overlapping stimulation of angiogenesis at the wound site. The down-regulation of *adam19* and *neurexin II* in *puma-/-* lungs may indicate that resolving inflammation may lead to angiogenesis and potentially to wound repair.

P.58. HCMV-Infected Monocytes Show Altered Arachidonic Acid Metabolism.

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Background: Human cytomegalovirus (HCMV) cause severe disease in immunocompromised patients and is associated with a number of inflammatory diseases in the immunocompetent. HCMV has a monocyte tropism and infects peripheral blood monocytes to promote the wide spread dissemination and life long infection. Furthermore, infection of monocytes and their differentiated counterparts, macrophages are directly associated with the development of viral-mediated disease. In order to understand how HCMV infects monocytes and utilizes these cells for viral spread and persistence, we have focused on understanding how infection alters lipid metabolism and the arachidonic acid pathway. By identifying how HCMV infection of monocytes alters lipid metabolism we expect to provide clues to how HCMV can utilize these important immune cells for spread and persistence.
Methods: Our lab has focused on an “omics” based approach to identify the genes and gene products associated with arachidonic acid metabolic pathways that are altered following HCMV infection of monocytes. We have investigated the changes in the expression of key mediators of the arachidonic acid pathway and have used inhibitors and siRNA to demonstrate their function in the setting of HCMV infection.
Results: We found that the mRNAs for the enzymes responsible for the production of arachidonic acid metabolites are upregulated in HCMV infected monocytes. For example, mRNA and protein of prostaglandin-endoperoxide synthase 2 is induced in monocytes following infection and that siRNA knockdown affects HCMV induced monocyte to macrophage differentiation and polarization. In addition, we have identified that the EGFR and PI3K signaling pathways are key to this induction as inhibitors to these pathways can abrogate the HCMV-mediated induction of these key enzymes.
Conclusion: HCMV alters the arachidonic acid metabolic pathway by utilizing /PI3K signaling and contributes to HCMV pathogenesis through the altered differentiation of the cells responsible for organ dissemination and long term infection. These data also provide new molecular evidence for how HCMV promotes productive infection of monocytes and macrophages and offers clues to HCMV-mediated pathogenesis.

P.59. HCMV transcriptionally regulates the differentiation of monocytes to macrophages.

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Monocytes play an essential role in human cytomegalovirus (HCMV) dissemination to multiple organ sites during primary infection. Furthermore, monocyte differentiation into macrophages is required for productive HCMV replication. Previously, we reported that HCMV infection induces monocyte differentiation that through transcriptomic profiling show features of both M1 and M2 macrophages. Functionally, this HCMV induced differentiation promoted long term survival and long term viral release (for 4+ months). Despite our understanding of the early events associated with HCMV induced differentiation of $CD14^+$ monocytes into macrophages, we have not strongly characterized the nature of their phenotype, nor the timing and regulation of the long term aspects of differentiation. Here, we define these HCMV-induced macrophages over time. In high human serum culture condition Mock infected-monocytes are differentiated into $CD14^+$/ $CD11c^+$ (Macrophage like cells) and $CD14^-$/$CD11c^+$ (Dendritic like cells) cells whereas HCMV-infected monocytes are differentiated into only $CD14^+CD11c^+$ cells. Additionally, transcriptional analysis shows that antiviral and type I interferon signaling related genes are significantly induced in HCMV infected monocyte overall times comparing to Mock infected monocytes. Also, transcriptional change is stabilized 2 weeks after infection, suggesting differentiation are occurred during the period. In low human serum, HCMV-infection significantly initiates the early steps of $CD14^+$ monocyte to macrophage differentiation (into $CD14^+$/$CD11c^+$ macrophages) even though

long term survival of monocytes/macrophages is not possible. HCMV infection induced expression of CD14, CD16, HLA-DR and CD68 on monocytes. Pharmacological inhibition assay showed that integrin and endosomal toll-like receptor signaling are involved in the monocyte differentiation. Also, blockade of these signaling suppressed the mRNA expression of IRF4 and MAFB, known as major transcriptional regulators for monocyte differentiation into macrophage and dendritic cells. Our finding reveals that HCMV infection initiates monocyte differentiation into macrophages like cells expressing activation surface markers via integrin and endosomal TLRs signaling.

