The South Eastern Branch of The American Society for Microbiology

Annual Meeting

September 6-8, 2024

Tampa FL

Welcome Remarks

It is my absolute pleasure to welcome you all to the Southeastern Branch of the American Society for Microbiology Annual Conference here in beautiful Tampa, Florida. Whether you're joining us for the first time or you're a seasoned attendee, we're thrilled to have you with us for what promises to be an exciting and enriching three days.

The goal of our branch meetings is to focus on microbiology trainees—those of you who are stepping into this incredible field with fresh perspectives, energy, and curiosity. As trainees, you are the future of microbiology, and it is your innovation and passion that will push the boundaries of our understanding of microorganisms, their impact on human health, and the environment.

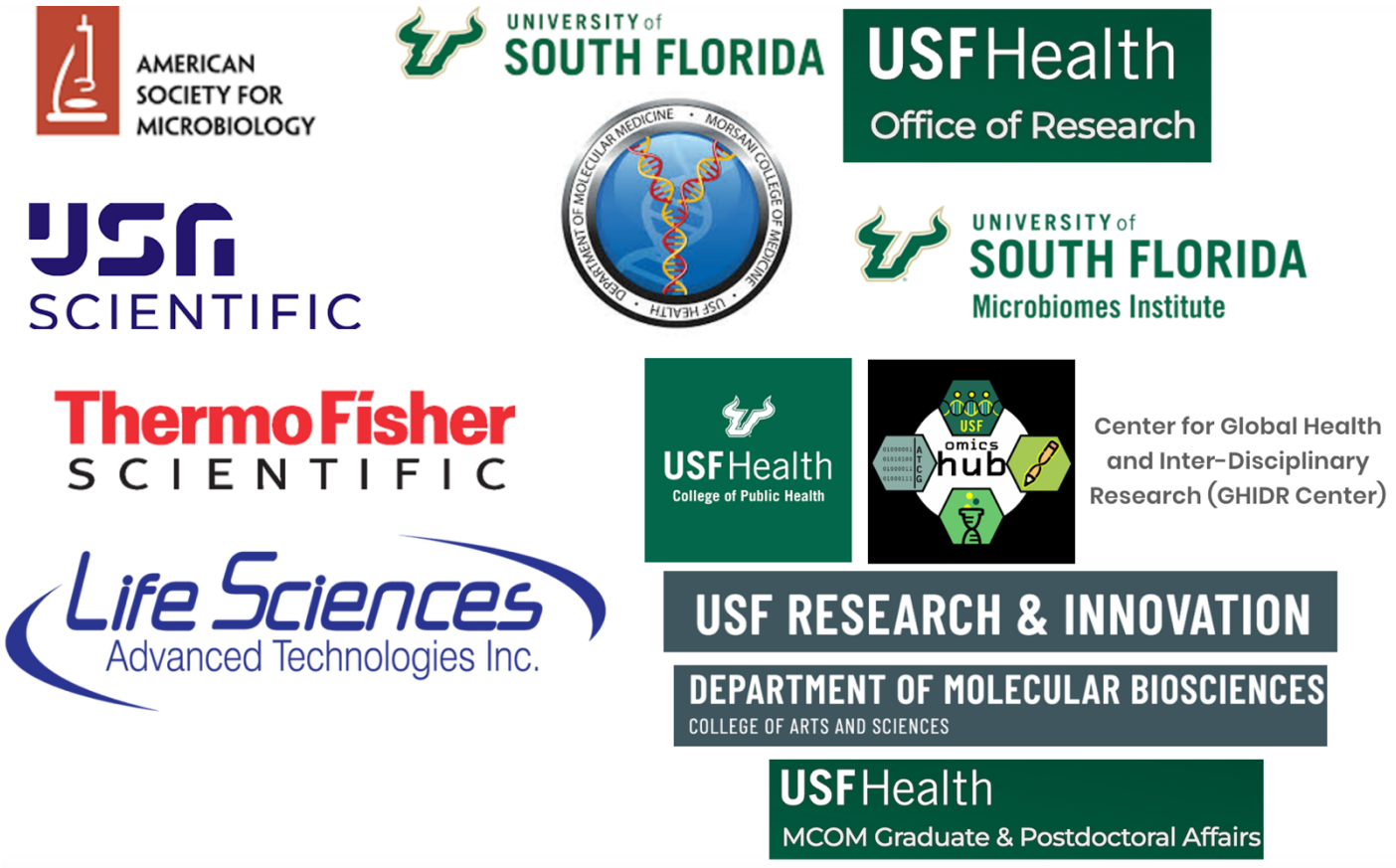
Over the next three days, you'll have the opportunity to engage with thought leaders in microbiology, learn about cutting-edge research, and connect with peers who share your passion. I encourage you to take full advantage of the sessions, workshops, and networking opportunities. Ask questions, exchange ideas, and challenge each other to think bigger.

Tampa offers a vibrant backdrop to our discussions, and I hope you take some time to explore the city, but most importantly, to reflect on the potential your work holds for the world.

Thank you for being here. Together, we are shaping the future of microbiology, and I can't wait to see the great things that will come from this gathering.

Dr Sophie E Darch
(SEB President 2023-24)

Thank you to our sponsors!



Additional thanks go to:

Alexa Gannon, Oriana Williams and Caroline Miller (Darch Lab) for going above and beyond in helping their busy PI organize this event!

Kiara Berrios and colleagues at the CAMLS venue for their support and help coordinating this event.

Schedule

Friday 6th September

12:00 pm – 1:00 pm	Registration and poster setup
1:00 pm – 1.30 pm	**Welcome remarks** *Dr Sophie Darch, PhD (Current SEB President, Assistant Professor, University of South Florida)* *Dr Robert Deschenes, PhD (Associate Dean, College of Medicine Molecular Medicine. Professor & Chair of Molecular Medicine, Morsani College of Medicine, University of South Florida)*
1:30 pm – 2:30 pm	**Keynote and ASM Distinguished Lecturer** *Dr Sam Brown PhD (Professor, Georgia Institute of Technology)* "Can we Reverse the Rise of Antibiotic Resistance?"
2:30 pm – 3:15 pm	**Clinical case study** *Dr Kirtan Amin, MD (Infectious Diseases Fellow, Morsani College of Medicine, University of South Florida)* *Dr Andreas Seyfang, PhD (Professor, Morsani College of Medicine, University of South Florida)* "Pass Me the Olive Oil, Fever and Rash in an Immunocompromised Host."
3:20 pm – 4:00 pm	**Flash poster presentations A** Haley Davenport Jared Smith Oriana Williams Priscila Halicki Ryan Williams Sierra George Alyssa DeSantis Alexia Pearah Barrett Maloney
4:00 pm – 5:00 pm	**Poster Session A**
4:00 pm – 6:00 pm	**Welcome Reception**

Saturday 7th September

8:30 am – 9:00 am	Registration
9:00 am – 9:05 am	*Welcome and announcements*
9:05 am – 10:00 am	**Session I** *Dr Tara Randis MD (Associate Professor, Chief of Neonatology, Morsani College of Medicine, University of South Florida)* "Neonatal Gut and GBS: The Invader at the Doorstep"
10:00 am – 10:15 am	*Mary-Elizabeth Jobson (University of South Florida)* "Mechanistic Insight into RNA-Mediated Control of Virulence in *Staphylococcus aureus*
10:15 am – 10:30 am	*David Ayala-Velastegui (University of Georgia)* "Characterization of *Salmonella* complex populations by Nanopore amplicon sequencing on poultry farms and carcass samples.
10:30 am – 11:00 am	**Coffee Break**
11:05 am – 11:20 am	**Session II** *Laura Shelton (University of Central Florida)* "Studying *Chlamydia* secreted effectors using *Drosophila melanogaster* as an *in vivo* cell biology platform"
11:20 am – 11:35 am	*Alexa Gannon (University of South Florida)* "*Pseudomonas aeruginosa* aggregate formation in the CF lung"
11:35 am – 11:50 am	*Sarah Forrstrom (Emory University)* "The battle of bacteriophages and bile acids against *Klebsiella pneumoniae* in a fecal microbiota transplantation recipient"
12.00pm – 1:00 pm	**Lunch**
1:15 pm – 1:30 pm	**Session III** *Ridhwana Appiah (University of Central Florida)* "Investigating Natural Proline-rich Antimicrobial Peptides (PrAMPs) Activity Towards *Klebsiella pneumoniae*
1:30 pm – 1:45 pm	*Greeshma Bharathan (Auburn University)* "Ferric Uptake Regulator (Fur) plays a significant role in the survival of *Salmonella* Typhimurium on meat"
1:45 pm – 2:00 pm	*Mohamed Marouf (University of South Florida)*

	"Identification of Novel Vaccine Candidates Against Falciparum Malaria Using an Integrated Approach of in Silico and Wet Lab Analysis"
2:00 pm – 2:15 pm	*Alysha Ismail (Emory University)* The Trade-Off Dilemma: Impact of Sub-Inhibitory Antibiotics on *Staphylococcus aureus* Growth, Resistance, and Persistence
2:15 pm -2:30 pm	*Jana Wieschollek (University of South Florida)* Unusual regulation of genes necessary for carbon dioxide fixation by marine chemolithoautotroph *Thiomicrospira pelophila*
3:00 pm – 3:25 pm	**Flash poster presentations B** Rachel Kemp Trevor Mello Amber Richards Caroline Miller Soumyadeep Chakraborty Navita Kohaal
3:30 pm – 4:00 pm	**Coffee break**
4:00 pm – 5:00 pm	**Poster Session B**

5:15 pm: Scavenger hunt

Sparkman Wharf discounts available/live music

(*Details will be made available at the conference*)

Sunday 8th September

8:30 am – 9:00 am	Registration
	Session IV
9:05 am – 9:20 am	*Anna Schulz (University of Central Florida)* "Distinct Metabolites Protect the Lyme Disease Pathogen *Borrelia burgdorferi* from Macrophage-Mediated Killing"
9:20 am – 9:35 am	*Esther Palmer (University of Georgia)* Persistence of multi-serovar Salmonella populations in surface water proximal to a cow-calf operation
9:35 am – 9:50 am	*Salvatore Scaffidi (University of South Florida)* "Identification of novel factors contributing to staphylococcal cell envelope integrity and surface protein septal trafficking
9:50 am – 10:05 am	*Claire Bowman (Emory University)* Understanding *C. elegans* Decision-Making in Complex Foraging Environments
10:05 am – 10:20 am	*Calvin Mackey (Florida State University)* *"*Potential of Universal Microbial Dynamics in Socially Isolated Male Prairie Voles"
10:30 am – 11:00 am	**Coffee Break**
	Session V
11:05 am – 11:20 am	SEB updates
11:20 am – 11:35 am	*Stephanie Guerra (Emory University)* "Examining the paradoxical relationship between host inflammation and *S. pyogenes* infections"
11:35 am – 11:50 am	*Kira Ruiz-Houston (University of South Florida)* "MraZ and DnaA coordinate DNA damage response in *Bacillus subtilis* to arrest cell division.
12:00 pm – 1:00 pm	**Lunch**
1:15 pm – 2:00 pm	*Branch elections, awards and closing remarks*

Poster Numbers

*Odd numbers will present Friday 6th September, even numbers will present Saturday 7th September (4-5 pm)

Microbial Communities

Presenting Author	Poster Number
Veronica Gosnell	1

Structural Microbiology

Presenting Author	Poster Number
Alexa Gannon	2
Barrett Maloney	9
Alexia Pearah	5
Abigail L. Laury	4
Sabin Poudel	6
Emily Soucy	8
Rashmi Gupta	3
Sarah Forsstrom	7

Microbial Ecology

Presenting Author	Poster Number
Jana Wieschollek	18
Amber K. Richards	16
Bryce Redfern	11
Trevor Mello	20
Lucy Elizabeth Turner	12
Claire Bowman	19
Satya Spandana Boddu	10
Esther G. Palmer	17
Jared C. Smith	15
Amy T. Siceloff	14
Ryan L. Williams	13

Host-Pathogen Interactions

Presenting Author	Poster Number
Parker Smith	24
Emilee Mustor	23
Mohammad Soban Khan	22
Anna M. Schulz	25
Rachel L. Kemp	26
George F Aranjuez	29
Mary-Elizabeth Jobson	21
Peyton Panos	27
Stephanie Guerra	30
Laura Shelton	28

Oriana M. Williams	31

Clinical/Diagnostic Microbiology

Presenting Author	Poster Number
Kirtan Amin	34
Sachitaa Senthilkumar	32
Emily Cason	33

Genomics and other Omics

Presenting Author	Poster Number
Calvin Mackey	41
Jason Carroll	39
Connor G. Norris	35
Samuel Smith	38
Alyssa DeSantis	43
Kayla MacDonald	36
Prem Patel	42
Tsai-Tien Tseng	40
Carlos David Ayala Velastegui	37

Evolutionary Dynamics

Presenting Author	Poster Number
Carmen Alvarez	45
Turner R. MacInnis	44

Virulence and Vaccines

Presenting Author	Poster Number
Shaohui Wang	50
Nicholas First	46
Aditya Anand	51
Dipak Kumar Raj	54
Ahmad Rushdi Shakri	56
Jhasketan Badhai	53
Mohamed Marouf	47
Jayanth Reddy Tallapalli	52
Lubem Agbendeh	55
Soumyadeep Chakraborty	48
Xingmin Sun	49

Antimicrobial Resistance

Presenting Author	Poster Number
Christina M. DeBarro	57
Caroline D. Miller	58
David deGuzman	59

Sofia Assent	60
Ridhwana Appiah	61
Hunter Sheffield	62
Leigh I. Codiniera	63
Alysha S. Ismail	64
Priscila C. B. Halick	65
Lian Jacobs	66
Haley C. Davenport	67
Navita Kohaal	68
Isaac Estrada	69

Molecular Biology of Microbes

Presenting Author	**Poster Number**
Maria Fernanda Cubero	86
Erika Valdespino	84
Ran Zhang	80
Yaosheng Jia	78
Carmella Gaucher	73
Asher King	70
Emily Felton	76
Kira M. Ruiz-Houston	74
Vivian M. Cruz-López	77
Lauren E. Bonefont	72
Greeshma Bharathan	82
Selina M. Walker	79
Salvatore J. Scaffidi	81
Kirstie Tandberg Francis	71
Aisha Madi	85
Sierra George	83
Richard Agyen	75

Keynote Lectures

ASM Distinguished Lecturer
Dr. Sam Brown, Ph.D.
School of Biological Sciences
Georgia Institute of Technology

Can we Reverse the Rise of Antibiotic Resistance?
Antibiotic resistance seems like an un-avoidable consequence of the widespread use of antibiotics to prevent and treat bacterial infections. In this lecture I will review the challenge of resistance evolution from clinical, epidemiological and experimental perspectives. I will then assess a range of proposed strategies that hold the potential to slow or even reverse the rise in antibiotic resistance. Specific strategies include the use of multiple drugs (cocktails, cycling, mixing), diagnostics, and non-conventional therapies (e.g. anti-virulence drugs, phage therapies). I will focus on diagnostic-informed, patient-specific control strategies that have the potential to effectively treat patients now and into the future

Dr. Tara Randis MD
The Pamela and Leslie Muma Endowed Chair
Associate Professor and Chief Division of Neonatology
Adjunct Associate Professor Department of Molecular Medicine
Morsani College of Medicine, University of South Florida

Neonatal Gut and GBS: The Invader at the Doorstep
Group B Streptococcus (GBS) is a leading cause of infectious morbidity and mortality in infants. Infants are particularly vulnerable to invasive disease following GBS gastrointestinal colonization. In adults, GBS is confined to the intestinal lumen due to a robust intestinal barrier. Interactions between the microbiota and a tightly regulated mucosal immune response help maintain this barrier's integrity. However, newborns, especially those born preterm, face a higher risk of invasive GBS disease due to reduced mucosal barrier function, dysregulated intestinal inflammatory responses, and an immature intestinal microbiome. This discussion will review key GBS virulence factors important for host colonization and disease progression and examine the unique aspects of the neonatal innate immune response that increase their susceptibility to this pathogen. Additionally, we will explore the significant challenges encountered in developing protective strategies.

SCIENTIFIC ABSTRACTS

Microbial Communities

Bioinformatic Strategy to Design a Novel Multi-Epitope Vaccine Against *Clostridioides difficile* Infection

V. Gosnell, S. Chakraborty, L. Agbendeh, X. Sun
University of South Florida, Morsani College of Medicine, Department of Molecular Medicine, Tampa, USA

Abstract
Clostridioides difficile is a Gram positive, spore-forming, anaerobic bacteria that is the principal cause of antibiotic-associated diarrhea worldwide. The spores of this opportunistic pathogen invade epithelial cells lining the stomach, allowing them to colonize the gastrointestinal tract of the host. The most common treatment for *Clostridioides difficile* infection (CDI) is the use of antibiotics, which disrupts homeostasis of the gut. This exacerbates the infection by providing an ideal environment for the pathogen to thrive. There is no FDA-approved vaccine for CDI, and there is a desperate need for one.

The major virulence factors that induce an inflammatory response to CDI are the secreted exotoxins: Toxin A and Toxin B. This study used Bioinformatics methods to analyze data on Toxin A (TcdA) of *C. difficile*. By retrieving the amino acid sequence of the *tcd*A gene from the National Center for Biotechnology Information (NCBI), we were able to determine the epitopes that would induce a cellular and humoral mediated immune response. Numerous rounds of screening led to the selection of 4 cytotoxic T lymphocyte (CTL) epitopes, 3 helper T lymphocyte (HTL) epitopes, and 4 B-cell lymphocyte epitopes. trRosetta and AlphaFold3 were used to predict the three-dimensional structure of the novel sequence. This structure was then refined using ModRefiner and validated using PROCHECK. Molecular docking simulations were performed using HADDOCK, clusPro, and LZerD servers to determine the potential conformations of complexes formed between the vaccine construct and Toll-like receptors 2 and 5 (TLR2, TLR5), major histocompatibility complexes I and II (MHCI, MHCII), and B-cell receptor (BCR) complexes. Additionally, immune simulation using C-ImmSim, population coverage using the Immune Epitope Database (IEDB), as well as *in silico* gene cloning with Snapgene was performed. The results of this study conclude that this novel vaccine candidate is stable *in silico* and will theoretically produce a potent immune response against *C. difficile* infection.

Structural Microbiology

Exploring aggregation genes in a *P. aeruginosa* chronic infection model

A.D. Gannon[a], J. Matlack[b], and S.E. Darch[a]

[a]Department of Molecular Medicine, Morsani College of Medicine, University of South Florida, Tampa, Florida, USA.
[b]Department of Molecular Oncology, H. Lee Moffitt Cancer Center and Research Institute, Tampa, Florida, USA.