P.60. HCMV Manipulates Syntaxin-6 for Successful Trafficking and Subsequent Infection of Monocytes

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Background: Human cytomegalovirus (HCMV) exhibits a complex host-pathogen interaction with peripheral blood monocytes. We have recently identified a unique intracellular trafficking pattern that HCMV utilizes to achieve access to the monocyte nucleus and ultimately to productive infection. In this study, we show that HCMV increases the amount of the retrograde trafficking protein Syntaxin 6 (Stx6) and that that increased Stx6 is functionally important in the viral intracellular trafficking pathway.
Methods: We infected primary human peripheral blood monocytes, ARPE-19 cells and HEL fibroblasts with the HCMV strain TB40/E and measured Stx6 protein level over a time course. We then used siRNA targeting Stx6 to knock down Stx6 in these cells and determined the effects on viral trafficking and productive infection.
Results: Infection of primary monocytes, ARPE-19 cells, and HEL fibroblasts led to increased Stx6 at the protein level at all of the time points examined. Knock down of Stx6 in monocytes significantly inhibited viral trafficking, while knock down of Stx6 in other cell types did not change the time frame of productive infection. This suggests that while Stx6 is increased in all infected cell types, yet is only functionally important in the intracellular and nuclear translocation steps in monocytes.
Conclusion: This study shows that the upregulation of Stx6 is functionally important for nuclear translocation of HCMV in monocytes. While Stx6 is significantly increased in all cell types tested, knock down of Stx6 did not impact productive viral infection in epithelial cells (ARPE-19 cells) or fibroblasts. This indicates that while Stx6 upregulation may be common, Stx6 is uniquely important for nuclear translocation in monocytes.

P.61. Investigating the inhibitory effects of human papillomavirus E7 on Epstein-Barr virus replication

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Background: A subset of HPV-positive oropharyngeal squamous cell carcinoma (HPV+OSCC) tumors are co-infected with a second tumor virus, Epstein-Barr virus (EBV). Our previous studies have shown that HPV16 E7 interferes with EBV replication in differentiated epithelia. HPV inhibition of EBV replication promoted a latent, oncogenic EBV infection, which would contribute to HPV+OSCC progression. HPV E7 facilitates the degradation of retinoblastoma protein (pRb), a cellular factor unknown to be required for EBV replication. We hypothesize that HPV16 E7 mediated degradation of pRb inhibits EBV replication.
Methods: For these studies, HPV16 E6/E7 positive/negative human foreskin keratinocytes (HFKs) and normal oral keratinocytes (NOKs) were grown in organotypic raft culture. Organotypic rafts are a physiologically relevant model that recapitulate differentiated, stratified epithelial tissues. HPV-positive/negative raft tissues are infected with EBV-positive Burkitt's lymphoma cells to establish co-infection. EBV replication is measured by qPCR.
Results: To study pRb requirement for EBV replication, we have transfected NOKs with siRNA to pRb and confirmed that knockdown of pRb can last for the duration of raft culture. pRb protein levels were reduced in HPV16 E6/E7 positive keratinocytes, as expected. Current studies are ongoing to determine if pRb is required for EBV replication in differentiated epithelia.
Conclusions: Our results suggest that we can use pRb siRNA transfected keratinocytes in organotypic raft culture to determine if pRb is required for EBV lytic replication in differentiated epithelia. If pRb is required for EBV replication, future studies will look to determine if HPV E7 binding and/or degradation of pRb inhibits EBV replication.

P.62. Directional Release of Reovirus from a Polarized Lymphatic Endothelial Cell Line

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Introduction**:** Mammalian orthoreovirus (reovirus) is a segmented dsRNA virus that infects a wide range of hosts. In neonatal mice, reovirus disseminates systemically via the blood and lymphatics to every major organ system in the body. Recent data from our laboratory identified lymphatic endothelial cells as a cell type that mediates reovirus systemic spread. An important aspect of endothelial cell physiology is the formation of polarized layers that line lymphatic vessels. Polarized cells contain distinct apical and basolateral surfaces due to formation of tight junctions between the cells, function to maintain the integrity of vessels, and prevent leakage of luminal contents into the surrounding tissue. Previous studies indicate that reovirus is preferentially released from the apical surface of polarized cells. We hypothesized that reovirus would be preferentially released from the apical surface of polarized lymphatic endothelial cells.
Methods**:** SV40-immortalized lymphatic endothelial cells (SVECs) were grown on Transwell inserts to allow formation of

polarized monolayers with distinct apical and basolateral surfaces. To confirm polarization of the cell monolayers, localization of the tight junction protein zona occludens-1 (ZO-1) was assessed by immunofluorescence staining, and the transendothelial electrical resistance (TEER) was measured. Release of reovirus was determined by infecting polarized SVECs via the apical or basolateral surfaces and measuring the amount of infectious virus present in each compartment.
Results**:** In SVECs grown under polarizing conditions, the ZO-1 protein localized to the cell periphery. The transendothelial electrical resistance (TEER), an indicator of barrier establishment, was increased under polarization conditions. Reovirus infection at the apical or basolateral surface of polarized SVECs led to an increase in viral titers in the apical, but not the basolateral compartment.
Conclusions**:** Lymphatic endothelial cells have the capacity to form polarized monolayers in culture as evidenced by tight junction formation, and increased and stabilized TEER measurements. Reovirus is preferentially released from the apical surface of polarized lymphatic endothelial cells.

P.63. Role of NLRP3 inflammasome in MRSA uropathogenesis

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Background**:** Methicillin resistant *Staphylococcus aureus* (MRSA) is a human commensal and an opportunistic pathogen that is resistant to β-lactam antibiotics. MRSA is an emerging cause of complicated urinary tract infections (UTI) associated with urinary catheterization. Since MRSA UTI can rapidly develop into potentially life-threatening invasive infections that are unresponsive to antibiotic treatment, a better understanding of MRSA uropathogenesis is warranted. The main objective of my project is to define the role of NLRP3 inflammasome in the urinary immune defenses against MRSA infection. NLRP3 inflammasome is a cytoplasmic, multi-protein signaling complex. The activation of NLRP3 by bacterial molecular patterns results in pyroptosis and the release of pro-inflammatory cytokines, IL-1β and IL-18.
Methods**:** We infected WT and *Nlrp3*$^{-/-}$ mouse models of ascending UTI via transurethral catheterization of 5x10^{7} CFU of MRSA strain USA300. At 6 and 24 h post infection (hpi), we compared the bacterial burden as well as the expression of cytokines and chemokines in bladder, kidneys, and spleen.
Results**:** At 24 hpi in comparison to their WT counterparts, *Nlrp3*$^{-/-}$ mice exhibited significantly reduced bacterial burden in urinary bladder and reduced dissemination to kidneys and spleen. However, we did not observe significant differences in bacterial burden in WT and *Nlrp3*$^{-/-}$ mice at 6 hpi. As expected, compared to the WT, *Nlrp3*$^{-/-}$ mice had significantly reduced expression of the pro-inflammatory cytokines, IL-1β, and IL-6 and neutrophil chemoattractant chemokines, CXCL1, and CXCL2 6 hpi. Conclusion**:** Overall, our results indicate the host-detrimental nature of NLRP3 inflammasome-mediated urinary immune response to MRSA and raise the possibility that in the future, NLRP3 inhibitors may be therapeutically harnessed for a more effective treatment of MRSA UTI.

P.64. Effect of N-acetylmuramyl-L-alanine amidase on the biological activity of peptidoglycan nucleotide-binding oligomerization domain (NOD) agonists.