Bacterial aggregates are observed in both natural and artificial environments. In the context of disease, aggregates have been isolated from both chronic and acute infections. *Pseudomonas aeruginosa* (*Pa*) aggregates contribute significantly to chronic infections, particularly in the lungs of people with cystic fibrosis (CF). Unlike the large biofilm structures observed *in vitro*, *Pa* in CF sputum forms smaller aggregates (~10-1000 cells), and the mechanisms behind their formation remain underexplored. This study aims to identify genes essential and unique to *Pa* aggregate formation in a synthetic CF sputum media (SCFM2). We cultured *Pa* strain PAO1 in SCFM2 and LB, both with and without mucin, and used RNA sequencing (RNA-seq) to identify differentially expressed genes. The presence of mucin revealed hundreds of significantly differentially expressed (DE) genes, predominantly downregulated, with 40% encoding hypothetical proteins unique to aggregates. Using high-resolution microscopy, we assessed the ability of mutants to form aggregates and identified 13 that were unable to form WT aggregates. Notably, no mutant exhibited a completely planktonic phenotype. Instead, we identified multiple spatial phenotypes described as 'normal,' 'entropic,' or 'impaired.' Entropic mutants displayed tightly packed, raft-like structures, while impaired mutants had loosely packed cells. Predictive modeling linked the prioritized genes to metabolic shifts, iron acquisition, surface modification, and quorum sensing. Co-culture experiments with wild-type PAO1 revealed further spatial heterogeneity and the ability to 'rescue' some mutant phenotypes, suggesting cooperative interactions during growth. This study enhances our understanding of *Pa* aggregate biology, specifically the genes and pathways unique to aggregation in CF-like environments. Importantly, it provides insights for developing therapeutic strategies targeting aggregate-specific pathways.

#3

Mycobacterium abscessus persistence in the face of *Pseudomonas aeruginosa* antagonism.

R. Gupta[a], M. Schuster[b], and K.H. Rohde[a]

[a] Division of Immunity and Pathogenesis, College of Medicine, University of Central Florida, FL
[b] Division of Microbiology, College of Science, Oregon State University, OR

Chronic bacterial infections are responsible for significant mortality and morbidity in cystic fibrosis (CF) patients. *Pseudomonas aeruginosa* (*Pa*) and *Mycobacterium abscessus* (*Mab*) both cause difficult to treat persistent pulmonary infections. Co-infection by these two bacterial pathogens leads to severe disease and poor clinical outcomes. To explore understudied interactions between these two CF pathogens, we employed microbiological and molecular- based approaches. In a planktonic co-culture model, growth of *Pa* continued unimpeded, and it exerted bacteriostatic effect on *Mab.* Strikingly, exposure of *Mab* grown in monoculture to cell-free spent supernatant of *Pa* resulted in a dramatic, dose-dependent bactericidal effect. Initial characterization indicated that this potent *Pa*-derived anti-*Mab* cidal activity was mediated by a heat-sensitive, protease-insensitive soluble factor of >3kDa size. Further analysis demonstrated that expression of this mycobactericidal factor requires LasR, a central regulator of *Pa* quorum sensing (QS). In contrast, Δ*lasR Pa* was still able to exert a bacteriostatic effect on *Mab* during co-culture, pointing to additional LasR-independent factors able to antagonize *Mab* growth. However, the ability of *Mab* to adapt during co-culture to counter the cidal effects of a LasR regulated factor suggested complex interspecies dynamics. Dual RNAseq analysis of *Mab-Pa* co-cultures revealed significant transcriptional remodeling of *Mab*, with differential expression of 68% of *Mab* genes compared to minimal transcriptional changes in *Pa*. Transcriptome analysis reflected slowed *Mab* growth and remodeling of carbon and energy metabolism akin to a non-replicating persister-like phase. A tailored *Mab* response to *Pa* was evident by enhanced transcript levels of genes predicted to interfere with alkylquinolone QS signals or provide protection against respiratory toxins and hydrogen cyanide. This is the first study to provide a transcriptome-level view of genetic responses governing the interplay between two important CF pathogens. This will provide insights into interspecies interaction mechanisms which can be targeted to disrupt their communities in a CF lung to improve patient clinical performance. Moreover, identification of a novel antimicrobial natural product with potent cidal activity against *Mab* will provide a chemical biology tool for identifying new drug targets in *Mab*

Understanding patterns of growth and morphology of Salmonella serovars Kentucky, Enteritidis, and Typhimurium in competition

A.L. Laury, A.T. Siceloff, and N.W. Shariat
Poultry Diagnostic and Research Center, University of Georgia, Athens, Georgia

Salmonella enterica subsp. enterica remains a leading cause of illness in the United States with about 1.35 million cases, 26,500 hospitalizations, and 420 deaths annually. There are over 1,500 serovars within subsp. enterica, as defined based on their surface (O) and flagellar (H) antigens; each serovar may display unique phenotypes based on their different genotypes. Conventional Salmonella isolation relies on picking colonies from a selective indicator agar for further characterization; this approach is limited since multiple serovars may exist within a population. For a 95% probability of identifying two serovars, which must exist in equal proportions, six colonies would have to be selected and characterized. Salmonella isolation could be streamlined and improved with greater understanding of colony phenotypes displayed by different serovars, such that it would be easier to pick multiple serovars based on the individual morphologies. This study sought to characterize the growth dynamics and resulting morphologies of three select Salmonella serovars when grown alone and in the presence of another serovar. Salmonella serovar Kentucky is the most frequently identified serovar in domestic poultry production, although it is infrequently attributed to human clinical cases. Alternatively, serovars Enteritidis and Typhimurium are also often found in poultry but account for half of all human infections. To observe growth in competition, 10 isolates from clinical, poultry, or water sources for the three serovars were grown individually, diluted to a standard 1,000 CFU/mL, then allowed to grow in a two-serovar mixed culture before being plated onto XL-N (Xylose-lysine with novobiocin) media for observation. Each mixed culture and subsequent plating were conducted in triplicate to assess the reproducibility and consistency of the growth patterns. Additionally, each isolate was grown individually to establish a baseline of growth characteristics. Serum agglutination testing was used for identification of differing phenotypes via monovalent specific 'O' antisera. This work highlights the effort to associate specific phenotypes and growth mechanisms with Salmonella serovars that sit at the forefront of modern microbiology and public health concerns.

#5

Candida albicans Impacts GBS Virulence

A.N. Pearah[1], A.K. Lindon[2], K. Dominguez[2], N. JL. Edwards[2], R. Handel[3], C.N.Z. Aparicio[1], S.E. Darch[2], T.M. Randis[1,2]

[1]Pediatrics, Morsani College of Medicine, University of South Florida, Tampa, FL

[2]Molecular Medicine, Morsani College of Medicine, University of South Florida, Tampa, FL

[3]Morsani College of Medicine, University of South Florida, Tampa, FL

Group B Streptococcus (GBS) is a leading cause of infant sepsis worldwide. Colonization of the gastrointestinal tract is a critical precursor to late-onset (LO) disease in exposed newborns. Previous data demonstrate that *Candida albicans* promotes GBS colonization of host tissues including the bladder and vagina. However, its impact on GBS colonization of intestinal tissues and GBS pathogenesis remains unknown. Our laboratory has a well-established mouse model of LO GBS disease. We adapted this model to study GBS and *C. albicans* co-colonization in the newborn host and found greater GBS burden in both the small intestine and colon when *C. albicans* was present. The goal of this study was to identify the mechanism responsible for this enhanced GBS colonization. GBS and *C. albicans* were co-cultured in Tryptic Soy broth for 4 hours at 37°C. Bacterial RNA was extracted and converted to cDNA for real time PCR using the comparative CT Method (ΔΔCT Method) with *recA* as the reference gene. Candidate genes hypothesized to contribute to LO GBS pathogenesis were amplified using previously validated primers. 2 biological replicates for each condition with 6 technical replicates were analyzed. We noted a significant upregulation of *cylE, spb1, lmb*, and *cfb* expression (all $p < 0.001$, unpaired t test) in GBS when incubated with C. *albicans* compared to GBS monoculture. Increased expression of these genes suggests that the presence of *C. albicans* increases GBS ability to survive and colonize the neonatal gut. Further examination of the underlying mechanism will be explored by studying whether heat-killed *C. albicans* and the supernatant of *C. albicans* also affect GBS gene expression which would identify specific components inducing changes in the GBS transcriptome. Understanding the mechanism of GBS and *C. albicans* co-colonization of human infants may reveal novel risk factors and therapeutic treatments for late onset GBS disease.

Keywords: Group B Streptococcus, Candida *albicans,* neonatal sepsis, cylE, real time PCR

Abstract: SEB-ASM

Impact of Spray Vaccination in Tracheal and Cecal Microbiome of Broiler Chicks

S. Poudel and D. Bourassa

**Department of Poultry Science, Auburn University*

The respiratory route is one of the primary routes for transmission of bacterial and viral diseases in poultry. Spray vaccination is commonly conducted at the hatchery to induce immunity. The objective of this experiment was to identify the impact of spray vaccine on tracheal and cecal microbiome of broiler chicks. On day of hatch birds from the vaccinated group received a spray vaccine (Poulvac Bron GA08 and Poulvac Bron Mass) for infectious bronchitis, the upper respiratory tract disease caused by gammacorona virus. Tracheal and cecal samples were collected from day-old broiler chicks and DNA was extracted. Genomic DNA was PCR amplified for complete bacterial 16s rRNA gene V1-V9 hypervariable region and sequenced using MinIon (Oxford Nanopore). Reads were base-called and demultiplexed using MinKnow and analyzed using EPI2ME platform and R-packages. The alpha diversity indexes (Shannon index, observed richness, and Pielou evenness) indicates that there was no difference (pair-wise Wilcox $p > 0.05$) in richness and evenness in tracheal and cecal microbiome between vaccinated vs non-vaccinated. However, differences between the vaccinated vs non-vaccinated birds in trachea samples were significant (adonis $p = 0.018$) for beta-diversity (Bray-Curtis), whereas it was non-significant for cecal samples (adonis $p = 0.425$). While comparing the bacterial composition at species level in trachea of non-vaccinated and vaccinated chicks *Streptococcus pluranimalium* (34.04%) and *Enterococcus faecalis* (40.93%) was the most abundant species; respectively. DESeq2 analysis shows, 27 species exhibiting higher abundance and 18 species showing lower abundance in vaccinated birds compared to non-vaccinated birds. In cecal samples of both non-vaccinated and vaccinated chicks, *Enterococcus faecalis* was the most abundant bacterial species (68.37% and 48.11%, respectively). However, DESeq2 analysis shows only 4 species were significantly less abundant in vaccinated compared to non-vaccinated chicks. In conclusion, this result indicates that the spray vaccination of infectious bronchitis vaccine to chicks at the hatchery significantly shifted the tracheal microbiome composition however, the impact was minimal in the cecal microbiome. The future implication of this research is that tracheal microbiome has the potential to be modulated potentially influencing the transmission and colonization of pathogenic microorganisms that transmit via aerosol or respiratory route.

Key words: microbiome, trachea, ceca, spray vaccine, and broiler chicks

#7

The battle of bacteriophages and bile acids against *Klebsiella pneumoniae* in a fecal microbiota transplantation recipient

Authors: S. Forsstrom[1], B. Berryhill[1], T. Gil Gil[1], J. Manuel[1], M.H. Woodworth[2], B. Levin[1]

[1] Department of Biology, Emory University; Atlanta, Georgia, 30322, USA.
[2] Department of Medicine, Division of Infectious Diseases, Emory University School of Medicine; Atlanta, Georgia, 30322, USA.

Fecal Microbiota Transplantation (FMT) can alter the enteric microbial community structure and function and reduce risk of recurrent *C. difficile* infection. In addition to bacteria, these communities include an abundance of bacteriophages (phages), bile acids, and other small molecules. In a focused analysis of a participant in a recent clinical trial, metagenomic analysis revealed that this patient's enteric microbiome was dominated by an extended-spectrum beta-lactamase-producing *Klebsiella pneumoniae* with a relative abundance of approximately 93%. Following FMT, all *Klebsiella* species in this patient disappeared. The mechanism for this exclusion is unknown.

A recent clinical trial on FMTs measured the signal intensity of reference standard annotated bile acids in stool and determined that FMT success was associated with an increase in the concentration of secondary bile acids post-FMT. Another recent study found that exposure to bile acids increases the sensitivity of Gram-negative bacteria to bacteriophages. We postulate that phages and bile acids together contribute to FMT efficacy. To test this hypothesis, we isolated both *K. pneumoniae* and *Klebsiella* phages from the patient's microbiome and the FMT used for treatment. We found that phage resistance dominates in the patient's microbiome prior to FMT. We are currently determining how bile acids may reprogram bacterial membrane surface phage receptor density and the effect this may have on phage sensitivity. Our preliminary results indicate that exposure to bile acids decreases the level of lipopolysaccharides and increases phage sensitivity. We hypothesize that exposure to bile acids, which are expelled from bacteria via multi-drug efflux pumps, cause upregulation of these large transporters embedded in the bacterial membrane, taking away space for lipopolysaccharides which confer phage resistance. We anticipate that increased efflux pump production leads to a decrease in lipopolysaccharides found in the bacterial membrane, which thereby increases sensitivity to phage, and ultimately contributing to the success of FMTs.

#8

E. Soucy[1,3], A. Palmer[1,2], Characterization of Carnivorous Plants for Phytoremediation in Space Agriculture
Affiliations: Biomedical Engineering and Sciences (1), Ocean Engineering and Marine Sciences (2), Aerospace, Physics, and Space Sciences (3), Florida Institute of Technology, Melbourne, FL

Further exploration to the Moon and Mars, will require the establishment of In Situ Resource Utilization (ISRU) agricultural systems. Heavy metals found in Martian and lunar regolith, however, present serious concerns for regolith-based agriculture as a means to ensure food security. These metals can prove toxic to the plants themselves and may accumulate in these crops and pose a threat to settlers. As a result, these regoliths will require pre-treatments prior to their use as a substrate. Phytoremediation presents a solution which may eliminate potential toxins and condition the regolith, helping convert it to soil. I propose carnivorous plants, such *as Utricularia gibba,* offer a partial remedy. In addition to being well-equipped to oligotrophic and low nitrogen environments, *U. gibba* is capable of accumulating several heavy metals, including Cr^{3+} and Mn^{2+}, which are found in both lunar and Martian regoliths in significantly high concentrations that they pose notable concerns to plant growth and human health. We hypothesize that several *Utricularia* species and their associated microbiomes will be able to successfully capture heavy metals from lunar and Martian regolith and provide support for the growth of edible plants once the regolith has been properly conditioned. In the present study, we explore the microbiomes of *U. gibba*, *U. subulata*, and *U.* vulgaris, for the purpose of characterizing the less-studied *U. subulata*, a terrestrial species, in order to understand how regolith stress impacts it.

#9

Title: Pre-extraction treatment methods for isolating microbial DNA from ground beef samples

Authors: B. Maloney, I. Gafanha, A. Belk
Affiliations: Department of Animal Science, Auburn University

Meat remains a major contributor for foodborne illness and the focus of much microbial research. Despite the critical need to investigate the microbial communities of meat products, there remain methodological challenges in conducting relevant experiments. One of these issues is our ability to reliably and consistently isolate microbial DNA from these systems. Meat samples are considered a low biomass microbial environment, containing primarily DNA from the host species. The mammalian DNA can be removed bioinformatically, but it is still difficult to achieve sufficient microbial coverage, and the added cost to sequence unnecessary reads can be prohibitive in many studies. Additionally, meat products often have high fat contents, making it hard to extract enough high-quality DNA required for downstream applications. Our goal was to identify the most effective pre-extraction sample treatment method for isolating bacterial DNA. Meat products were first inoculated with the ZymoBIOMICS® Microbial Community Standard. We investigated six potential treatments: two groups with direct extraction from the product- one with the mock community and one without, two-step centrifugation of the product and peptone buffer, swabbing the surface of the product with an SDS extraction buffer, PBS rinsate and centrifugation, and treatment with proteinase K. DNA was extracted using Zymo Research Quick-DNA™ Fecal/Soil Microbe Microprep Kits. DNA quantification was done with an Invitrogen Qubit™ Flex Fluorometer, with preliminary results showing that treatment with Proteinase K is the most effective method for isolating DNA, with a mean concentration of 121.56 µg/m. Two-step centrifugation was determined to be the least effective method with a mean concentration below 1 µg/mL. Following extraction, samples were sequenced for shotgun metagenomics as a commercial service using and Illumina NovaSeq X Plus as well as using an Oxford Nanopore MinION Mk1B and the Oxford Nanopore Native Barcoding Kit 24 V14 Kit. Following quality trimming, reads were aligned using Kraken2 and statistically compared to the expected mock community results. Using these data, we will determine the method that most accurately captured the microbial community profile.

Microbial Ecology

#10

Bistability in dynamics of worm-microbe interactions

S.S.Boddu, K.M. Martini, I. Nemenman, N. Vega

Departments of Biology and Physics, Emory University, Atlanta USA

Microbial community composition across nominally identical hosts is highly variable. Sources of this variation are not well understood. To explore the variability, we let the roundworm *C. elegans* be colonized with single bacterial species from its native gut microbiome. We then observed the temporal dynamics of the bacterial population in many individual hosts. We observe a bistable distributions of population sizes across individual hosts. Our analysis of the temporal evolution of these distributions after perturbations suggests that demographic noise and stationary host heterogeneity alone cannot account for the observed variation. To account for this bistability, we suggest that the bacterial growth rate in the hosts should have multiple stable fixed points or alternatively the host has stochastic state switching.

Structural specificity of *Sinorhizobium* exopolysaccharides in infection thread formation on *Medicago* plant hosts

B. Redfern
Florida State University, Biological Science, Tallahssee, FL

Legumes can overcome soil nitrogen deficiency by forming a symbiosis with N_2-fixing rhizobia. In many symbiont-host pairs, the association begins with root hair (rh) curling induced by rhizobia-produced Nod factor and trapping of rhizobia within a rh curl. In associations between species of the *Sinorhizobium* genus and the plant genus *Medicago*, the actions of both Nod factor and expolysaccharide (EPS) are required for bacterial invasion via tubular invaginations in rhs called infection threads (ITs). Concomitant with invasion, the plant forms a nodule that will house the invading *Sinorhizobium,* in which they will differentiate into N_2-fixing bacteroids. There are structural differences in EPSs produced by different rhizobial species, and structural modifications to EPS can affect host invasion. The mechanism(s) of EPS/host interaction and the bases for the structural specificity of EPSs are poorly understood even though IT formation is a crucial step in achieving successful symbiosis.
EPSI/succinoglycan produced by *S. meliloti* 1021 (*Sm*1021) is absolutely required for IT extension into the developing nodule primordium on *M. truncatula* A17 and R108, and is required for **efficient** IT formation on alfalfa (*M. sativa*) cv. Iroquois. *Sm*1021 revertants to wild type of the *expR* regulatory gene gain the ability to make EPSII/galactoglucan as well. EPSII can mediate IT development in place of succinoglycan on Iroquois, albeit at lower efficiency. One possibility is that *Sm* EPSII can substitute for succinoglycan on Iroquois because even a low-quality EPS can function in the very robust interaction between these partners. A second possibility is that alfalfa Iroquois has a mechanism for perceiving EPSII that is lacking in *M. truncatula*. I have found that EPSII can also substitute for succinoglycan in the alfalfa cultivars GT13R+ and *M. sativa* L. subsp. *falcata*. I have also found that succinoglycan-deficient mutants of, *S. medicae* WSM419 can invade *M. truncatula* A17 albeit at a lower efficiency than wild type. The role of EPSII in this invasion is being determined.