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Purpose**:** Acetylmuramyl-L-alanine amidase (NAMAA) cleaves bacterial cell wall peptidoglycan (PGN) reportedly reducing its pro-inflammatory activity. We have detected NAMAA like activity in human retinal pigmented epithelium (HRPE) and human corneal epithelial cells (HCEC). The current study investigates whether HRPE and HCEC lysates inhibit the apoptogenic activity of Nod1 and Nod2 agonists in rabbit kidney tubule cells.
Methods**:** Using FITC-labeled MDP (^{FITC}-MDP; Nod2 agonist; N-acetylmuramyl-L-alanyl-D-isoglutamine), HRPE and HCEC lysates and agarose gel electrophoresis, NAMAA activity was measured densitometrically by cleavage of the FITC-muramic acid moiety. The inhibitory effect of HRPE and HCEC NAMAA on cytopathy (Caspase-3 and DNA fragmentation) induced by Nod1 agonist (C12-iE-DAP, γ-D-glutamyl-meso-diaminopimelic acid) and Nod2 agonists was detected in rabbit kidney cells.
Results**:** HRPE and HCEC lysate (60-80 µg protein/ml) cleavage of ^{FITC}-MDP (Nod2 agonist) was time-dependent; cleaved >90% of 500 ng ^{FITC}-MDP in 24 h. C12-iE-DAP (Nod1 agonist), but not MDP (Nod2 agonist), incubated in HRPE and HCEC lysates exhibited loss of apoptogenic activity (i.e., reduced capacity to induce caspase-3 mediated cell death and DNA ladders).
Conclusion**:** The current results demonstrate an innate immunity in retinal and corneal epithelial cells that can potentially act against bacterial infection. In that, HRPE and HCEC contain enzymatic activity that cleaves Nod2 (MDP) and (C12-iE-DAP) NOD1 agonists, but only inhibited NOD1 agonist induced apoptogenic activity.

P.65. HIV-1 Tat Interacts with Selective Estrogen Receptor Modulators to Influence Morphine-Conditioned Place Preference

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Background: Opioid abuse is a common route of transmission for human immunodeficiency virus (HIV), involved in 13.8% of new infections among women and 8.3% of new infections among men. We have found that the HIV-1 regulatory protein, trans-activator of transcription (Tat), can potentiate the rewarding properties of cocaine or morphine in male mice. Tat also

potentiates cocaine reward in female mice and these effects are estrous cycle-dependent. But how Tat interacts with sex steroid targets to influence opioid reward is not well-understood.
Methods/Hypothesis: We hypothesized that estradiol would potentiate Tat-mediated opioid reward and that these effects would be differentially modulated by estrogen receptor α (ERα)- and β (ERβ)-acting selective estrogen receptor modulators (SERMs). Ovariectomized Tat-transgenic mice were treated with vehicle, a physiological regimen of estradiol (0.09 mg/kg, QOD for 12 d), the ERα-selective SERM, propylpyrazole triol (PPT; 0.09 mg/kg, QOD for 12 d), or the ERβ-selective SERM, diarylpropionitrile (DPN; 0.09 mg/kg, QOD for 12 d) and assessed for morphine reward in an unbiased conditioned place preference (CPP) paradigm. Complementary in vitro studies were performed on primary C57BL/6HNsd mixed glial cells that were treated with vehicle, Tat (100 nM), morphine (500 nM), estradiol, PPT, and/or DPN (0.01-10 nM) and assessed for reactive oxygen species (ROS) formation via CM-H2DCFDA assay.
Results: All mice demonstrated morphine-CPP and induction of Tat alone did not influence this effect. However, the combination of Tat and estradiol significantly increased morphine-reward. The ERβ-selective SERM, DPN, did not influence basal morphine-CPP; however, the ERα-selective SERM, PPT, attenuated morphine-CPP in both Tat-transgenic mice and their wildtype counterparts. In primary mixed glia, Tat significantly increased the formation of ROS (~10-15% above baseline) and physiological concentrations of estradiol (0.01-1 nM) significantly attenuated this effect with or without morphine co-exposure. Of interest, the highest estradiol concentration (10 nM) significantly interacted with morphine to increase ROS production (~20% above baseline), irrespective of Tat. Similarly, DPN or PPT attenuated Tat-mediated ROS; however, these effects were notably greater with PPT. Like estradiol, both DPN and PPT significantly interacted with morphine to increase ROS, but the effects occurred at lower concentration for DPN (1 nM) and to greater effect (~30% above baseline) than PPT (at 10 nM, ~11% above baseline).
Conclusion: Thus, estrogen receptors may serve as important targets for Tat/opioid interactions.

P.66. HIV-1 Tat Protein and Oxycodone Dysregulate Adrenal and Gonadal Endocrine Axes and Promote Affective and Cognitive Dysfunction in Mice

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Background: Human immunodeficiency virus (HIV) is associated with co-morbid affective and neurocognitive disorders that afflict ~50% of infected individuals. One factor that may contribute to neuropathology is the HIV regulatory protein, trans-activator of transcription (Tat), which promotes neuroinflammation and neurotoxicity that can be exacerbated by opioids. We and others have observed steroid hormones, such as estradiol and/or progesterone, to attenuate Tat-mediated neurotoxicity in cell culture; however, their interactions with opioids and their protective effects in a whole-animal model are unknown.
Methods/Hypothesis: We hypothesized that doxycycline-inducible expression of Tat in transgenic mice would interact with the opioid, oxycodone, to induce psychomotor, anxiety-like, depression-like, and cognitive behavior and to dysregulate adrenal and gonadal steroid hormones.
Results: When administered acutely, oxycodone (3 mg/kg) increased psychomotor behavior in an open field and induction of HIV-1 Tat protein significantly potentiated these effects. Tat expression also potentiated the anxiolytic-like effects of oxycodone, increasing entries into the center of the open field, largely among females in the diestrous phase of the estrous cycle. Tat increased depression-like behavior among proestrous, but not diestrous, females. When administered repeatedly, oxycodone (3 mg/kg, QD for 5d) interacted with Tat protein to decrease cognitive performance in a novel object recognition test among all mice with the exception of proestrous control mice.
Conclusion: These data suggest that induction of Tat potentiates psychomotor and anxiety-like effects of acute oxycodone and either Tat induction or repeated oxycodone perturb cognitive performance. Manipulations also influenced adrenal and gonadal endocrine status in a cycle-dependent manner. Tat or oxycodone increased circulating corticosterone in all mice acutely. Tat increased circulating estradiol and progesterone among diestrous mice and decreased estradiol among proestrous mice. Oxycodone increased estradiol and progesterone among diestrous mice when administered acutely, but only elevated estradiol when administered repeatedly. Thus, neuroendocrine function may be an important target for HIV-1 Tat/opioid interactions.

P.67. A new humanized mouse model mimics humans by lacking α-Gal epitopes and secreting anti-Gal antibodies

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Background: The α-Gal is a carbohydrate epitope that is synthesized by the α1,3 Galactosyltransferases enzyme (α1,3GT) on glycolipids, glycoproteins, and proteoglycans within the Golgi of the cell. Humans have a natural mutation in the gene that encodes α1,3GT enzyme and therefore they do not express terminal α-Gal epitopes. In addition, the loss of immune tolerance to this epitope in humans resulted in production of abundant natural antibody called anti-Gal antibodies. In contrast, mice have intact α1,3GT gene and active α1,3GT enzyme, therefore they express α-Gal epitopes and lack anti-Gal antibodies. The natural anti-Gal antibodies can be used as an adjuvant to enhance processing of vaccine epitopes to antigen presenting cells