#12

L.E. Turner, K. Cutshaw, E. Soyke, M. Taylor, J. Ahrens, J. Labishak, B. Lee, R. Quick, T. Mello, and A. Palmer:
Investigating eukaryotic cell-cell communication using reverse genetics on model organism *Chlamydomonas reinhardtii.*

As the human population becomes increasingly larger, there becomes a need for highly efficient industrial systems that take up increasingly less space. Microorganisms, many of which are versatile and can withstand harsh environments, may be the solution to supporting humanity. A trait of these organisms that can help revolutionize industrial systems is quorum sensing (QS), a cell-cell communication method in which phenotypes within a population of unicellular organisms are regulated by cell density. In prokaryotes, the mechanisms of QS have been thoroughly studied; however, much remains to be discovered about QS in unicellular eukaryotes. Learning more about these signaling systems can lead to biotechnological revolutions in life-sustaining industries such as agriculture and medicine. To better understand eukaryotic QS, we studied the unicellular algae *Chlamydomonas reinhardtii* which appears to regulate motility in a cell density-dependent manner in the most common lab strain of cc124. However, *Chlamydomonas reinhardtii* has numerous genetically distinct environmental isolates due to the diverse habitats this species occupies. These ecotypes of *Chlamydomonas reinhardtii* make it a natural candidate for a reverse genetics approach to identifying critical QS mechanisms. Here, we present a reverse genetics approach to identify the algae's QS mechanism by screening multiple strains of *Chlamydomonas reinhardtii* for QS phenotypes and comparing them to the QS-positive strain cc124. Identifying the QS mechanism in this model organism can lead to greater utilization of *Chlamydomonas reinhardtii* in industrial microbiology and help us develop tools for identifying QS mechanisms in other eukaryotes for biotechnological applications.

#13

Title: Identifying and Characterizing Freshwater Microbes Affected By Agricultural Runoff

R. Williams

R. Williams, University of West Alabama, 4236 County Road 54 West

G. Britt

G. Britt, Auburn University, Auburn, AL 36849

R. Tollerson II

R. Tollerson II, Auburn University, Auburn, AL 36849

Williamsr5305@uwa.edu
tollerson@auburn.edu
gdb0019@auburn.edu

Abstract: In freshwater ecosystems, algal blooms occur when there has been an increase in microbe growth from utilizing the nutrients from agricultural runoff. When agricultural runoff is present in the freshwater ecosystem, this will allow specific freshwater microbes to utilize the nutrients which in turn could cause them to produce toxins that can be harmful to that aquatic ecosystem, wildlife, and humans. Within this project, we are interested in how freshwater bacterial communities are able to react to the nutrients from agricultural runoff. In addition, we aim to identify what specific microbe's function is that corresponds with algal blooms or the process of algal blooms decomposition. The main objective of this research is to characterize and identify the microbes that are present in freshwater ecosystems and then see which microbes can utilize the nutrients from agricultural runoff using culture methods, microscopy, and 16S rRNA sequencing. Our findings have found that freshwater microbes can be selected on specific types of media containing phosphorus and/or nitrogen; thus, allowing us to sequence those bacteria to understand their role in the freshwater ecosystem and how they can accomplish such a feat from their genome. Overall, this project will help us gain an understanding of the function of bacterial communities that are present in freshwater ecosystems, and how some of those bacterial communities produce toxins and possibly be pathogenic when they utilize the extra nutrients from agricultural runoff.

Keywords: Algal blooms, 16S rRNA sequencing, genome, freshwater ecosystems, agricultural runoff, microscopy.

#14

Longitudinal Salmonella Surveillance in Commercial Poultry Production with Rodent Control

A.T. Siceloff[1], S.P. Nolan[2], and N.W. Shariat[1]
[1]Poultry Diagnostic and Research Center, University of Georgia, Athens, Georgia
[2]Nolan Integrated Pest Control and Management (NIPCAM) Group, Watkinsville, Georgia

Despite significant reduction of *Salmonella* incidence during processing, poultry products remain a considerable source of foodborne salmonellosis. Successful *Salmonella* control during poultry production requires an appropriate surveillance platform and a focus on robust on-farm biosecurity. Conventional *Salmonella* monitoring relies on the identification of a single serovar from a sample, which causes some serovars to remain undetected. To overcome this limitation, deep serotyping with CRISPR-SeroSeq is a next-generation sequencing approach that determines the relative abundance of serovars within mixed populations. This study was designed to assess the prevalence of multiserovar *Salmonella* populations in broiler breeder flocks, and to highlight the importance of maintaining rodent control to limit *Salmonella* introduction. Across two commercial complexes (A, B), the young chickens (pullets) were sampled at weeks 14 and 21, then the mature chickens (breeders) sampled every four weeks. In total, 15 pullet (five farms) and 13 breeder houses (seven farms) were sampled over a 65-week production period. Two boot sock pairs were collected from each house and cultured for *Salmonella* (n = 394). Rodents (mice plus roof and Norway rats; n = 355 carcasses across 49 composite samples) were captured from farms and tested for *Salmonella*, along with bait station swabs (n = 33). Overall *Salmonella* prevalence in pullets was 17% (11/64), although only houses in Complex B were positive (6/7 houses). In breeders, the overall prevalence was 30% (55/182) and 54% (80/148) in Complexes A and B, respectively. *Salmonella* prevalence and serovar diversity peaked between 34-38 weeks. Deep serotyping revealed 36% (53/146) of boot socks contained multiple serovars. The number of serovars differed between Complex A (five serovars) and B (14 serovars). In rodents, 35% (17/49) of composite samples and 9% (3/33) of bait station swabs were positive, and six serovars were identified. Three rodent serovars were also found in boot sock samples from the same farms/houses. This longitudinal characterization of *Salmonella* highlights differences across broiler complexes, which may be attributed to management practices as Complex A employs a 3rd party integrated pest control company while Complex B relies on growers to control rodents.

#15

Characterization of complex *Salmonella populations* in watersheds of the southeastern United States

J.C. Smith, A.T. Siceloff, S. Shirazi, N.W. Shariat
Department of Population Health, College of Veterinary Medicine, University of Georgia, Athens, GA 30602

Salmonella enterica is a leading cause of bacterial foodborne illness and although it is most commonly associated with animal hosts, it is frequently isolated from fresh water sources. It is unknown how *Salmonella* persists in fresh water environments, nor the extent to which *Salmonella* can be transmitted within creeks. Further, in a mixed serovar population, traditional *Salmonella* isolation is limited to identifying the most abundant serovars in a population, potentially allowing background serovars to remain undetected. This temporal study was designed to determine the incidence of multiserovar *Salmonella* populations in four different creeks. Over 24 months (November 2021 – October 2023), 10L water samples were collected from 19 sites across four creek watersheds (n=456) using modified Moore swabs. Each system was defined by a distinct land use, including animal agriculture (A and C), suburban developments (B), and a national forest (D). In addition to *Salmonella* isolation, deep serotyping was performed to characterize *Salmonella* serovar populations. The overall *Salmonella* prevalence was 68.9% (314/456) and differed significantly depending on the watershed and season ($p=0.046$ and $p<0.001$, respectively). *Salmonella* prevalence was highest in spring (93.0%, 106/114) and lowest in summer (47.4%, 54/114). Deep serotyping revealed that 88.8% (229/258) of positive samples collected contained multiple serovars (average 3.7 serovars/sample and a range of 1-13 serovars/sample). Of the 40 serovars detected, 14 are in the top 15 most often associated with human illness in the US. The incidence of multiserovar populations was also highest in spring (4.7 serovars/sample) and ranged from 77.5% in watershed A (55/71 samples) to 94.7% in watershed C (90/95). Shannon diversity was used to assess changes in population complexity, with downstream sites in watersheds A and D having more complex *Salmonella* populations than upstream sites. Additionally, the consistency of these populations over time were investigated using PCoA analysis of the Jaccard distance of each sample in a watershed, where watershed A had tighter clustering than other watersheds. These data demonstrate that *Salmonella* prevalence and serovar populations in freshwater are highly complex and that their stability can differ between watersheds.

#16

***Salmonella* serovar complexity is reduced on broiler carcasses and parts post antimicrobial intervention.**

A.K. Richards and N.W. Shariat
Department of Population Health, University of Georgia, Athens, Georgia

Approximately one in five salmonellosis cases is attributed to consumption of contaminated chicken. A limitation to traditional *Salmonella* surveillance is that often a single colony is picked from a positive sample and characterized. This favors the most abundant serovar in a sample, effectively masking other serovars that are also present. CRISPR-SeroSeq is a deep serotyping approach that can resolve this problem by identifying the relative frequency of multiple serovars in a sample. The objective of this project was to use deep serotyping to assess *Salmonella* serovar complexity during broiler processing, including before and after antimicrobial interventions, and to determine whether a surface swab of a carcass (using MicroTally mitts) could serve as an improved sampling method instead of rinsing the whole carcass. Three commercial broiler processing establishments were each visited two times. Rinses and mitts were used to sample both whole carcasses and parts, with a total of 40 rinses and 40 MicroTally mitts collected per plant visit. Additionally, to test the limits of the MicroTally mitts, they were used to swab 50 carcasses and 100 wings, rather than just one carcass (multi-MicroTally). Samples were collected at hot rehang (n = 25), post-chill (n = 25), pre-antimicrobial dip parts (n = 25), and post-antimicrobial dip parts (n = 25). Bacterial recovery was the greatest from the rinsing method (22.75%; 53/233), but the multi-MicroTally mitt was similar at 21.37% (25/117). The single MicroTally alone had slightly lower recovery at 18.46% (43/233). As expected, the highest *Salmonella* incidence was found after limited antimicrobial treatment during hot rehang (74.67%; 112/150), while the lowest was found after all interventions at post-chill (0.67%; 1/150). CRISPR-SeroSeq was performed on *Salmonella*-positive samples collected at hot rehang (n = 129), post-chill (n = 1), pre-dip parts (n = 8), and post-dip parts (n = 1). Multiple serovars were detected in 56.59% (73/129) rehang, 0% (0/1) post-chill, 25% (2/8) pre-dip, and 0% (0/1) post-dip samples. Eight different serovars were identified. These data demonstrate that processing interventions effectively reduce *Salmonella*, as serovar complexity was reduced in nearly all post-chill and post-dip samples.

#17

Title: Persistence of multi-serovar *Salmonella* populations in surface water proximal to a cow-calf operation

Authors: E.G. Palmer, J.C. Smith, N.W. Shariat
Affiliations: Departments of Microbiology and Population Health, University of Georgia, Athens Georgia

Contaminated freshwater is a significant risk factor for the spread of *Salmonella*, yet the persistence of *Salmonella* in surface water remains understudied. This study sought to identify whether environmental factors influence *Salmonella* transmission from a cow-calf operation to a proximal creek, and to evaluate *Salmonella* survival and movement downstream. A modified Moore swab was used to sample *Salmonella* from a creek at the base of a cow-calf operation. Creek samples and three composite cattle fecal samples were taken twice a month, once after significant rainfall, and once after three dry days. Weather and water variables (including conductivity, flow rate, pH, dissolved oxygen, total dissolved solids, water temperature) were recorded during each collection. *Salmonella* was isolated following standard enrichment procedures and positive samples analyzed by deep serotyping (DS) and whole genome sequencing (WGS). Overall *Salmonella* prevalence for water and fecal samples was 67.4% (83/123) and 6.0% (7/117), respectively. Dissolved oxygen and total dissolved solids were found to be negatively associated with *Salmonella* prevalence, while no weather variables were significantly correlated. DST analysis revealed that 91% (62/68) of positive samples contained multiple serovars. A total of 24 serovars were identified, with a range of 1-10 serovars per sample. Following rainfall, the average number of serovars per sample increased, from 3.6 serovars per dry sample to 5.0 serovars per wet sample. We observed a shift in the most abundant serovar as sampling sites progressed downstream; serovar Muenchen accounted for 36% of the isolates upstream of the farm to 18% and 12% both of the downstream sites, respectively. Meanwhile, serovar Montevideo accounted for 18% of the isolates upstream of the farm and 39% and 37% at the downstream sites. WGS analysis determined that highly related isolates (within 5 allelic differences), identified across several sites, and across several months, were observed multiple times. This could either be caused by reintroduction events or could represent the persistence of some strains within the water. These observations highlight differential serovar presence and persistence in freshwater, and this information can be used in risk analysis where surface water is used for produce irrigation.

#18

Unusual regulation of genes necessary for carbon dioxide fixation by marine chemolithoautotroph *Thiomicrospira pelophila*

J. Wieschollek[1], D. Walsh[1], Y.X. Zhao[1], A. Mislay[1], MCB4404L 2020,2022[1], K. Scott[1]
[1]University of South Florida

Microbial CO_2-fixation is the foundation of food-webs in a huge variety of habitats. Even when CO_2 is scarce, many autotrophic bacteria can fix dissolved inorganic carbon (DIC; CO_2, HCO_3^-) with high efficiency by utilizing carbon dioxide concentrating mechanisms (CCMs), consisting of carboxysomes and DIC-transporters. Autotrophic bacterium *Thiomicrospira pelophila* has carboxysomes, and encodes six potential DIC-transporters, more than is typical for bacteria with CCMs. We conducted experiments on how carboxysomes and multiple transporters are integrated into a functioning CCM in *T. pelophila*. *T. pelophila* was grown in chemostats, under DIC-limitation or ammonia limitation, to induce its CCM. Potential DIC-transporter genes were heterologously expressed in *E. coli*, to determine capability for DIC transport. Silicone oil centrifugation was used to confirm DIC-uptake by DIC transporters. QRT-PCR was used to track transcript abundance of genes encoding CCM components, and electron microscopy was used to track carboxysome abundance. *T. pelophila* grew under low DIC conditions (>10 μM), evidence that it expresses a functional CCM. 4 out of 6 transporters were capable of DIC uptake when expressed in *E. coli* and DIC-uptake capabilities were confirmed by silicone-oil centrifugation for those transporters. Transcript abundances from carboxysome-related genes in *T. pelophila* do not differ under low vs. high DIC conditions, unlike other organisms with CCMs. Only one transporter was upregulated under low DIC conditions. A slight increase in carboxysome abundance was apparent under low DIC conditions; however, this increase is far smaller than in other autotrophs. The CCM of *T. pelophila* is unique as carboxysomes are universally present under different DIC concentrations and the large number of DIC-transporters. A possible explanation for these findings may lay in the lineage of *T. pelophila*. All close relatives are alkaliphiles. CO_2 is generally scarce in alkaline environments and thus always expressing carboxysomes might provide an advantage. Exploring new CCMs could be of particular interest to biomolecular-engineering-applications to use microorganisms to synthesize compounds of industrial importance from CO_2, as well as to increase crop yields.

#19

Understanding *C. elegans* Decision-Making in Complex Foraging Environments

C.V. Bowman[1], N.M. Vega[2,3], C.E. Alvarez[2]

[1]Emory College of Arts and Sciences, Atlanta, Georgia
[2]Emory University, Population Biology, Ecology, and Evolution, Atlanta, Georgia
[3]Emory University, Department of Physics, Atlanta, Georgia

Decisions made by the nematode *C. elegans* when foraging for bacterial food sources have been thoroughly studied in 2D environments using canonical choice assays; however, interactions between *C. elegans* and microbes in structured 3D environments such as soil matrices are currently unclear. Additionally, the scales at which *C. elegans* are able to make foraging decisions in patchy environments are not well understood, and the consequences for microbial ecology are currently unknown. This project seeks to better understand the spatial scales of decision making and worm-microbe interactions by creating patterned bacterial lawns with various strain combinations and imaging the movement of *C. elegans* and the bacterial distributions on these lawns over time. These results provide a greater awareness of the foraging choices made by *C. elegans* in latticed bacterial lawns compared to separated spots of bacteria and serve as foundational data to further investigate the consequences of spatially structured environments on microbial populations.

#20

Title: Quorum sensing in the model algal species *Chlamydomonas reinhardtii*

Presenting Author: Trevor Mello

Authors List: Trevor Mello, Dr. Andrew Palmer

Affiliations: Department of Ocean engineering and Marine Sciences, Florida Institute of Technology, Melbourne, FL

The phenomenon known as quorum sensing (QS) has been extensively studied in bacteria for many years due to the insight it gives us into microbial ecology, host-microbial associations, and the evolution of coordinated (i.e. 'social') behaviors. QS has also been identified in some fungal species, suggesting the phenomenon may persist among other eukaryotes. When the model algae species *Chlamydomonas reinhardtii* reaches a cell density of 5x10^6 cells/mL, there is a noticeable increase in the average swimming speed of the cells, which has been attributed to a QS-like phenomenon. Interspecies variation in this response could provide valuable insight into the QS mechanism in this model unicellular eukaryote. Two strains of particular interest are cc4886 and cc4889. These light sensitive strains require different environmental conditions and still contain distinct characteristics of the common lab strain. The process by which these specific strains grow is completely different than the common lab strains of cc124. This, however, does not change the fact that they also experience an increased swimming speed when at a high cell density. Along with physical traits, these different lab strains can show different phenotypic changes at higher cell densities. Enabling us to take a closer look at the different QS reactions between each lab strain of the same species.