(APCs). However, wild-type mice and all existing humanized mouse models cannot be used to test the efficacy of vaccines expressing α-Gal epitopes because they express α-Gal epitopes and lack anti-Gal antibodies.
Methods: In vitro fertilization (IVF) was performed to cross the C57BL/6 α1,3GT knockout (GTKO) mouse strain with NSG mouse strain. We verified mutations in the SCID^{Prkdc,} X-linked *IL2Rγ^{null}*, α-1,3GT genes in the parental and hybrid strains by PCR analysis. Next, we evaluated the relative proportion of C57/BL6 and NOD background present in the genomic DNA by analyzing 142 single-nucleotide polymorphisms (SNPs) in the NSG/α-Gal^{null} mice. Immunohistochemistry and flow cytometry analysis were used to evaluate expression of α-Gal epitope. To generate NSG/α-Gal^{null} mice with human immune cells, human CD34^{+} cells were engrafted into sub-lethally irradiated NSG/α-Gal^{null} mice, 5-6 wk of age. Reconstitution of immune cells was measured by analyzing the expression of different human immune cell-surface markers by flow cytometry. To determine the activity of the human anti-Gal antibody in the humanized NSG/α-Gal^{null} mice, plasma from Hu-NSG/α-Gal^{null} mice was tested for the presence of human anti-Gal antibodies by Enzyme-Linked Immunosorbent Assay (ELISA) in animals with demonstrated human cell engraftment.
Results: We confirmed that the NSG/α-Gal^{null} mice carry the mutant α-1,3GT gene in Prkdc, IL2Rγ, and α-1,3GT alleles. We found that the NSG/α-Gal^{null} have an average of 75.5% NSG SNP alleles. In addition, we confirmed that the NSG/α-Gal^{null} mouse lacks α-Gal epitopes similar to GTKO mouse. In determining whether the NSG/α-Gal^{null} mice spontaneously produce murine anti-Gal antibody as a result of their nonfunctional α1,3GT gene, we found that plasma from GTKO mice displayed modest murine anti-Gal antibodies, whereas NSG parental and NSG/α-Gal^{null} mice displayed no anti-Gal antibody. The levels of baseline murine anti-Gal antibody in NSG/α-Gal^{null} mice serum did not increase in response to boosting with α-Gal epitopes as was seen with C57BL/6 GTKO mice. After engraftment of human hematopoietic stem cell, flow cytometry analysis revealed the presence of human CD45^{+} lymphocytes, which were composed of human CD20^{+} B cells and the appropriate ratio (2:1) of CD4/CD8 in the human T cells population up to 30 weeks post-engraftment. The sera obtained from all humanized NSG/α-Gal^{null} mice were found to contain human anti-Gal antibodies.
Conclusion: The new Hu-NSG/α-Gal^{null} mouse model can be used in various applications for examples, in in xenotransplantation studies and pre-clinical studies to evaluate of viral and tumor vaccines based on α-Gal epitopes. The new humanized mouse model (Hu-NSG/α-Gal^{null}) is currently the only available humanized mouse model with such features.

P.68. Peptidoglycan NOD1 and NOD2 Agonists Induce Caspase-Mediated Rabbit Kidney Cell Death

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Purpose**:** To determine if the pro-inflammatory bacterial cell wall peptidoglycans (PGN), shown to signal through binding to intracellular nucleotide-oligomerization domains (NOD), can induce caspase-mediated cell death (apoptosis) in rabbit kidney tubule epithelial (RK13) cells as a model for renal inflammation following bacterial infection.
Methods**:** Commercially available PGN fragments containing gamma-D-glutamyl-meso-diaminopimelic acid (5 NOD1 agonists) or L-alanyl-D-isoglutamine (7 NOD2 agonists) and 2 NOD1/2 agonists were incubated with RK13 cells. Cytopathy was identified by cytological changes, caspase-3 activation was quantified by colorimetric assay, and nuclear fragmentation was quantified by densitometric analysis of DNA ladder.
Results**:** All pro-inflammatory PGN shown to bind to NOD1 and/or NOD2 induced RK13 cell death, caspase-3 activation, and polymeric DNA fragmentation. Variations in 50% cytopathic dose and time to induce caspase-mediated DNA fragmentation/cell death were noted for Nod1 agonists and Nod2 muropeptides.
Conclusion**:** The pro-inflammatory bacterial cell wall PGN fragments produced via bacterial degradation can exert cytopathic effects in kidney (RK13) cells. The results support the concept that renal failure may be due to the cytopathic and pro-inflammatory effects of bacterial PGNs containing the D-isoglutamine (D-glutamic acid) moiety.