Host-Pathogen Interactions

#21

Mechanistic Insight into RNA-Mediated Control of Virulence in *Staphylococcus aureus*

M.-E. Jobson[1,2], B.R. Tomlinson[1], E.M. Mustor[1,2], E.A. Felton[1,2], A. Weiss[1], C.C. Caswell[3], L.N. Shaw[1,2]

[1]Department of Molecular Biosciences, University of South Florida, Tampa, Florida, United States

[2]Center for Antimicrobial Resistance, University of South Florida, Tampa, Florida, United States

[3]Department of Biomedical Sciences and Pathobiology, Center for One Health Research, VA-MD College of Veterinary Medicine, Virginia Tech, Blacksburg, Virginia, United States

At over 1,200-nt in length, SSR42 is the longest and most abundant noncoding RNA in the *S. aureus* cell, second only to RNAIII. It is highly conserved across strains and exhibits pronounced stability in stationary phase, however the mechanism behind its regulatory role has yet to be elucidated. Herein, we used transcriptomic and proteomic approaches coupled with *in vivo* reporters and biochemical techniques, revealing that SSR42 is a novel, primary regulator of proteolytic, hemolytic, and cytolytic activity in *S. aureus*. We demonstrate that SSR42 regulates myriad virulence factors post-transcriptionally through direct RNA:RNA interactions, facilitating stabilization or degradation of target transcripts. Using mutagenesis, we identified key SSR42 interaction regions with target mRNAs, including *aur, lukFS, lukAB, hlgBC,* and the *spls*. In the case of *lukAB*, this regulation is initiated in response to phagocytosis by neutrophils, where SSR42 is activated via PerR in response to oxidative stress, leading to RNA-mediated activation of *lukAB* in order to escape the phagosome. In addition to this host-specific response, we also found that SSR42 is part of a broader, more complex regulatory network, with its expression controlled by other global regulators of virulence, such as SarA, Rot, CodY, and Rsp. Finally, we demonstrate that SSR42 is absolutely required for full virulence of *S. aureus* in murine models of pneumonia and sepsis. Collectively, we present the characterization of SSR42 as a novel RNA-based regulator of virulence factor abundance that responds to host niche dynamics to facilitate *S. aureus* infection.

#22

Role of Human Tyrosine Phosphatase PTP1B in the intracellular development of *Chlamydia trachomatis*

M.S. Khan[1], R. Pelles[1], V.J. Wang[1], and T. Jewett[1]

1. Immunity and Pathogenesis Division, Burnett School of Biomedical Sciences, College of Medicine, University of Central Florida, Orlando, FL USA

Chlamydia trachomatis (Ctr), an obligate intracellular gram-negative bacterium, is responsible for a significant global disease burden, causing sexually transmitted infections and trachoma. Ctr follows a biphasic developmental cycle consisting of infectious elementary bodies (EBs) and non-infectious reticulate bodies (RBs). EBs promote entry into host cells with the help of Type III secretion system early effectors and take up residence within a membrane-bound compartment called an inclusion.

Due to its obligate nature, *Ctr* relies on the host cell for growth and development. Within the inclusion, *Chlamydia* manipulates various intracellular host signaling pathways and processes for nutrient acquisition, modulation of the host immune response, pro-survival signaling, and cellular apoptosis. Many of these processes are regulated through phosphorylation/dephosphorylation of both host and bacterial targets mediated by host kinases and phosphatases.

The role of host kinases in chlamydial pathogenesis has been extensively studied; however, the role of host phosphatases in Ctr development remains unexplored. With the evolving understanding that dephosphorylation is an active cellular process, and the growing interest in host phosphatases in intracellular metabolism, we aimed to investigate the role of the central tyrosine phosphatase PTP1B in *C. trachomatis* development. PTP1B is known to be associated with the upregulation of eukaryotic pro-survival signaling pathways.

We employed loss- and gain-of-function approaches for PTP1B in host cells to explore its potential role in the *Ctr* developmental cycle. Our experiments indicate that *C. trachomatis* harnesses host phosphatase PTP1B to promote development. PTP1B overexpression correlated with increased inclusion size, while its inhibition led to reduced inclusion size and area. We also demonstrate that PTP1B is capable of dephosphorylating the early chlamydial tyrosine phosphorylated effector Tarp.

Our future experiments will focus on elucidating the impact of PTP1B modulation on the generation of infection competent EBs as well as investigating chlamydial and host targets of PTP1B that promote *C. trachomatis* development.

#23

Dissecting the Impact of Extracellular Proteases on the Host Pathogen Interface

E.M. Mustor[1,2], A.M. Frey[1], D. Chaput[1], L.N. Shaw[1,2]
[1]Department of Molecular Biosciences, University of South Florida, Tampa, FL, USA
[2]Center for Antimicrobial Resistance, University of South Florida, Tampa, FL, USA

Staphylococcus aureus persists as a major human pathogen in community and healthcare settings, causing myriad infections ranging from endocarditis to pneumonia, osteomyelitis and bacteremia. It does this through a vast repertoire of virulence factors, which includes toxins, hemolysins and secreted proteases. These latter enzymes play a pivotal role in pathogenesis, cleaving host factors to engender disease. While few protease targets have been identified in isolation, a global characterization of the impact of proteolysis during infection has yet to be undertaken. Herein, we used degradomic approaches to capture protease mediated cleavage of host proteins at multiple host-pathogen interfaces. In so doing, we began to unravel how this bacterium can induce immune dysregulation through targeting of human leukocytes. Across two experimental models we uncovered a total of 332 substrates. From these targets, we found many aspects of neutrophil functionality being modulated including degranulation via cleavage of lysozyme C2, MMP14, myeloperoxidase, BPI, as well as gelosin and beta actin. Neutrophil adhesion was also affected via proteolysis of integrin beta, integrin alpha-L, kindlin-3, ICAM- 3, and moesin. Importantly, proteases may abrogate ROS production by targeting pyruvate kinase PKM, PKC-alpha and Rac2. We next mapped proteolysis by *S. aureus* during active infection using a murine model of osteomyelitis. Here, we noted 121 substrates that again included important neutrophil proteins such as integrin alpha M, myeloperoxidase, MMP8, NGP, PKM, and pro-cathepsin H, among others. Beyond leukocytes, additional targets indicated skeletal muscle deterioration via cleavage of troponin T, titin, vimentin, and several tubulin subunits. Meanwhile, we also garnered potential insight into the underlying mechanisms of the *S. aureus* 'trojan horse' effect whereby observed proteolysis of caveolin-1 may promote bacterial internalization and survival. Collectively, our work provides critical insight into the dynamics of *S. aureus* host-pathogen interaction and furthers our understanding of the role played by secreted proteases during infection.

#24

Pyocin killing of *Pseudomonas aeruginosa* populations isolated from cystic fibrosis sputum samples

Parker Smith[1], Joanna Arulraj[1], Isaac Estrada[1], Stephen P. Diggle[1]

[1]Georgia Institute of Technology

Cystic Fibrosis (CF) is a genetic disorder marked by heightened mucus viscosity in the lungs, leading to persistent bacterial infections. *Pseudomonas aeruginosa* (Pa), an opportunistic pathogen known for its biofilm-forming abilities, is frequently associated with CF lung infections. It is thought that patients with CF are only infected by one Pa strain in their lifetime, with this one strain adapting to the host's lung environment leading to high levels of genetic diversity in the Pa population in the lung. These populations competitively exclude other Pa strains from the lung environment and persist in the lung despite regular antibiotic treatments. One of the ways that Pa strains compete is through the production of bacteriocins known as pyocins, that kill other strains of Pa. Utilizing sequenced populations of genetically heterogeneous Pa strains previously isolated from CF sputum samples, and a collection of pyocins produced by lab strains and clinical isolates of Pa, we explore pyocin killing and resistance in Pa strains isolated from CF sputum samples. Using a high throughput screen, we find heterogeneity in the pyocin resistance of Pa strains in diverse populations isolated from CF sputum samples. Leveraging the sequenced genomes of these populations we explore the genetic mechanisms of resistance to pyocins. By elucidating the nuances of pyocin killing of Pa isolates from the CF lung, this work will contribute to a more comprehensive understanding of intraspecies competition between Pa strains in the CF lung environment, and the potential of pyocins to treat Pa infections in the CF lung.

Distinct Metabolites Protect the Lyme Disease Pathogen *Borrelia burgdorferi* from Macrophage-Mediated Killing

A.M. Schulz[1], S.A. Noureddine[1], M.W. Jewett[1]

[1]Division of Immunity and Pathogenesis, Burnett School of Biomedical Sciences, College of Medicine, University of Central Florida, Orlando, Florida, USA

Borrelia burgdorferi, like most bacterial organisms, requires a variety of nutrients such as riboflavin and its downstream effectors FMN and FAD. However, unlike most bacterial organisms, *B. burgdorferi* lacks the ability to synthesize riboflavin *de novo* due to its reduced genome. Instead, it requires a mechanism to allow for the uptake of riboflavin from the host environment. Previously identified gene, *bb0318,* encodes the predicted ATPase component of an ABC-type riboflavin transport system contained within a four-gene operon, *bb0316-bb0319*. A Δ*bb0318* mutant exhibits attenuated infectivity in mice, reduced survival in the presence of macrophages, increased ROS susceptibility, and reduced survival in ticks. Moreover, *B. burgdorferi* lacking *bb0318* demonstrates significantly reduced expression of the glycerol utilization system encoded by the *glp* operon, a metabolic system particularly relevant for the metabolic switch that occurs as *B. burgdorferi* moves from the tick vector to the mammalian host. It is well-established that the *glp* operon is also regulated by signal transduction second messenger, cyclic di-GMP. Thus, we hypothesized that there is a regulatory link between riboflavin transport and cyclic di-GMP level in *B. burgdorferi*. Despite the overlap between some *bb0318*-dependent and cyclic di-GMP-dependent phenotypes, surprisingly, cyclic di-GMP levels were not found to be altered in the Δ*bb0318* mutant. To elucidate the extent of regulatory convergence between *bb0318* and cyclic di-GMP, we decoupled riboflavin transport and cyclic di-GMP dependent regulation by introducing a constitutively expressed diguanylate cyclase to a Δ*bb0318* mutant to increase cyclic di-GMP levels. Elevated cyclic di-GMP levels were sufficient to override *bb0318* dependence of *glpD* expression. Furthermore, high levels of cyclic di-GMP rescued the susceptibility of the Δ*bb0318* mutant to macrophage-mediated killing by reducing the incidence of phagocytosis. In contrast, increased cyclic di-GMP levels did not improve the ability of the Δ*bb0318* mutant to resist ROS stress. Together, these data suggest that the phenotypes of the Δ*bb0318* mutant can be separated into those that are mediated by both riboflavin salvage and cyclic di-GMP signaling and those that are regulated by riboflavin salvage alone. Work to elucidate the riboflavin-mediated mechanisms which generate these phenotypes is still ongoing.

#26

Characterization of *Borrelia burgdorferi* Lipases: Implications for Fatty Acid Acquisition and Therapeutic Targeting of Lyme Disease

R.L. Kemp[1], M.W. Jewett[1]

[1]Division of Immunity and Pathogenesis, Burnett School of Biomedical Sciences, College of Medicine, University of Central Florida, Orlando, Florida, USA

Borrelia burgdorferi (*Bb*), the causative agent of Lyme Disease (LD), is characterized by its reduced genome and limited capacity for *de novo* synthesis of important metabolites. *Bb* heavily relies on scavenging nutrients from host environments, yet how it acquires these nutrients to aid its survival and pathogenicity remains largely unknown. Notably, *Bb* lacks the ability to synthesize fatty acids (FAs), vital components of the borrelial membrane and lipoproteins, and must employ mechanisms for fatty acid scavenging. Our lab previously demonstrated that gene *bb0562* encodes a lipolytic enzyme, BB0562. Deletion of *bb0562* attenuated *Bb* growth in low fatty acid environments, including the mammalian host. The *in vitro* growth defect of the mutant was restored with free fatty acid supplementation, but not triglycerides, suggesting that BB0562 may be critical for scavenging fatty acids from triglycerides. The genome of *Bb* has been shown to encode another lipase enzyme, BB0646. Deletion of gene *bb0646* significantly impaired borrelial infectivity and lipase activity, suggesting that BB0646 also contributes to fatty acid acquisition needed for survival. The combined contributions of these two lipases to *Bb* biology remains unknown. We hypothesize that together BB0562 and BB0646 function to supply *Bb* with the complete repertoire of fatty acids needed for survival. This project employs genetic and biochemical approaches to characterize the enzymatic activity and substrate specificity of these lipases. Primary amino acid sequence analysis revealed minimal sequence similarity between the two proteins; AlphaFold-predicted 3D protein structures suggested distinct structural differences. Taken together, these data suggest that BB0562 and BB0646 may contribute different functions to fatty acid acquisition. Furthermore, a *Bb* mutant lacking both *bb0562* and *bb0646* was found to be significantly attenuated for growth even in medium rich in fatty acids, suggesting that these lipases may be potential targets for LD treatment. In support of this notion, recombinant BB0562 and BB0646 proteins were found to be sensitive to inhibition by FDA-approved lipase inhibitors such as Orlistat. Future work is aimed at further characterizing the activities of BB0562 and BB0646, and exploring how lipase inhibition affects *B. burgdorferi* survival and infection of the mammalian host.

Why be virulent? Exploring the evolutionary advantages of Shiga toxin production in *Escherichia coli*

P. Panos, T. Gil-Gil, B. Berryhill, B. Levin
Department of Biology, Emory University, Atlanta, GA, USA

Microorganisms that colonize eukaryotic hosts are dependent on the host survival for their existence. Nevertheless, many colonizing bacteria produce toxins that increase the morbidity and mortality of their hosts. What is then the evolutionary advantage of producing host damaging toxins? In this study, we address this question with Shiga toxin-producing *Escherichia coli*. Such pathogenic *E. coli* cause an estimated 265,000 cases of illness annually in the United States. We postulate that Shiga toxin production provides an advantage to the bacteria during infection by promoting colonization and/or bacterial survival. Using a Shiga toxin-producing strain of *E. coli* and an isogenic Shiga toxin knockout mutant we compare the effect of Shiga toxin production on the relative fitness of these two strains of bacteria *in vitro*. These experiments have shown that there is no relative fitness advantage to the Shiga toxin-producing strain when co-cultured at the same initial density. Additionally, neither strain increased in frequency when introduced to a population dominated by the other strain. We next performed parallel competition experiments using an *in vivo* infection model, the larvae of the wax moth *Galleria mellonella*. These experiments are still underway. By the time of the conference, we should have definitive results as to whether the Shiga toxin producing *E. coli* generate a higher morbidity and mortality than the Shiga toxin knockout, and if the production of this toxin provides a selective advantage to *E. coli*.

#28

Studying *Chlamydia* secreted effectors using *Drosophila melanogaster* as an in vivo cell biology platform

L. Shelton and G.F. Aranjuez, PhD
Immunity and Pathogenesis Division, Burnett School of Biomedical Sciences
College of Medicine
University of Central Florida
Orlando, FL 32827 USA

Chlamydia trachomatis (*Ct*) is an obligate intracellular bacterial pathogen and the leading STI in the United States. Most prevalent among 15-24 year olds, the CDC has estimated that approximately 4 million people in the U.S. are infected with *Chlamydia* every year, with the number of reported infections surpassing 1.6 million in 2022. *Ct* uses a wide array of secreted protein effectors to promote host cell entry, establish a replicative vacuolar niche called an inclusion, and release via cell lysis. The secreted effectors have many functions including disarming the host cell defenses and preventing early apoptosis. The *Ct* effector CpoS is found on the inclusion membrane and has been shown to play a role in preventing premature host cell death. Additionally, CpoS binds to the host protein Rab35, a small GTPase involved in vesicle trafficking. How the CpoS-Rab35 interaction leads to a block in host cell apoptosis has not been determined. To address this gap, we used *Drosophila melanogaster* as an in vivo cell biology platform to study CpoS-Rab35 interaction in vivo. Transgenic expression of CpoS in the whole fly results in lethality. Restricted CpoS expression in the *Drosophila* wing circumvents lethality and results in reduced wing dimensions. Interestingly, the reduced wing dimensions were also observed when expressing Rab35 S22N “on” mutant protein, but not when overexpressing WT Rab35 or expressing Rab35 Q67L “off” mutant protein. Because these phenotypes look alike, we hypothesize that Cpos shifts Rab35 to an “on” state. We plan to test this hypothesis by performing genetic interaction experiments and assessing downstream effects. This project also highlights the utility of using *Drosophila* as an in vivo model to study effector-host interactions.

T3SS secreted effector of the intracellular bacteria Chlamydia intersects with the host Hippo pathway

O. Patel, D. Patel, T.J. Jewett, and G.F. Aranjuez
Immunity and Pathogenesis, College of Medicine, University of Central Florida, Orlando, FL, USA

Chlamydia trachomatis is an obligate intracellular bacterial pathogen and the leading bacterial STI in the United States. Upon entry into the host cell, Chlamydia establishes a parasitophorous vacuole called an inclusion which serves as a replicative niche during its development. The last stage of infection involves host cell lysis and the release of new Chlamydia infectious particles. At every stage of development, Chlamydia manipulates the host cell biology using various secreted bacterial effectors. The early effector Tarp is important for Chlamydia entry through its ability to polymerize and bundle F-actin, mediated by its well-characterized C-terminus. In contrast, not much is known about the function of Tarp's N-terminus (N-Tarp), despite its evolutionary conservation across all Chlamydia species. To address this, we utilized Drosophila as an in vivo cell biology platform to study N-Tarp-host interactions. Drosophila development is well-characterized such that developmental phenotypes can be traced back to the perturbed molecular pathway. Initial characterization revealed that N-Tarp expression in the dorsal thorax results in abnormal duplication of mechanosensory bristles, reminiscent of altered host Hippo signaling. The Salvador-Warts-Hippo pathway is a conserved signaling cascade that regulates host cell proliferation and survival during normal animal development and in some forms of cancer. To explore this further, we studied N-Tarp function in larval imaginal wing discs, which are sensitive to perturbations in Hippo signaling. N-Tarp causes wing disc overgrowth and a concomitant increase in adult wing size, phenocopying overexpression of the co-activator Yorkie. Using transgenic reporters, N-Tarp also causes upregulation of Hippo target genes. Last, N-Tarp-induced phenotypes can be rescued by reducing the levels of Yorkie, or the Hippo target genes cycE and Diap1. Thus, we provide the first evidence that the N-terminal region of the Chlamydia effector Tarp intersects with the host Hippo signaling pathway. Chlamydia-infected cells have long been observed as highly resistant to extrinsic and intrinsic apoptosis cues. Chlamydia delays host cell apoptosis for the duration of its developmental cycle, though the exact mechanism remains unknown. Our findings implicate the N-terminal region of Chlamydia effector Tarp as a means to manipulate the host Hippo signaling pathway, which directly influences cell survival.