P.69. Human papillomavirus type 16 E5 inhibits interferon signaling and supports episomal viral maintenance

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Background**:** Human papillomaviruses (HPVs) persistently infect keratinocytes of the squamous epithelia and cause benign lesions and several malignancies, including cervical cancer. Viral oncogenes are critical for immune evasion and facilitation of productive viral life cycle. One of these oncogenes is a transmembrane protein E5, which has been shown to regulate multiple growth signaling pathways.
Methods**:** Human foreskin keratinocytes (HFKs) harboring the wild type genomes and HPV16 genomes containing the a translational stop codon (E5 stop) were used in these studies. Cells were treated with various inhibitors, siRNA, and growth

factors followed by protein, RNA, and DNA analysis.
Results**:** We found that interferon kappa transcript levels *were* suppressed in the presence of E5. This suppression by E5 involved both the epidermal growth factor receptor (EGFR) and suppress transforming growth factor beta (TGFβ) 2 signaling pathways. Additionally, we observed that HPV16 genomes lacking E5 intergraded at a higher rate when compared to the wild type.
Conclusion**:** This work has unveiled previously unknown function of the HPV16 E5 oncoprotein in the suppression of interferon responses. This suppression is targets keratinocyte-specific IFN. We have also established a link between the loss of E5 and the subsequent loss of genome maintenance and stability, resulting in increased genome integration

P.70. Assessing the oncolytic potential of influenza viruses

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Background: Glioblastoma (GB) is the most common malignant primary tumor of the adult brain. Despite recent advances, GB remains difficult to treat, usually involving an aggressive combination of surgery, radio-, and chemotherapy, and typically has a poor long-term survival rate. Viruses are exquisitely adapted to recognize cells and deliver "cargo", typically their own genomes and replication machinery. Recently**,** the use of viruses targeting cancers (viral oncotherapy) has shown great promise in the treatment cancers, with the licensing of genetically modified oncolytic viruses derived from herpesvirus and adenovirus being licensed in the USA and China respectively, and unmodified enterovirus being licensed in Latvia, Georgia, and Armenia.
Methods: We have tested a panel of influenza A, B, and C viruses for their ability to inhibit the growth of GB cell lines.
Results: Several influenza viruses demonstrate the ability to reduce the growth of some, but not all, GB cell lines tested. Ongoing studies will determine whether the growth inhibition observed is due to cell killing, and whether the viruses are effective in cells resistant to temozolomide (TMZ/Temodar), the "standard of care" drug, to which patients frequently develop resistance.

P.71. Toll-like receptor agonist, Imiquimod, affects Leukocyte Immune-type receptor expression in Channel Catfish, *Ictalurus Punctatus*