#30

Examining the paradoxical relationship between host inflammation and *S. pyogenes* infections

S. Guerra[1] and C. LaRock[1]

[1]Department of Microbiology and Immunology, Emory University, Atlanta, GA

Streptococcus pyogenes infections are a major burden to the healthcare system in the United States and globally. While most infections are mild and superficial, fatal consequences arise if these infections become invasive and infiltrate epithelial barriers, leading to severe necrotizing fasciitis. Antibiotic treatment can be compromised during necrotizing fasciitis due to inflammation, cell death, and tissue destruction, which impairs access of antibiotics at the site of infection. Therefore, these invasive infections often require surgical interventions to avoid the rapid spread of infection. Unlike most pathogens, *S. pyogenes* thrives in inflammatory environments by altering virulence factor expression through the two-component system CovRS. CovRS detects the host defense peptide LL-37, released during skin cell inflammation, as a potent signal for virulence factor regulation. One major factor regulated by CovRS is the streptococcal exotoxin called SpeB, which promotes invasion of deeper tissues. Intracellularly, SpeB cleaves the host pore-forming protein Gasdermin A (GSDMA) to trigger inflammatory cell death in skin cells. The goal of this project was to examine how GSDMA-mediated cell death and LL-37 release can modify CovRS toxin regulation. We modeled this interaction *in vitro* by infecting human skin keratinocytes (NHEK) with *S. pyogenes*. Quantitative reverse transcription (RT-qPCR) was used to measure changes in expression of SpeB, Gasdermin A, LL-37, and other inflammatory regulators. In conjunction, cell death was measured by Lactic Dehydrogenase release Assay (LDH) to detect changes in cytotoxicity. Lastly, the impact of LL-37 on virulence factor expression was evaluated directly using fluorescent reporters. Our results demonstrate that increasing GSDMA expression sensitizes NHEK to cell death and LL-37 release, which can be detected by the bacteria through CovRS, altering bacterial virulence gene regulation. These data suggest that *S. pyogenes* induces inflammation and cell death, which can promote further pathogenesis.

#31

Exploring the spatial structure and transcriptome of *Pseudomonas aeruginosa* aggregates tolerant to host factors

O.M. Williams[1], S.E. Darch[1]

[1]Department of Molecular Medicine, University of South Florida, Tampa, FL, USA

Pseudomonas aeruginosa (Pa) is an opportunistic pathogen that colonizes the airways of the lungs contributing to inflammation, tissue damage, and chronic infection in people with cystic fibrosis (CF). *Pa* can organize itself into aggregates consisting of 10-1,000 cells. When in aggregate formation *Pa* exhibits increased resistance to antibiotics and higher tolerance to host immune cells. Polymorphonuclear leukocytes (PMNs) are one such immune cell that is often observed at high levels in the airways during chronic CF infections. PMNs act as a first line of defense for the immune system, working to clear pathogens through classical phagocytosis or neutrophil extracellular trap (NET) deployment. Both forms of PMN-mediated bacterial killing utilize the serine protease neutrophil elastase to inflict damage to the bacterial cell. However, the effects of elastase on aggregate spatial organization and regulation of aggregate specific genes remain to be determined. We hypothesize that elastase will influence *Pa* aggregates spatial structure and differentially express a unique subset of genes compared to planktonic cells. To understand how elastase impacts aggregate organization and aggregate specific gene regulation *Pa* grown in a synthetic sputum media with the addition of mucin (SCFM2) was incubated with human neutrophil elastase (HNE) and imaged using confocal laser scanning microscopy (CLSM) and followed by RNA sequencing (RNAseq). We observed a significant inverse relationship between concentration of HNE added to cultures and aggregate volume over time. This trend suggests that while HNE can contribute to a reduction in aggregate volume, cells are still metabolically active despite an immune challenge. Additionally, we have determined a subset of genes that are differentially expressed when *Pa* aggregates are in the presence of HNE. Unique trends of gene expression that directly correlate to concentration of HNE present in the culture were also observed. Understanding the spatial requirements and genetic regulation of aggregates when in the presence of host immune products will provide valuable insight into *Pa* pathogenesis. Furthermore, determining the contribution of immune products in shaping aggregate persistence will contribute to understanding multiple aspects of the host immune response seen in the airways of individuals with chronic *Pa* infections.

Clinical/Diagnostic Microbiology

#32

Title: Phenotypic Characterization of *Staphylococcus aureus* Strains from Carriage and Disease among Pediatric Populations

Authors: Senthilkumar S.[1], Johnston C.[1], Cadilla A.[2], Azarian T.[1]

Affiliations: [1]*Univ. of Central Florida, Orlando, FL;* [2]*Nemours Children's Health, Florida, Orlando, FL*

Abstract:

Staphylococcus aureus (SA) is a gram-positive bacterial pathogen that is associated with invasive and non-invasive infections in humans and is a leading cause of healthcare associated infections. SA is carried asymptomatically in approximately 20-30% of the populations with the anterior nares the most common sight of colonization. The bacteria often cause skin and soft tissue infections as well as more severe infections such as osteomyelitis, endocarditis, and bacteremia. SA strains are diverse, possessing a variety of virulence and antibiotic resistance determinants that make them more likely to cause disease and difficult to treat. In hospital studies, β-hemolysis and biofilm formation are phenotypic properties that present a viable way to characterize the virulence of encapsulated SA strains. Here, we aimed to determine whether phenotypic characterization of SA strains from carriage and disease among pediatric populations differ between pre- and post- COVID-19 pandemic periods. To this end, we obtained historical SA isolates collected from carriage and disease among patients seeking care at a children's hospital in Orlando, FL from November 2019 to April 2023 as part of an ongoing biobanking project. SA was isolated using BBL CHROMagar selective media and confirmed using BBL Staphyloslide Latex Test. One SA isolate from each positive participant was selected for phenotypic testing. Hemolysis and crystal violet assays were performed to detect clear hemolysis, polysaccharide, and biofilm presence.

#33

Evaluating the origin of *Salmonella* within commercial broiler live-production and how prevalence and load shift temporally

E.E. Cason and N.W. Shariat

University of Georgia, Department of Population Health

With up to 93% of broiler houses being *Salmonella* positive, understanding how this pathogen persists and changes temporally in poultry live-production is critical to improve food safety. The objective of this study was to evaluate how *Salmonella* prevalence and load change over time in commercial broiler houses. Eight houses from four farms belonging to two broiler complexes were sampled using swabs of chickboxes, pooled fecal grabs, bootsocks, and bootsock-rollers at 0, 3, 8, 15, 22, 29, 36, 43, 50, and 57 days post-placement. Samples were evaluated by culture and by molecular-based detection and *Salmonella* positive samples were quantified using a commercially available kit. All incoming chickboxes were *Salmonella* culture positive (n=8), while bootsocks and bootsock-rollers taken pre-placement showed that a combined 63% and 50% houses were positive by culture and qPCR, respectively. Following chick placement, all houses were positive for *Salmonella* during all time points. A Chi^2 McNemar's test was used to compare qPCR and culture prevalence within sampling methods. In overall bootsock (p=0.020), bootsock-roller (p=0.002), and fecal grab (p=0.046) prevalence results, qPCR was found to outperform culture-based *Salmonella* detection. In this study, fecal grab prevalence was highest by both qPCR and culture, compared to bootsocks and bootsock-rollers. Quantification values ranged from 31.8-50.5 Ct, with lower values indicating higher *Salmonella* loads. *Salmonella* load peaked on day 29, when the average overall Ct values of bootsocks, bootsock-rollers, and fecal grabs were 40.0, 40.1, and 40.6, respectively. *Salmonella* prevalence remailed high and load decreased overall as flocks approached market-age. Ultimately, we found that *Salmonella* shedding in commercial broiler production varied with flock age and between detection method. On day 29, *Salmonella* prevalence and load peaked in 5/8 and 4/8 houses, respectively, suggesting this could be the optimal time for sample collection to determine the *Salmonella* status of a flock. A future goal is to use this data to evaluate how *Salmonella* status in live-production compares to that at processing. Further, improved understanding of *Salmonella* during production can guide the application of targeted interventions and determine when the most useful surveillance samples should be collected.

#34

Pass Me the Olive Oil, Fever and Rash in an Immunocompromised Host.

Authors

K. Amin[1], Y. Pasikhova[2], J. Messina[3], J.H. Katzman[1]

Affiliations

University of South Florida, Department of Infectious Disease, Tampa Fl, USA [1]

Moffit Cancer center, Department of Pharmacy, Tampa Fl, USA[2]

Moffit Cancer Center, Department of Pathology, Tampa Fl, USA[3]

Abstract:

We present a case of 43 y/o male with myelofibrosis s/p matched related donor allogeneic hematopoietic stem cell transplant 3 weeks prior presented due to fever along with night sweats, fatigue, weakness, rhinorrhea and new onset back rash x 1 day. The patient was staying locally after his recent discharge from the hospital after the transplant. The patient was hemodynamically stable however had a temperature of 38.2°C. Skin exam revealed many scattered scaly hyperpigmented macules without tenderness or swelling. Piperacillin-tazobactam was started. Complete blood count showed pancytopenia with a white blood cell count of 1.04 k/uL, an absolute neutrophil count of 0.91 k/uL, hemoglobin 6.9 g/dL, and platelet count of 23 k/uL. The chemistry panel was unremarkable. The patient's back rash was scraped and sent for a potassium hydroxide (KOH) fungal smear, and the initial smear returned positive for yeast. At the same time, the microbiology lab was alerted to add olive oil for growth due to suspicion of tinea versicolor. There was no evident growth for two weeks. Given the patient's immunocompromised state, he underwent a skin biopsy on day 3, which showed numerous fungal spores and hyphae in the stratum corneum consistent with tinea versicolor. The patient was started on topical selenium sulfide 2.5% and resolved after 2 treatments. The remainder of his infectious workup was negative, and his symptoms abated after 1 week of amoxicillin-clavulanate. The patient was on micafungin prophylaxis during the neutropenia period, where *Malassezia* species are known to be resistant. Also, there was no indication of continuing antifungal prophylaxis post-engraftment. There was a delay in the growth of *Malassezia* in the microbiology lab, as the organism is known to be fastidious. Empiric treatment with a topical ketoconazole 2% shampoo or selenium sulfide is indicated for limited disease without needing culture or skin biopsy unless other dermatoses are in the differential. The differential diagnosis of a rash in an immunocompromised patient is broad. Common illnesses may have different presentations among immunocompromised patients and can sometimes be severe. The net state of immunosuppression and the context of the rash are essential in deciphering the etiology.

Genomics and other Omics

#35

Developing a molecular diagnostic assay for *Campylobacter jejuni* and *Campylobacter coli*

C.G. Norris[1,2], A.T. Siceloff[2], and N.W. Shariat[2]

[1]Department of Epidemiology and Biostatistics, College of Public Health, University of Georgia, Athens, Georgia, USA. [2]Department of Population Health, College of Veterinary Medicine, University of Georgia, Athens, Georgia, USA.

Campylobacter ssp. are leading causes of bacterial gastroenteritis, with *C. jejuni* and *C. coli* causing the most human cases. With regulatory agencies adopting whole genome sequencing as part of regular bacterial foodborne pathogen screening, a large collection of bacterial sequence data is now publicly available in online repositories such as Pathogen Detection on NCBI, which contains >124,000 *Campylobacter* genomes. We aimed to use this database to develop a qPCR assay to differentiate between *C. jejuni* and *C. coli,* which would serve as a diagnostic tool to inform management strategies and improve public health. We curated a dataset of 1,854 *Campylobacter* genomes (Subset A) by using the software Phylogenetic Diversity Analyzer (PDA) to identify representative assemblies from SNP clusters within the NCBI database. We further filtered Subset A using PDA to form Subset B to reduce the computational power required for more complex analyses. Subset B contains 417 *Campylobacter* genomes, of which 236 are *C. jejuni* and 181 are *C. coli*. Initial gene targets for this assay were identified using a pairwise alignment of the reference strains of each species from NCBI (*C. jejuni subsp. jejuni* NCTC 11168 and *C. coli* FDAARGOS_735) using Mauve. Genes with defined protein identities that were found within one species but not another were identified as potential candidates. A local alignment search of these genes, using BLAST, was conducted with the genomes in Subset B to determine the allelic frequencies in each species. Additional gene targets were identified using a pan-genome analysis of Subset B using Roary, based on presence in >90% of the genomes of one species and absent in 100% of the other species. Primer and probe sets were made using the PrimerQuest tool from Integrated DNA Technologies. These assays were tested *in silico* with genomes from Subset A. Future directions will include testing these molecular assays on 50 *Campylobacter* isolates, with the goal of rapidly being able to identify *Caampylobacter*-positive samples.

#36

Identification of the BeAn 58058 Virus in Tuberculosis Patient Microbiomes

K. MacDonald[1], D. McGrath[2], and T.-T. Tseng[1]

[1]Department of Molecular and Cellular Biology, Kennesaw State University, Kennesaw, GA USA

[2]Center for Disease Control and Prevention, Atlanta, GA USA

Next-generation sequencing (NGS) has advanced our understanding of microbiomes, particularly in patient microbiomes. The goal of this study is to elucidate viral compositions from tuberculosis patients to further understand related microbiomes. Anonymized patient data was retrieved from Xu *et al*., 2022 at Tianjin Medical University General Hospital in China, suspected or diagnosed with pulmonary tuberculosis from 2018 through 2021. Bronchoalveolar lavage fluid (BALF) samples were subjected to NGS using the Illumina NextSeq 550 platform and are available as PRJNA847681 in NCBI BioProject. Sequencing reads were trimmed and underwent quality control with fastp. Kraken2 with the “viral” database was then used to identify viral members within this data set. Samples from the above data set contain viral sequences occupying 0.2% to 0.7% for the entire metagenome. Most surprisingly, sequences from the BeAn 58058 virus can contribute up to 90% within the individual viromes. On average, 69.8% of sequences within these viromes were contributed by the BeAn 58058 virus. Sequences from *M. tuberculosis* occupied 33.5% of all bacterial DNA in severe cases of TB, where BeAn 58058 accounted for an average of 70.75% among all viral genetic material. The BeAn 58058 virus was first isolated from *Ozyzomys* sp. rodents in the Amazon region of Brazil. It is an unclassified *Oryzopoxvirus* according to NCBI Taxonomy within the *Poxviridae* family. As a zoonotic orthopoxvirus, the BeAn 58058 virus can infect a wide range of hosts, both wild and domestic animals, as well as humans. This virus has been found in patients of chronic obstructive pulmonary disease (COPD) from Tshwane, South Africa. COVID-19 patients in the Campania region of Italy in 2020 were also found to be infected with BeAn 58058. Our study is among the first to report the presence of BeAn 58058 in lung microbiomes from TB patients. Our finding potentially implies that some patients with TB may also be infected with BeAn 58058. These findings highlight the need for additional research towards the role of BeAn 58058 within the tuberculosis microbiome.

#37

Characterization of *Salmonella* complex populations by Nanopore amplicon sequencing on poultry farms and carcass samples.

D. Ayala-Velastegui, A.T. Siceloff, N.W. Shariat
Department of Population Health, University of Georgia, Athens, GA, 30602.

Abstract

Salmonella is a leading bacterial foodborne pathogen in the United States, with an estimated 1.35 million human illnesses annually. *Salmonella* is composed of 2600 serovars, but only a small subset of these have been associated with significant human illness. Therefore, it is necessary to detect and differentiate the serovars of concern to better understand the risk of salmonellosis from food products and the environment. Previous research has identified that *Salmonella* can exist in complex populations of mixed serovars, and traditional methods miss this complexity by picking too few colonies. Thus, molecular methods that capture multiple serovars at once have improved our understanding of *Salmonella* ecology in different animals and environments. The objective of this project was to develop a new molecular approach to profile multiserovar populations that is quicker than earlier approaches. A previous *in silico* experiment identified two *Salmonella* virulence genes whose sequences can be interrogated to distinguish between different *Salmonella* serovars. A database was built from 35 different serovars (641 genomes), including those most commonly found to cause human illness, plus serovars most often associated with food animal production. Here, Oxford Nanopore sequencing was performed on PCR amplicons of these genes and multiserovar populations were analyzed. Field samples obtained from different poultry sources (farm, processing plants) were used. These samples had previously been characterized using CRISPR-SeroSeq, which relies on Illumina sequencing of native CRISPR amplicons. Both genes were amplified, sequenced and the raw reads were trimmed. To overcome limitations due to high error rates with Nanopore sequencing, a kmer approach was used to identify SNPs that are associated with different serovars. Kmer counts were used to determine the relative frequency of different serovars within each sample. The data was comparable to the profiles identified by CRISPR-SeroSeq, with the exception that the order of resolution was less (1% versus 0.5%). This experiment shows that this new approach can be used to accurately call multiserovar populations. It is a faster technique that can be implemented in different fields for routine screening of *Salmonella* complex populations.

#38

Metagenome Assembled Genome of a Novel Phage from a Small-Scale White Cheese Processing Facility

S. Smith[1], and T.-T. Tseng[2]

[1]Department of Chemistry and Biochemistry
[2]Department of Molecular and Cellular Biology

Kennesaw State University, Kennesaw, GA USA

Next-generation sequencing (NGS) has been providing new opportunities in finding new phages from metagenomic data sets. The surface metagenome from a small-scale white cheese processing facility was originally sequenced by FoodOmics Laboratory at Hacettepe University using Illumina NovaSeq 6000 and deposited in NCBI Sequence Read Archive (SRA) under the accession number of PRJNA951084. One specific run from the above experiment, SRR24234328, was downloaded from NCBI SRA and subjected to the following data mining protocol with the goal of identifying new phages. First, Metaviralspades was used for *de novo* assembly of sequencing reads after adapter trimming with fastp to generate contigs and scaffolds, which may contain metagenome assembled genomes (MAGs) for phages. Contigs and scaffolds were then subjected to identification of mobile genetic elements, including plasmids and viruses, by geNomad. GeNomad was also utilized for taxonomic assignments of viral genomes. One scaffold thus generated contained 43,851 nucleotides in length and a G + C content of 52%. Metaviralspades also reported a coverage of 1,256.523580 for this contig. Prediction with geNomad revealed this scaffold as a member of the *Autographiviridae* family in the class of *Caudoviricetes*. Similarity search with BLASTN against nt_viruses revealed 90.41% identity to *Enterobacter* phage SDFMU_Pec as the most related entry, which is also a member of *Autographiviridae*. Among 74 coding sequences (CDSs) predicted by phold, the most noteworthy categories include: nucleotide metabolism with 12 CDSs, lysis with three CDSs, tail with four CDSs and phage head/packaging with nine ORFs, as well as a large terminase subunit of 616 amino acids. CheckV reported that the assembly is at a completeness of 100% with the presence of direct terminal repeats (DTR). CheckV also reported that the minimum information about an uncultivated virus genome (MIUVIG) quality is “High-Quality.” This MAG is therefore considered as a complete phage genome. In conclusion, this study suggests the discovery of a novel phage, which potentially infects the genus of *Enterobacter*, from the surface microbiome in a small-scale white cheese processing facility.

#39

Phosphoregulation of GpsB in Staphylococcus aureus

L. McKnight, A. King, J. Carroll, B. Boyer, L. Hammond and P. Eswara
USF MBS

GpsB is a highly conserved, Firmicutes-specific cell wall synthesis protein. Our lab has demonstrated that GpsB plays an important role in the cell cycle of *S. aureus*, including directly regulating FtsZ polymerization and interacting with many divisome proteins. Yet, little is known regarding the regulatory mechanisms behind GpsB. Ser/Thr kinases are found to phosphorylate GpsB in a multitude of species, including *S. aureus* GpsB. Phosphoproteomic analysis identified 6 serine/threonine residues that are phosphorylated in *S.aureus* GpsB. In this study, using *B. subtilis* as a genetic tool, we can investigate the importance of GpsB phosphorylation. To investigate this, we generated phosphomimetic and phosphoablative mutants of GpsB by mutating several of the identified residues to glu or ala respectively. In previous studies, we demonstrated that overexpression of *S. aureus* GpsB is toxic to *B. subtilis* cells. We utilized this observation to probe the functionality of phosphomutants, and observed that phosphomimetic mutants, but not phosphoablative mutants, rendered GpsB non-toxic. This likely indicates that the phosphomimetic mutants render GpsB non-functional. This indicates GpsB is functional in an unphosphorylated state and ser/thr kinase mediated phosphorylation likely turns off the function of GpsB. To further examine this outcome, we generated individual point mutants of all 6 phosphoresidues to discern which site could be chiefly responsible for this phenotype, and therefore play a major role in GpsB regulation. Ongoing studies in the lab further investigate the impact of GpsB phosphomutants directly in *S. aureus*. Overall, we aim to uncover the significance of GpsB phosphorylation and illuminate its molecular role in cell cycle regulation in *S. aureus* and other organisms.

#40

Identification of a Novel Jumbo Phage from Cacao Fermentation

D. McGrath[1], K. MacDonald[2], S. Smith[2], A. DeSantis[2], and T.-T. Tseng[2]
[1]Center for Disease Control and Prevent, Atlanta, GA USA
[2]Department of Molecular and Cellular Biology, Kennesaw State University, Kennesaw, GA USA

The fermentation of cocoa beans is a spontaneous process and critical as the starting material for chocolate production. Lima *et al*. in 2022 generated raw sequencing data with specific time points for this fermentation process and was deposited under BioProject PRJNA552479. SRR9640353 from PRJNA552479 was retrieved at NCBI Sequence Read Archive (SRA), while representing the microbiome of cocoa bean fermentation after 48 hours. After quality control and adapter trimming by fastp, metaviraspades was used for *de novo* assembly of metagenome-assembled genomes (MAGs). One generated contig was predicted as a circular viral genome by geNomad with 362,172 nucleotides, which satisfies the definition of a jumbo phage. According to CheckV, the viral genome represented by this contig is 100% complete and assembly is high-quality. This contig was then subjected to open-reading frame (ORF) prediction by PHANOTATE and annotation with prodigal as implemented by pharokka. Among a total of 635 predicted ORFs, 518 ORFs were assigned as “unknown function”. Other predicted ORFs were assigned into categories of “connector” with two ORFs, “DNA, RNA and nucleotide metabolism” with 41 ORFs, “head and packaging” with 22 ORFs, “lysis” with two ORFs, “host takeover” with nine ORFs, “tail” with 12 ORFs, “transcription regulation” with one ORF, “tRNAs” with two ORFs, and “other” with 28 ORFs. A tRNA-His guanylyltransferase, which carries out 3′-5′ addition of nucleotides to nucleic acid substrates and commonly found in jumbo phages, was also predicted within this genome. Four ORFs were predicted as “terminase large subunit,” with three showing similarities to potential homologs from *Agrobacterium* phage Atu_ph07, which is a jumbo phage. One tyrosyl-tRNA synthetase, which has also been found in genomes of several jumbo phages, was predicted by PHANOTATE. Jumbo phages have recently gained attention because of the ability to be optimized into a type of personalized therapy against specific pathogens. Our study is among the first to report the presence of a jumbo phage in the cocoa bean microbiome. These findings highlight the need for additional research towards the role of jumbo phages in complex microbiomes during fermentation processes.

#41

Potential of Universal Microbial Dynamics in Socially Isolated Male Prairie Voles

Mackey, C.S PhDc[1], Chun E PhD[2], Brown, A.N. PhD[3], Washburn, B.K. PhD[3], Liu Y PhD[4], Wang Z PhD[4], Jones K.M PhD[1]

1. Florida State University, Department of Biological Sciences, Tallahassee Fl, USA
2. Boston College, Morrissey College of Arts and Sciences, Boston MA, USA
3. Florida State University, Department of Biological Science Core Facilities, Tallahassee Fl, USA

Florida State University, Department of Psychology, Tallahassee Fl, USA

Statement of Purpose: This study aims to investigate the potential effectiveness of both re-socialization of isolated animals as well as the effectiveness of coprophagic behavior as intervention methods for prolonged social isolation stress in a prairie vole model.

Methods: Male voles, who had been pre-acclimated with a cage mate were subject to an 8-week intervention study where they began as either socially isolated or kept in a cohoused environment for an initial period of four weeks. After these initial four weeks, animals subject to isolation were then either returned to a cohoused environment with their previous cage mate, maintained in isolation while being provided fresh stool samples from a non-experimental isolated animal, or maintained in isolation with no further manipulations. Collected stool samples were prepared in triplicate for 16s amplicon sequencing of the V3-V4 region and denoised using the DADA2 pipeline in Qiime2. Resulting microbiome data at the Genus level was analyzed for network associations initially using the SpiecEasi and SPRING R packages and then through NetCoMi using as similar parameters as possible to obtain network comparison and differential association analytics.

Results: Initial analysis of network associations with SpiecEasi demonstrated evidence that the intervention methods for the social isolation stress used in this study had an effect in alleviating the effects of social isolation stress on the gut microbial compositions of the male prairie voles. However, key taxa that were highlighted as important for the networks were displayed sparse, low abundance pattern in the initial raw count data bringing results into question. Follow up analysis using SPRING, which handles sparsity more directly than SpiecEasi, did demonstrate a similar, though less pronounced trend to the initial results where general density and connectivity metrics were more similar between the cohoused control and treatment groups and although the treatment groups still displayed significant similarity differences, comparative metrics from NetCoMi provided evidence that treatment had an effect on the presence of key microbes in the functional network such as the Clostridium methylpentosum group as well as had an impact on key associations between microbes in the networks such as with the Parasutterella genus which is known to have potentially clinically relevant impacts on microbially derived gut metabolites.

Conclusions: Preliminary analysis of the network associations not only provides evidence of the efficacy of the intervention treatments in either changing or buffering changes to the functional associations as well as provided some potential exploratory pathways for further metabolic analysis.

#42

Impact of the 919 Syrup on the Intestinal Microbiome of Postpartum Stressed Female Mice

P. Patel[1], B. Jabri[1], S. Nguyen[1], D. McGrath[2], and T.-T. Tseng[3]

[1]Ponce Health Sciences University, St. Louis, MO, USA
[2]Center for Disease Control and Prevention, Atlanta, GA, USA
[3]Kennesaw State University, Kennesaw, GA, USA

Postpartum depression (PPD) poses a significant mental health issue for many new mothers, impacting not only their well-being but also potentially influencing infant development. Although treatment options for this disease exist, the current slate of medications does not come without risks. While selective serotonin reuptake inhibitors (SSRIs) are commonly prescribed for PPD, concerns over side effects, such as autism for future offsprings and weight loss, prompted exploration of possible alternative treatments. This study investigated the effectiveness of a Chinese herbal medicine, 919 TJ syrup, in female mice shortly after giving birth. Traditional Chinese medicine, like 919 TJ, had in previous studies demonstrated benefits in mood improvement and other symptoms associated with PPD. Given the emerging research on the gut microbiome's role in mental health, this study explored a potential link between 919 TJ's influence on the gut flora. Raw sequencing reads were downloaded from the European Nucleotide Archive (ENA) with the accession number of PRJEB44528 for this study. Metagenomic analysis of fecal samples from mice treated with 919 TJ revealed shifts in microbial composition, suggesting a possible connection between specific microorganisms and PPD symptoms. Methodologically, fastp and Kraken2 were used for data preprocessing and taxonomic classification, with Bracken for abundance estimation, and R for data visualization. Results demonstrated that 919 TJ significantly alters the microbiome by influencing the abundance of *Bacteroides acidifaciens* and firmicutes. These findings indicate the potential of 919 TJ to impact gut flora composition greatly, consequentially alleviating PPD symptoms. 919 TJ opens the door for further research and possible medical treatment options for women suffering from PPD.

#43

Survey of Bacteriophages in the Tibetan Glaciers

A. DeSantis[1] and T.-T. Tseng[1]

[1]Department of Molecular and Cellular Biology, Kennesaw State University, Kennesaw, GA, USA

Analysis of the genetic variation found within the microbial communities of the Tibetan Plateau utilizing next generation sequencing (NGS) has increased our ability to understand the evolution of life in extreme environments. This study aims to identify novel viral genomes from a study by Liu *et al*. in 2022 with a BioProject number of PRJNA813429. The aforementioned study collected sequence data from three glacial habitats found in the Tibetan Plateau: cryoconite, snow, and ice, through the utilization of the Illumina HiSeq 2500 platform. In our workflow, quality control and adapter trimming of sequencing reads were first performed using fastp. This was then followed by *de novo* assembly with metaspades and metaviralspades. The most prevalent viral genomes identified from SRR18577021 within PRJNA813429 were bacteriophages falling under the class of *Caudoviricetes*. A contig from SRR18577021 was subjected to open-reading frame (ORF) prediction by PHANOTATE and annotation with prodigal as implemented by pharokka. A total of 32 ORFs were predicted, with five ORFs in the category of “head and packaging.” With a predicted large terminase subunit from the category of “head and packaging” as a query sequence, BLAST search revealed several potential homologs from sphingomonads. This phage therefore likely has the host specificity towards the genus of *Sphingomonas*. Its predicted tail length tape measure protein was found to be similar with members from *Caudoviricetes*. Our discovery potentially revealed a phage that existed over 10,000 years ago, along with the presence of sphingomonads in the Tibetan Plateau. With certain sphingomonads causing nosocomial infections, new phages targeting the genus of *Sphingomonas* may provide therapeutic applications. Other potential therapeutic agents may also be hidden in these glaciers as phages for combating infections. Our findings in this study therefore expanded the understanding of the microbial composition found within the Tibetan Plateau. Further analysis will likely reveal and allow for the identification of additional viral species and provide insight on any possible effects which may pose to future societies.

Evolutionary Dynamics

#44

“What Monod does not tell us: A study of bacteria and phage dynamics at differing glucose concentrations”

T.R. MacInnis*, K.E. Adams*, A.S. Ismail, T. Gil-Gil, B.A. Berryhill, B.R. Levin

EcLF Laboratory, Emory University, Department of Biology, Atlanta, United States of America

In the early 1940s, Jacques Monod explored the relationship between the rate of growth of *Escherichia coli* and the concentration of limiting resources. He developed a mathematical model to describe how bacterial growth rates increase with resource concentration. Not considered by Monod or others is the relationship between the rate of replication of bacteriophages (phages) and the concentration of the resource available for the bacteria. Using *in vitro* experiments with *Escherichia coli* B/6, the phage T4, and mathematical computer-simulation models, this study investigates the relationship between a limiting resource concentration (glucose) and the dynamics of bacteria and phage. Our results demonstrate that the lag time, the time before the bacteria start growing, was the same at all concentrations. Additionally, the exponential growth rate and stationary phase densities were proportional to the glucose concentrations. Interestingly, our results indicate that even in the absence of glucose, low densities of bacteria can grow. We postulate that in the absence – or low concentrations – of glucose, bacteria use stored glycogen to fuel their growth. We are currently examining the phage dynamics at different concentrations of and in the absence of glucose. Our preliminary results show that with varying concentrations of glucose, the rate of replication of the phage and the maximum densities of phage populations are independent of the glucose concentration. By the time of the conference, we will have a boarder picture of the relationship between the concentration of the limiting resource and the dynamics of replication of bacteria and phage. By understanding this relationship, our work will contribute to understanding the application of phage to treat bacterial infections as different locations of the body have varying resource concentrations.

#45

Parallel Species Evolution in Microbial Communities

C. Alvarez[1], M. Taylor[2,3], M. Duckett[1,4], C. Bowman[2], N. Vega[2]
[1]Emory University, Graduate Division of Biological and Biomedical Sciences, Population Biology Ecology and Evolution, Atlanta, USA
[2]Emory University, Department of Biology, Atlanta, USA
[3]Goldbelt C6, LLC, Chesapeake, USA
[4]Exploratorium, San Francisco, USA

Environmental context plays a critical role in evolution, often leading to distinct evolutionary trajectories. However, the selective pressures driving evolution, particularly within community contexts, vary widely and their effects remain unclear. In this work, we evolved communities composed of combinations of seven bacterial genera common to soil microbiomes alongside the model organism *Caenorhabditis elegans* (*C. elegans)*. During the course of experimental evolution, we observed the appearance of an alternate morphotype with a similar phenotype in two unrelated genera *Microbacterium* and *Chryseobacterium*. Here, we characterize and compare traits of our two alternate morphotypes in relation to each other and their original counterparts. Additionally, we show how the presence of *C. elegans* correlates to the appearance of alternate morphotypes in these bacterial communities.

Virulence and Vaccines

#46

Eosinophils Augment the Mucosal Immune Response to Infection and Vaccination

N. First[1], E. Abdollahi[1], K. Parrish[1], M. Woolard[1], J. McLachlan[2], and M.C. Gestal[1]
[1]Department of Microbiology and Immunology, Louisiana State University Health Sciences Center – Shreveport, Shreveport, LA, USA. [2]Department of Microbiology and Immunology, Tulane University School of Medicine, New Orleans, LA, USA.

Eosinophils, traditionally associated with asthma, allergies, and parasitic infections, have recently been recognized as critical players in bacterial infections. This study investigates their role in immune responses against *Bordetella* spp., respiratory pathogens adept at evading host defenses. Despite vaccination advancements, mucosal pathogen infections like Pertussis, caused by *Bordetella* pertussis, remain significant public health challenges, resulting in over 150,000 deaths annually, primarily among children and neonates. The resurgence of Pertussis, despite available vaccines, underscores the urgent need for improved vaccines that provide long-term immunity.

We previously identified a sigma factor, *btrS* that regulates *B. bronchiseptica* immunosuppressive pathways. A mutant strain, Δ*btrS*, leads to rapid bacterial clearance and long-term sterilizing immunity against all three classical *Bordetella* species, suggesting a potential pathway for enhancing immune responses through targeted genetic modifications.

When investigating immune responses to Δ*btrS*, we observed a significant increase in eosinophil numbers, along with a rapid and robust influx of B cells and T cells to the lungs. This was accompanied by an augmented Th1/Th17 cellular response, elevated levels of secretory and mucosal IgA, and the formation of bronchus-associated lymphoid tissue within the first seven days of infection. Eosinophils emerged as critical drivers of this response, raising important questions about their role in generating protective immune responses to vaccination.

Our results revealed that eosinophil-deficient mice fail to generate robust protective responses to vaccination with both the current acellular pertussis vaccine and the whole-cell vaccine. The lack of protection was particularly pronounced in the lungs and even more so in the nose. These findings offer promising insights for enhancing vaccine efficacy against *Bordetella* spp. and other respiratory pathogens. Given the resurgence of Pertussis and the limitations of existing vaccines, this research holds significant promise for improving long-term immunity and public health outcomes.

Title: Identification of Novel Vaccine Candidates Against Falciparum Malaria Using an Integrated Approach of in Silico and Wet Lab Analysis

List of Authors:
M. Marouf, J. Tallapalli, A.N. Rubidoux, M.V. Gupta, Y.H. Elgodamy, S. Kamojjala, S. Anand, A. R. Shakri, J. Badhai, A. Anand, A. Ramkallawan and D.K. Raj
Affiliations:
Division of Infectious Disease and International Medicine, Department of Internal Medicine, University of South Florida, Florida, USA
Department of Pathology and Laboratory Medicine, Warren Alpert Medical School, Brown University, Providence, RI, USA

ABSTRACT
Objective: Malaria is one of the most endemic diseases in modern history, with an estimated 249 million cases and 608 thousand deaths in 2022. Despite robust control measures like insecticide-treated bed nets and antimalarial drugs, malaria continues to disproportionately affect young children and pregnant women. Current research efforts are focused on developing new vaccines, with over 40% of identified vaccine candidates in the malaria parasite containing tandem repeats in their genes. This study aims to identify vaccine targets through in silico analysis of P. falciparum genes with tandem repeats, contributing to the broader goal of achieving a 90% reduction in malaria cases by 2030 as part of the WHO's global elimination strategy.
Methods: The P. falciparum genome was scanned for genes containing tandem repeats. These genes were further down-selected using in silico analysis for antigen prediction. Software tools were used to identify B-cell & T-cell epitopes, regions of hydrophilicity, low complexity sequence, MHC II binding epitopes, protein 3D structure, low genetic diversity, transmembrane domains, and signal peptides. From this analysis, 112 genes were selected for further analysis and 29 fragments were randomly selected from 22 gene , and 11 fragments were inserted into a DNA vaccine vector (pVR2001), immunized into mice. Polyclonal antibodies were harvested, and the efficacy of each antigen was evaluated using growth inhibition assays (GIAs). Immunofluorescence confocal microscopy identified the localization of antigen-antibody reaction.
Results: Among the 11 anti-sera tested, 2 were found to inhibit P. falciparum growth in culture by 75-98%. These 2 anti-sera significantly inhibited the parasite in GIAs and recognized proteins of the appropriate size from parasite protein extracts.
Conclusions: This investigation demonstrates that reverse vaccine identification techniques can successfully identify potential vaccine targets. Of the 11 antigens used to vaccinate mice, 2 showed potential for use as a vaccine against falciparum malaria. This study serves as proof-of-concept for a strategy that can be replicated with other distinct primary characteristics to identify viable blood-stage vaccine candidates.

#48

A Novel Multi-epitope Vaccine against *Clostridioides difficile* Surface & Spore Proteins

S. Chakraborty; A. Roy; X. Sun

Department of Molecular Medicine, Morsani College of Medicine, University of South Florida, Tampa, Florida, USA

Clostridioides difficile is a gram positive, spore-forming, obligatory anaerobic bacteria that causes diarrhea and colitis within infected individuals. One of the major problems with *C. difficile* infections (CDI) is the high rates of recurrences, which are mainly caused by persistent *C. difficile* colonization. Vaccination for prevention of CDI could be cost-effective over a range of C. difficile risk. However, there is no commercialized vaccine available. Various surface layer lipoproteins LP1 and LP2 [1], cell wall binding protein Cwp2 [2] and Cwp84 [3]; spore proteins CotA [4] were reported to be immunogenic and confer protectiveness against *C. difficile* infection. The goal of this project is to develop epitope-based vaccines targeting C. difficile colonization. A multi-epitope novel vaccine was generated by integrating potential epitopes from the *C. difficile* surface proteins by immunoinformatic approach. Both T cell and B cell epitopes were screened and 12 Cytotoxic T Lymphocytes (CTL) epitopes, 6 Helper T Lymphocytes (HTL) epitopes, and 11 B-Cell epitopes were finally selected. These epitopes were linked together with appropriate linkers to develop an antigenic and immunogenic chimeric fusion vaccine. Structural modelling and refinement helped to develop a stable structure of the chimeric protein. Molecular docking of the fusion protein with immune cell receptors ensured the interaction and binding stability to elicit immune response. Further studies will be conducted in-vivo to establish its efficacy in the host.