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Background: Leukocyte immune-type receptors (LITRs) are a diverse, polymorphic, and polygenic family of immunoregulatory receptors that are unique to teleost fish. They are phylogenetically related to receptors encoded within the mammalian leukocyte receptor complex (LRC) and to Fc receptors (FcRs). Also, like these receptors, activating LITRs signal through association with adaptor proteins, which contain immunoreceptor tyrosine-based activation motifs (ITAMs). In contrast, inhibitory LITRs signal through immunoreceptor tyrosine-based inhibitory motifs (ITIMs) and/or immunoreceptor tyrosine-based switch motifs (ITSMs) located in their cytoplasmic tails. Since it was previously demonstrated by flow cytometry that channel catfish virus (CCV) infection of catfish clonal G14D T cells induced changes in LITR expression, we expected that stimulation with imiquimod, a virus-associated toll-like receptor 7 (TLR7) agonist, would induce a similar response.
Methods: Following imiquimod stimulation, LITR expression was examined by flow cytometry on G14D and 3B11 (catfish clonal B cells) using monoclonal antibodies (mAbs) 125.2, CC34 and CC41. In addition, reverse transcription polymerase chain reaction (RT-PCR) was performed to examine expression of activating and inhibitory LITRs following stimulation.
Results: Interestingly, stimulation with imiquimod significantly upregulated certain activating LITRs in G14D T cells and 3B11 B cells indicating that at least some LITRs may function as target ligands.
Conclusion: This study shows that at least some LITRs are involved in anti-viral pathways within catfish B and T cells. Knowing more about how LITRs function should lead to a better understanding of how innate and adaptive immune mechanisms work together.

P.72. Vectorborne Diseases in Louisiana: 2008-2018

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Background: Over the past 10 years, Arthropodborne Viral Disease Network (ArboNET), Centers for Disease Control and Prevention (CDC), and Louisiana Department of Health (LaDH) have reported vectorborne disease cases from Louisiana.
Methods: We reviewed vectorborne disease cases and other applicable information occurring since 2008 using ArboNET, CDC, LaDH and other sources. For each disease, a timeline was constructed to show case occurrence.
Results: Eighteen vectorborne diseases were surveyed and include: Babesiosis, Chagas Disease, Chikungunya, Dengue, Eastern Equine Encephalitis, Ehrlichiosis, Hantavirus Pulmonary Syndrome, LaCrosse, Lyme Disease, Malaria, Murine Typhus, Plague, Rocky Mountain Spotted Fever, St. Louis Encephalitis, Tularemia, West Nile, Yellow Fever, and Zika. Sixteen of the diseases were reported by LaDH, except Plague and Yellow Fever. Vectors include fleas, mosquitoes, kissing bugs,

rodents, and ticks. The five most reported diseases were West Nile, Malaria, Zika, Dengue and Lyme Disease.
Conclusion: This study shows that the number of reported vectorborne disease cases in Louisiana is increasing. Further investigations are underway to determine the number of imported cases as well as environmental and other factors contributing to this increase in disease cases.

P.73. Evaluation of a Recombinant Varicella Vaccine Expressing Simian Immunodeficiency Virus Antigens

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Background: A vaccine to control the global HIV/AIDS epidemic is urgently needed. In this study, the ability of a recombinant varicella vaccine expressing SIV antigens to induce humoral responses to SIV in immunized nonhuman primates (NHPs) was evaluated by immunoblot analysis.
Methods: Four NHPs were initially immunized with a simian varicella virus (SVV) vaccine expressing the SIV gag and env antigens (rSVV-SIVgag/env) followed by boost immunization with SIV gag and env protein. A control group was immunized with a recombinant SVV vaccine expressing a respiratory syncytial virus glycoprotein (rSVV-RSVG). Serum was derived from animals at various times post-immunization and analyzed for antibody responses to the SIV antigens by immunoblot assay.
Results: Rhesus macaques immunized with the rSVV-SIVgag/env vaccine followed by SIV gag/env protein developed antibodies to the SIV capsid (*gag*) and envelope (*env*) antigens as determined by immunoblot analysis. The humoral response was most prominent following the protein boost inoculation. There was a relatively higher antibody response to the *env* antigen. compared to the g*ag* antigen.
Conclusion: A combination of HIV antigenic targets will likely be necessary for successful immunization against HIV/AIDS. We are now developing a recombinant vaccine that will express SIV rev, tat, and nef regulatory genes, for addition to the vaccination schedule utilized in this study. The results attained in this study demonstrate that a recombinant varicella vaccine expressing multiple HIV antigens could be a potential candidate for an HIV vaccine.

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1992	Mississippi State University, University, MS
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2016	University of Louisiana, Lafayette, LA
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