References:

1. Kovacs-Simon A., Leuzzi R., Kasendra M., Minton N., Titball R.W., Michell S.L.; Lipoprotein CD0873 is a novel adhesin of *Clostridium difficile*. J Infect Dis. 2014 Jul 15;210(2):274-84. doi: 10.1093/infdis/jiu070. Epub 2014 Jan 29. PMID: 24482399.
2. Wang S., Zhu D., Sun X.; Development of an Effective Nontoxigenic *Clostridioides difficile* - Based Oral Vaccine against C. difficile Infection. Microbiol Spectr. 2022 Jun 29;10(3): e0026322. doi: 10.1128/spectrum.00263-22. Epub 2022 May 18. PMID: 35583336; PMCID: PMC9241731.
3. Pechine S., Deneve C., Le Monnier A., Hoys S., Janoir C., Collignon A.; Immunization of hamsters against *Clostridium difficile* infection using the Cwp84 protease as an antigen. FEMS Immunol Med Microbiol. 2011 Oct;63(1):73-81. doi: 10.1111/j.1574-695X.2011.00832. x. Epub 2011 Jul 20. PMID: 21707776.
4. Permpoonpattana P., Phetcharaburanin J., Mikelsone A., Dembek M., Tan S., Brisson M.C., La Ragione R., Brisson A.R., Fairweather N., Hong H.A., Cutting S.M.; Functional characterization of *Clostridium difficile* spore coat proteins. J Bacteriol. 2013 Apr;195(7):1492-503. doi: 10.1128/JB.02104-12. Epub 2013 Jan 18. PMID: 23335421; PMCID: PMC3624542.

#49

A novel peptidoglycan hydrolase, plays pleiotropic roles in *Clostridioides difficile* R20291

D. Zhu, S. Wang, X. Sun *
Department of Molecular Medicine, Morsani College of Medicine, University of South Florida, Tampa, FL, USA.

Correspondence: sun5@usf.edu

Clostridioides difficile is a Gram-positive, spore-forming, toxin-producing anaerobe known to cause nosocomial antibiotic-associated intestinal disease. While the production of toxin A (TcdA) and toxin B (TcdB) is central to the pathogenesis of *C. difficile*, the mechanism of TcdA and TcdB release from cells remains unclear. In this study, we identified and characterized a novel cell wall hydrolase, Cwl0971 (CDR20291_0971), from *C. difficile* R20291, which plays a role in bacterial autolysis. Using CRISPR-AsCpfI, we generated a gene deletion mutant (R20291Δ0971), demonstrating significantly delayed cell autolysis and enhanced cell viability compared to R20291. Furthermore, purified Cwl0971 exhibited hydrolase activity against Bacillus subtilis cell walls. Notably, deletion of gene 0971 impaired TcdA and TcdB release due to reduced cell autolysis in the stationary/late phase of growth. Additionally, sporulation in the mutant strain was significantly reduced compared to the wild type. In vivo experiments using a mouse infection model revealed decreased fitness of the Cwl0971-deficient strain compared to the parent strain. Overall, Cwl0971 plays a crucial role in cell wall lysis, impacting cell viability, toxin release, sporulation, germination, and pathogenicity of R20291. These findings suggest that Cwl0971 could serve as a promising target for therapeutics and prophylactics against *C. difficile* infections.

Cwp2 protects mice against *Clostridioides difficile* infection

S. Wang [1], X. Sun [1]*

[1] Department of Molecular Medicine, Morsani College of Medicine, University of South Florida, Tampa, FL, USA.

Abstract

Clostridioides difficile (*C. difficile*) infection (CDI) is a disease that causing inflammation by *C. difficile* produced toxin. Another important factor that impact host infection and colonization is the protein array on the surface of *C. difficile.* Cell wall protein (Cwp) 2 was predicted to be good vaccine antigen. In this study, we use Cwp2 as vaccine antigen to protect against CDI in mice. Immunization of mice with Cwp2 induce potent IgG/A antibody responses against Cwp2. Anti-Cwp2 can protected mice against *C. difficile* infection and decrease *C. difficile* spores and toxin levels in the feces after infection. Then, we found that both anti-Cwp2 sera inhibited the binding of *C. difficile* vegetative cells to HCT8 cells. These results imply that Cwp2 protein may represent an effective vaccine candidate for the prevention from *C. difficile* infection (CDI).

#51

Title:

"Evaluation of an ABC transporter, PfABCB6, as a therapeutic target against falciparum malaria.

List of Authors:
A. Anand [1], H.E. Park [2], J. Badhai, A.R. Shakri [1] and D.K. Raj [1,2]
Affiliations:
[1]Division of Infectious Diseases and International Medicine, University of South Florida, Florida, USA
[2]Department of Pathology and Laboratory Medicine, Warren Alpert Medical School, Brown University, Providence, RI, USA

Abstract

"Malaria remains a persistent and significant threat, causing sickness and claiming lives in tropical and subtropical regions across the globe. The urgent need for new treatment approaches is underscored by the emergence of resistance to nearly all existing antimalarial drugs. The malaria parasite, *Plasmodium falciparum*, depends entirely on the host for nutrients as it resides within the host's red blood or liver cells."

Genes encoding transporters make up 2.5% of the parasite genome, underscoring their importance in parasite biology. However, the biological roles of these transporters remain largely unknown, with only a few of the transporter proteins involved being extensively studied. Our current research aims to characterize the PfABCB6 a member of the ABC transporter family in *P. falciparum* and investigate its involvement in multidrug resistance and blood stage development.

A transgenic parasite generated in our laboratory through targeted deletion of ABCB6 in *P. falciparum* exhibited growth impairment and showed resistance to artemisinin and quinine. We generated anti-PfABCB6 polyclonal antibodies using two recombinant fragments from the gene on a DNA vaccine platform and ten oligopeptide antigens in a murine model. The polyclonal antibodies generated against one of the DNA vaccine fragments (FII) and two oligopeptides (P1 & P3) show robust antiparasitic activity in in vito Growth inhibition assays (GIA). The GIA activity remains consistent across various strains (3D7, D10, W2, DD2, and Indo) of P. falciparum, indicating minimum stain variation impact on anti-*Pf*ABCB6 mediated parasite neutralization activity. Confocal microscopy and stage-specific Western blotting were performed using Anti-PfABCB6 generated against the DNA vaccine, revealing the protein localization and expression on the membrane of mature schizonts.

Our research suggests that antibodies targeting these transporters could be a game-changer, effectively hindering blood-stage development and opening up a new avenue of research for drug development by competition-binding assays using commercially available chemical libraries and anti-PfABCB6 antibodies. Furthermore, the potential of developing a multi-target blood-stage malaria vaccine using PfABCB6, combined with other known recombinant antigens fused into a single recombinant product, could be a significant advancement. In conclusion, our findings highlight the importance of ABCB6 as a target for antimalarial drugs and vaccine development against *falciparum* malaria.

#52

Title: Characterization of a novel vaccine candidate *against falciparum* malaria identified by an integrated approach of in silico and wet lab analysis

List of Authors:
J.R. Tallapalli[1], M. Marouf[1], A.N. Rubidoux[2], M.V. Gupta[2], A. R. Shakri[1], J. Badhai[1], A. Anand[1], A. Ramkallawan[2] and D.K. Raj[1,2].

Affiliations:
[1]Division of Infectious Disease and International Medicine, University of South Florida, Tampa, Fl, USA
[2]Department of Pathology and Laboratory Medicine, Warren Alpert Medical School, Brown University, Providence, RI, USA

Abstract:
Malaria is a leading cause of death in children living in areas where the disease is endemic, especially children between the ages of 6 months and five years. Earlier studies in unicellular eukaryotic parasites suggest that genes containing tandem repeat sequences have a significantly higher likelihood of possessing antigenic properties against the pathogen, similar to the malaria parasite.
As part of an ongoing project in our lab, we scanned the entire genome of Plasmodium falciparum to identify potential antigens for vaccine development against malaria, using tandem repeats as a primary tool. Our laboratory utilized XSTREAM software to identify genes with tandem repeats, which have proven high-accuracy predictions. Further downstream selection of genes involved analysing signal peptides, transmembrane domains, hydrophilicity, the complexity of amino acid sequences, SNPs, PEXEL regions, and gene function analysis.

We selected around 112 genes from the above analysis and randomly selected 29 fragments from 22 genes for further study in wet lab experiments. All the gene fragments were codon-optimized for expression in mouse models and cloned into DNA vaccine vector pVR2001 vectors. The initial results indicate an excellent potential of a gene with gene ID Pf3D7_0401900 as an anti-malarial vaccine. Western blot analysis using the in vitro cultured P. falciparum extract detects the relevant protein and confirms the protein expression in the parasite. Growth inhibition assays (GIAs) were performed in vitro with anti-sera produced from DNA-vaccinated mice against the Pf3D7_0401900 antigen, showing robust inhibition of various parasite strains. Immunofluorescent confocal microscopy shows the antigen's localization on the merozoites' surface. The antigen can be targeted by a vaccine during the egress and invasion of P. falciparum during blood stage development responsible for the disease in the human host.

Our research suggests that the Pf3D7_0401900 gene shows strong potential for vaccine development against the malaria parasite. However, our experimental data are still preliminary, so we must continue our research and conduct further studies to evaluate the antigen's suitability for vaccine development. This ongoing work is an important step forward in our mission to prevent malaria in humans.

#53

Title: Evaluation of Immunodominant Fragments of PfGARP for Vaccine Development Against Malaria

List of Authors:
J. Badhai [1], J. Jung [2], H.E. Park [2], Y. Elgodamy [2], A. Anand [1], A.R. Shakri [1] and D.K. Raj [1,2]
Affiliations:
[1]Division of Infectious Diseases and International Medicine, University of South Florida, Florida, USA
[2]Department of Pathology and Laboratory Medicine, Warren Alpert Medical School, Brown University, Providence, RI, USA

ABSTRACT
Malaria affects almost half the world's population and causes more than half a million deaths annually. Children with underdeveloped immune systems in malaria-endemic areas have the highest mortality rate, but no vaccine candidates are explicitly identified for this group. Global efforts to control the disease have had limited success, with no blood-stage vaccine being approved yet. Therefore, there is an urgent unmet need to discover new vaccine candidates or improve the efficacy of known antigens to develop a new generation of malaria vaccines for children.
Our previous studies discovered a promising vaccine candidate called PfGARP using phase display screening of malaria-resistant children's serum and a *P. falciparum* T7 Phage-based cDNA library. Our work led to the identification of Pf Glutamic Acid Rich Protein A (PfGARP-A) and publication of a comprehensive, full-length Research Article in Raj et al. ***Nature***, 2020. published in *Nature* by Raj et al. (2020) During the screening phase, we found antigens from the C-terminus regions of PfGARP, which overlapped with each other and only interacted with antibodies from the plasma of malaria-resistant children. These findings suggest that the lower molecular weight recombinant PfGARP (rPfGARP) antigens may contain the crucial domain for functional antibodies and could generate growth inhibition activity similar to PfGARP-A.
In our current approach, we immunized the mice (n=5/antigen) with DNA vaccine containing the smaller fragments of the immunodominant regions: PfGARP-B, PfGARP-C, PfGARP-D, and a 324 bp N-terminus fragment of PfGARP (PfGARP-E as negative control), and then harvested polyclonal serum. Our experimental data demonstrate that polyclonal serum generated against PfGARP-B and PfGARP-C in mice specifically interacts with *P. falciparum* in western blot, flow cytometry, Immunofluorescent microscopy, and shows significant parasite-killing activity in the growth inhibition assay (GIA) comparable to PfGARP-A.
In conclusion, the ongoing project holds significant promise. It is poised to provide core supporting data for the further development of the lower molecular weight PfGARP antigens. This advancement could reduce production costs and enhance compatibility with other blood-stage proteins as fusion antigens, thereby bringing us closer to the goal of an efficacious malaria vaccine for humans.

Immunization with PfGBP130 induces antibodies that block P. falciparum parasites from invading red blood cells.

List of Authors:
D.K. Raj [1,2], Y. Johnson [2], A.R. Shakri [1], S. Pond-Tor [2], A. Jnawali [2], T. Najrana [2], H. Wu [2], J. Badhai [1], M.G. Alameh [3], D.Weissman [3], E. Kabyemela [4], P. Duffy [5], M. Fried [5], J. Kurtis [2]

Affiliations:
[1] Division of Infectious Disease and International Medicine, University of South Florida, Florida, USA
[2] Center for International Health Research, Rhode Island Hospital, Warren Alpert Medical School, Brown University, Providence, RI, USA
[3] Department of Medicine, University of Pennsylvania, Philadelphia, PA, USA.
[4] Muhimbili University of Health and Allied Sciences, Dar es Salaam, Tanzania
[5] Laboratory of Malaria Immunology and Vaccinology, National Institute of Allergy and Infectious Diseases, National Institutes of Health, Rockville, MD, USA

ABSTRACT

Even after decades of effort, *Plasmodium falciparum* malaria continues to be a leading cause of death in children. Effective public health interventions are constrained by the absence of a very effective vaccine and the emergence of parasites that are resistant to frontline therapeutic medication. Current malaria vaccines, RTS, S, and R21, are only moderately effective pre-erythrocytic vaccines and do not confer sterile protection. This situation necessitates the development of innovative novel blood-stage vaccine candidates that will attenuate clinical disease and incorporate effective blood-stage components into the current pre-erythrocytic vaccines.

Our whole parasite proteome screening method led us to a promising discovery of novel vaccine candidates. In a parallel phase display screening, we identified PfGBP130 as a parasite protein that triggers a specific immune response in children resistant to *P. falciparum* infection. This protein, not recognized by antibodies from susceptible children, holds great potential as a novel blood-stage vaccine candidate. We formulated PfGBP130 as lipid-encapsulated mRNA, DNA plasmid, and recombinant protein-based immunogens. Stage-specific western blot and confocal microscopy using Anti-PfGBP130 indicate that the protein is localized to the RBC membrane of mature trophozoites/early schizonts and on the merozoite of mature schizonts up to egress. We evaluated the efficacy of murine polyclonal anti-PfGBP130 antisera to inhibit parasite growth in in vitro growth inhibition assay (GIA). Mice that were immunized with PfGBP130-A (amino acids 111–374), which was the region identified in our differential screen and was formulated as a DNA plasmid or a lipid-encapsulated mRNA but not as a recombinant protein, produced antibodies that hindered red blood cell invasion *in vitro*. Furthermore, mRNA that encoded the entire ectodomain of PfGBP130 (amino acids 89–824) also produced antibodies that inhibited parasite growth.

Our research is guided by the understanding that pre-erythrocytic vaccines do not provide complete protection, necessitating the inclusion of effective blood stage components. Our data demonstrating that anti-PfGBP130-A antibodies generated by lipid-encapsulated mRNA-based/DNA vaccination can attenuate parasite growth is a testament to the potential of our antigen/delivery platform.

#55

DEVELOPMENT OF NOVEL MULTI-EPITOPE FUSION VACCINE AGAINST *CLOSTRIDIOIDES DIFFICILE* INFECTION USING IMMUNOINFORMATIC APPROACH

Agbendeh, L.N.;[*1,2] Chakraborty, S.;[2] Tomatsidou, A.;[2] Roy, A;[2] Wang, S.;[2] Sun, X.[1,2]

1Department of Chemistry, College of Arts and Sciences, University of South Florida, Tampa, Florida, USA; [2]Department of Molecular Medicine, Morsani College of Medicine, University of South Florida, Tampa, Florida, USA

Clostridioides difficile infection (CDI) is the leading cause of pseudomembranous colitis – a nosocomial antibiotic associated diarrhea. One of the major problems with CDI is the high rates of recurrence which are primarily caused by the persistence of *C. difficile* spores in the intestine. Alternative approaches are crucial for preventing infections, treating patients, and avoiding recurrences, beyond traditional antibiotic treatments. Recently, the FDA approved the first oral fecal microbiota drug for the prevention of CDI recurrence. However, there are no vaccine licensed against CDI. There are various proteins from *C. difficile* reported to have antigenic properties, among them two spore exosporium proteins CdeC and CdeM, have been reported to be critical for
C. difficile spore adhesion/colonization.

This study aimed to develop a novel vaccine, named CdeCM, by employing immunoinformatic techniques to design and build a fusion of multiple epitopes. Protein sequences of CdeC and CdeM were analyzed and validated using standard bioinformatics tools for discovering antigenic and immunogenic epitopes. The predictions identified 7 cytotoxic T-cell lymphocyte (CTL), 3 helper T lymphocyte (HTL), and 6 B-cell potential epitopic regions capable of inducing immunogenic response. These epitopes were fused together with required linkers to develop the final fused vaccine CdeCM, which was modelled, refined, and validated. Molecular docking and molecular dynamics (MD) stimulation of CdeCM with Toll-like receptors (TLRs), B-cell receptor (BCR) complex and major histocompatibility complex (MHC) complexes, further assured its stability and binding ability to confer immune response. The in-silico analysis indicated the chimeric vaccine is stable and can confer a robust immune response in the host. In addition, the proteins of interest and the fusion protein were cloned and expressed to study their immune stimulation and protectiveness against CDI.

#56

Title: Antibodies against PfCDPK-5 inhibit the growth of *P. falciparum* in the blood stages of the parasite by disrupting schizont egress.

A.R. Shakri [1], J. Badhai [1], A. Anand [1]B. Sharman [2] A. Jnawali [2], T. Najrana [2], M.G. Alameh [3], D. Weissman [3], E. Kabyemela [4], P. Duffy [5], M. Fried [5], J. Kurtis [2] J. Dvorin[4] and D.K. Raj [1,2]

[1] Division of Infectious Disease and International Medicine, Department of Internal Medicine, University of South Florida, Tampa, FL, USA.

[2] Center for International Health Research, Rhode Island Hospital, Warren Alpert Medical School, Brown University, Providence, RI, USA.

[3] Department of Medicine, University of Pennsylvania, Philadelphia, PA, USA.

[4] Boston Children's Hospital, Boston, MA, USA.

[5] Laboratory of Malaria Immunology and Vaccinology, National Institute of Allergy and Infectious Diseases, National Institutes of Health, Rockville, MD, USA.

Malaria affects almost one-half of the world's population and causes more than 500,000 deaths annually. Global efforts to control the disease have had limited success, and no blood-stage vaccine has yet been approved for clinical use. Therefore, there is an urgent need to discover new vaccine candidates. A vaccine against childhood malaria is a priority because children below five years are highly vulnerable to the disease.

This study is uniquely focused on gaining an immunological understanding of anti-PfCDPK-5 antibodies and their role in preventing parasite maturation and egress. In our ongoing antigen discovery studies, we have pioneered a high-throughput differential whole proteome screening method. This method has allowed us to identify targets of antibodies that protect children from severe malaria, such as Schizont Egress Antigen-1 (Raj *et al*. Science 2014) & Glutamic Acid Rich Protein PfGARP (Raj *et al*. Nature 2020). In a parallel screening approach, we identified PfCDPK-5 as a potential vaccine candidate. Notably, we found antibodies against the PfCDPK-5 protein only in the sera of malaria-resistant children, not in those of susceptible children.

The polyclonal antibody generated by recombinant DNA vaccine shows significant growth inhibition activity in *in vitro* assays and animal challenge experiments. The confocal microscopy demonstrates the protein localization on the merozoite of the rupturing schizonts. Our data indicate that immunization with PbCDPK-5 recombinant antigens can trigger a strong antibody response, protect mice from parasitemia, and significantly extend their lifespan following a lethal *P. berghei* ANKA challenge. We developed a rPfCDPK5 vaccine on the mRNA platform that showed robust antiparasitic activity in GIA and assessed the vaccine's potential *in vivo* using *P. berghei* parasites in C57BL/6 mice models. We demonstrated the effectiveness of anti-PfCDPK5 antibodies by passively immunizing humanized mouse models and challenging them with the human malaria parasite *P. falciparum*.

In this study, we have successfully validated PfCDPK-5 as a vaccine candidate using integrated translational approaches that leverage high-throughput molecular techniques and *in vitro* functional assays. We are confident that the robust data generated in this project will significantly advance the development of a blood-stage vaccine against human malaria.

Antimicrobial Resistance

Harnessing the Power of Genetics: Surface Display of Antimicrobial Peptides to Probe *Klebsiella pneumoniae* Membrane Interactions

C.M. DeBarro[1], S. Assent[1] J. Randall[2], B. Davies[2], R. Fleeman[1]

[1]University of Central Florida, Orlando, FL, 32827, United States
[2]The University of Texas at Austin, Austin, TX, 78712, United States

Klebsiella pneumoniae infections are almost completely drug resistant and associated with a high rate of mortality. Antimicrobial peptides can provide us with a more thorough understanding of how to treat these infections. Our lab employs a genetic screening platform that allows us to characterize sequence activity relationships of antimicrobial peptides. To begin to understand the characteristics needed for protegrin-1 activity we utilized our genetic platform and tested a defined peptide library on the surface of a colistin resistant strain of *K. pneumoniae*. Our screening revealed mutations in different structural regions of the peptide that resulted in its decreased activity. We validated our next generation sequencing results by direct cloning of select mutated sequences into our genetic screening system. Intriguingly, our analysis revealed a reduction in activity upon a single mutation of an arginine to proline at position 9 within the sheet region. Furthermore, we saw a complete elimination of activity when this mutation was accompanied by an arginine to leucine mutation within the β-turn region. Synthesized peptides were tested for minimal inhibitory concentrations against a panel of *K. pneumoniae* isolates and our results correlated with our genetic screen. Continued structural analysis revealed the shortening of the beta sheets observed when substituting a proline for arginine at position 9 was not found when substituting a proline for arginine at position 11 in the β-turn region, which also retained its antimcirobial activity upon mutation. The protegrin-1 pore formation predicted using AlphaFold2 demonstrated this position 9 proline substitution also resulted in the collapse of the pore formed upon membrane insertion, while the position 11 substitution maintained its integrity. These findings validate previous reports that structural integrity plays an important role in protegrin-1 antimicrobial activity and suggest a reduction in charge can be more tolerated than beta sheet disruption. In conclusion, we found that a single proline substitution in protegrin-1 secondary sequence can decrease its antimicrobial activity towards a colistin resistant *K. pneumoniae* isolate. This is important insight into the importance of structure of host defense peptides that can kill isolates that capitalize on decreasing their membrane charge to gain colistin resistance.

Single-cell characterization of *Pseudomonas aeruginosa* heterogeneity due to antibiotic treatment using high-resolution biotechnologies

C.D. Miller[1], M. Asgari[2], S.E. Darch[1]

[1]Department of Molecular Medicine, Morsani College of Medicine, University of South Florida, Tampa, FL USA
[2]Department of Medical Engineering, College of Engineering, University of South Florida, Tampa, FL USA

Microfluidic systems are robust and versatile tools that utilize the properties of fluid mechanics to govern fluid flow at the microscale. The precise and consistent control possible using droplet-based microfluidics allows for microbial applications such as particle encapsulation. Novel techniques such as this to encapsulate and further study the physiology and interactions between bacterial cells are needed to understand clinically relevant, opportunistic pathogens such as *Pseudomonas aeruginosa*. The interactions between individuals and groups of *P. aeruginosa* cells are of significant interest due to their highly virulent behavior and persistence in immunocompromised patients. Thus, droplet-based microfluidics presents an opportunity to isolate *P. aeruginosa* and observe the impact of environmental changes at the microscale. A flow-focusing microfluidic device was used to manipulate the relative discontinuous (deionized water or fluorescently labeled gelatin solution with *P. aeruginosa* cells) and continuous (mineral oil and surfactant solution) phase flow rates to control droplet generation and diameter (μm). Droplets were transferred to incubation chip microwells (e.g., 20 μL) and observed using confocal laser scanning microscopy (CLSM) and atomic force microscopy (AFM) in the presence and absence of antibiotics (tobramycin and colistin). Combining these approaches (i.e., CLSM and AFM) allows us to extract key visual and physical spatial information (e.g., organization, physical forces within and between aggregates, and the localized force spectroscopy maps that denote the resistance to deformation at each specific site within the aggregate) about engineered *P. aeruginosa* aggregates and their heterogeneity due to strain and/or antibiotic treatment type. *P. aeruginosa* cells were then isolated, and single-cell RNA sequencing was performed using the Chromium Next GEM Single Cell 3’ RNA-sequencing platform to generate transcriptomic profiles of each droplet population. Differentially regulated genes were identified using bioinformatic approaches, both in the presence and absence of antibiotics. Among other high-resolution methodologies to encapsulate aggregates and single cells, droplet-based microfluidics is a promising tool to isolate and observe the development of aggregates from single bacterial cells in engineered spatial conformations. Here we show how this microfluidic system can be used as a high-resolution tool that allows for *P. aeruginosa* isolation on a single-cell level in biocompatible materials and can be coupled with microscopy and single-cell genomics to further understand heterogeneous bacterial response to antibiotic treatment.

#59

D. deGuzman, The University of Tampa, Tampa, FL
K.T. Francis, Ph.D., Mote Marine Laboratory, Sarasota FL

Isolation and Identification of Deep-Sea Sediment Microbes with Antimicrobial Activity Found in the Gulf of Mexico

The study of secondary metabolites, or natural products, from marine bacteria is vital in combating antimicrobial resistance facing our healthcare systems. Many of the antibiotics prescribed today are derived from actinomycetes bacteria, which are often found in terrestrial and marine sediment communities. This research aims to identify antimicrobial marine natural products from marine sediment communities as leads for drug discovery. Sediments were collected via multicores from deep benthic communities (458-1879 m) in the Gulf of Mexico in September 2023 and 1) plated immediately at sea using a variety of isolation medias or 2) desiccated for 10 months before plating to select for spore forming microorganisms. Morphologically unique colonies were selected for a given culture condition, streaked to purity, and screened using a soft agar pour-over assay to assess the antimicrobial activity of each isolate against four medically relevant pathogens: Methicillin-Resistant *Staphylococcus aureus* (MRSA), Vancomycin-Resistant *Enterococcus faecium* (VRE), drug resistant *Candida albicans*, and *Bacillus subtilis*. In sum, 40 of 134 bacteria (29.9% hit rate) isolated from deep-sea sediment communities showed activity against one or more pathogens. Two isolates, NF012-9 (5.5 ± 0.2 mm MRSA ZOI) and MC118-56 (7.6 ± 0.5 mm MRSA ZOI), were selected for larger scale fermentation and chemical extraction to further study the antimicrobial metabolites produced by these marine microorganisms. Crude extracts were fractioned using an automated SPE system to separate the extracts by polarity into 7 fractions. Extracts and fractions were evaluated for anti-MRSA activity using a Kirby-Bauer disk diffusion assay. 16S rRNA sequencing was performed to taxonomically identify active isolates, NF012-9 and MC118-56, as *Bacillus* spp. This project demonstrated the value of surveying the microbial communities of unique environments for antimicrobial activity in effort to identify new compounds for drug discovery.

#60

Identification of a Scaffold to Potentiate Pseudomonas aeruginosa Antimicrobial Activity

S. Assent, Q. Gao, R.W. Huigens III, and R.M. Fleeman

Antibiotic resistance is a growing issue in global healthcare. *Pseudomonas aeruginosa* is known for its extreme resistance to antibiotics. Intrinsic resistance mechanisms include low outer membrane permeability and efflux pumps. Decreased outer membrane permeability is unique to *P. aeruginosa* because it does not utilize diffusion porins found in other Gram-negative pathogens. In addition, *P. aeruginosa* contains several classes of efflux pumps with overlapping specificity adding to its volatility. Discovering an adjuvant compound to inhibit these intrinsic resistance mechanisms can allow for the rescue of multiple antibiotics. A unique multi-tier screening process was employed to screen over 1,600 compounds from a pharmacologically relevant Asinex chemical library to identify compounds that rescue tetracycline antimicrobial activity, a known efflux substrate. To ensure our screening method is robust, we utilized a parental strain of *P. aeruginosa* (PAO1 K2732) and a mutant strain (PAO1 K2733) that lacks 5 major RND efflux pumps. Our screening identified a compound with no antimicrobial activity alone but could rescue tetracycline activity. We then had the two diastereomers of our library hit synthesized and checkerboard assays were performed with the parental and efflux mutant. Importantly, while we observed these compounds allowed for similar rescue of tetracycline activity towards the parental isolate as in our Asinex screening, there was no increased killing of tetracycline when testing the compounds' effects on the efflux mutant. Checkerboard assays with erythromycin, kanamycin, and polymyxin B antibiotics suggest that the mechanism of efflux inhibition may be due to the compounds' membrane activities. Testing the membrane activities of our compounds using $DiSC_3$ membrane depolarization and O-nitrophenyl beta-galactosidase membrane leakage assays revealed they are depolarizing the inner membrane but not causing membrane leakage. Interestingly, we found the membrane of the efflux mutant without treatment was less polarized than the parental isolate, perhaps explaining the differential adjuvant potential observed in our testing. This research is important to provide a tool for understanding how to sensitize *P. aeruginosa* and aid the global healthcare community in developing therapeutics that combat infections in a post-antibiotic age of medicine.

#61

Investigating Natural Proline-rich Antimicrobial Peptides (PrAMPs) Activity Towards *Klebsiella pneumoniae*

R. Appiah, C. DeBarro, L. De Los Santos, and R. Fleeman
Burnett School of Biomedical Sciences, University of Central Florida

The rapid advancement of *Klebsiella pneumoniae* towards antibiotic resistance is a major concern. The extracellular polysaccharide capsule of *Klebsiella pneumoniae* protects the bacteria against host innate immunity. Our previous study showed antimicrobial peptides can disrupt the extracellular polysaccharide (EPS) capsule of *K. pneumoniae*. Further investigation revealed the host defense peptide bac7 (1-35), a proline-rich antimicrobial peptide (PrAMP) had the greatest ability to aggregate with *K. pneumoniae* EPS capsule and the most potent antimicrobial activity. However, the correlation between the multiple proline residues and other key features aiding in EPS and membrane interactions, and antimicrobial activity is poorly understood. This lack of understanding impedes the development of this class of antimicrobials as therapeutics for drug-resistant *K. pneumoniae*.

Here we investigated the antimicrobial activity of natural PrAMPs from diverse range of organisms against several strains of *K. pneumoniae* and also the interactions of the peptides with *K. pneumoniae* membrane and EPS capsule. Interestingly, we found proline rich antimicrobial peptides Apidaecin Cd3+, Tur1A, and PR-39 to be active against all strains tested, including hypervirulent strains, with a minimum inhibition concentration ≤ 1 ug mL^{-1}. These three most active peptides have in common a proline percentage greater than 37 with more than +5 charge. Combination activity of the active peptides with PepC, a less active peptide, revealed possible synergistic activity. The active PrAMPs depolarized the membrane of *K. pneumoniae* membrane suggesting interactions with the membrane might be facilitating the entry of the peptides. Additionally, biofilms treated with the active peptides significantly collapsed compared to controls. Our findings reveal potential antimicrobial peptides that have specific features which better interacts with *K. pneumoniae* EPS capsule and membrane, subsequently leading to bacterial death.

#62

Synergistic Effects of Polyphenols and Antibiotics Against Multi-Drug Resistant *Salmonella* Typhimurium DT104

H. Sheffield[1], K.E. Rodriguez Chinchilla[2], G. Bharathan[1], M. Hayden[1], A. Madi[1], J. Buhr[3], and S. Srikumar[1].
1 Department of Poultry Science, College of Agriculture, Auburn University, Alabama, AL 36830
2 Zamorano University, Honduras, C.A.
3 US National Poultry Research Center, Athens, Georgia

The rise of antimicrobial-resistant foodborne pathogens necessitates innovative approaches to enhance the efficacy of existing antibiotics. This study explores the synergistic effects of phyto polyphenols, specifically hydroxycinnamic acid and itaconic acid, with conventional antibiotics in inhibiting multi-drug resistant *Salmonella enterica* serovar Typhimurium, DT104 (DT104). We used DT104 due to its resistance to five antibiotics giving us the opportunity to test the multi antibiotic polyphenol synergy. Initially, the minimum inhibitory concentrations (MICs) of the polyphenols were determined individually using the standard 96-well plate microdilution protocol. Following this, a checkerboard assay was performed to assess potential synergistic interactions between each polyphenol and different antibiotics. Both hydroxycinnamic acid and itaconic acid did not show any significant anti-DT104 effect when tested individually. However, when tested in the presence of antibiotics, both polyphenols showed excellent anti-DT104 effects demonstrating synergy with all the tested five antibiotics resulting in a reduction in the individual antibiotic MIC. We also tested the practical application of our observation on food samples. One-gram portions of boneless, skinless chicken tenderloins were inoculated with DT104. The samples were then treated with hydroxycinnamic acid and itaconic acid, while control samples received no treatment (PBS). The treated and untreated samples were incubated (temp?) and then plated on XLD at 2-hour intervals over a 16-hour period to assess bacterial growth. Our results indicated a significant reduction in the growth of DT104 in chicken samples treated with polyphenols compared to the untreated controls. This reduction highlights the potential of polyphenols to enhance the effectiveness of antibiotics against multi-drug resistant pathogens in food matrices. It is important to note that even though individual polyphenols did not show any anti-DT104 effect in the MIC analysis, there were significant antimicrobial effects on chicken meat. This could be because of the hurdle principle wherein the meat itself produced some stress environments for DT104 in the presence of our polyphenols. These preliminary findings suggest that polyphenols can play a crucial role in mitigating the risk of foodborne illnesses caused by pathogenic bacteria.

#63

Evaluating Antibiotic Resistance Patterns in *Salmonella* Isolated from Surface Water

L.I. Codiniera, A.T. Siceloff, J.C. Smith, N.W. Shariat
Department of Population Health, College of Veterinary Medicine, University of Georgia, Athens, GA 30602

Salmonella enterica is a leading cause of bacterial foodborne illness in the United States, causing an estimated 1.35 million cases per year. The consumption of fresh produce accounts for almost 44% of these cases, more than chicken, pork, beef, and turkey combined. *Salmonella* is frequently found in surface water such as creeks, rivers, and ponds, where it poses a significant risk to food production when contaminated water is used for crop irrigation. Evaluating antibiotic resistance is important in risk assessment considering the clinical impact of Salmonellosis. For two years, we analyzed monthly *Salmonella* prevalence throughout 19 different sites across four different watersheds in the southeast. The objective of this study was to characterize *Salmonella* isolates by evaluating their resistance to ten clinically relevant antibiotics. Modified Moore swabs were used to filter and capture *Salmonella* from each water site, and *Salmonella* presence was determined by selective enrichment and isolation on XLT-4 agar. Following confirmation, the Kirby-Bauer disk diffusion assay was used to determine resistance to Ampicillin, Amoxicillin Cluvanate, Ceftriaxone, Gentamicin, Streptomycin, Tetracycline, Ciprofloxacin, Sulfamethoxazole/Trimethoprim, Chloramphenicol, and Nalidixic Acid. *Salmonella* prevalence over two years was 68.9% (314/456). A total of 314 isolates were analyzed and 11.5% (36/314) of isolates were resistant to one or more antibiotics tested, while 86.9% (273/314) were pan -susceptible. Of the isolates that were only resistant to one antibiotic class, 83% (15/18) were resistant to only Streptomycin with 53% (8/15) of these being found in watershed A. Further, 2.9% (9/314) of the isolates demonstrated multidrug resistance, indicating resistance to three or more antibiotic classes. Among them, 44.4% (4/9) of these isolates shared identical resistance profiles and were discovered in the same watershed across 20 months (February 2022 to October 2023), implying that this resistance profile persists in the same environment. One isolate was determined to be resistant to seven antibiotics. Understanding the persistence of *Salmonella* in surface water and the prevalence of antibiotic resistance is important for improving food safety and public health outcomes.

#64

Emory University

“The Trade-Off Dilemma: Impact of Sub-Inhibitory Antibiotics on Staphylococcus aureus Growth, Resistance, and Persistence”

A.S. Ismail, B.A. Berryhill, T. Gil-Gil, J.A. Manuel, A.P. Smith, F. Baquero, B.R. Levin

Antibiotic treatments aim to use drug concentrations that exceed the minimum inhibitory concentration (MIC) needed to halt bacterial replication. However, spatial and physiological variability within a patient's body can create antibiotic gradients, leading to areas where antibiotic concentrations fall below the MIC. Using *in vitro* experiments with *Staphylococcus aureus* and mathematical computer-simulation models, this study investigates the impact of sub-inhibitory antibiotic concentrations on the population dynamics of bacteria. With antibiotics of six different classes, we demonstrate that exposure to sub-inhibitory concentrations of these drugs significantly affects bacterial growth dynamics. Exposure to sub-inhibitory concentrations of antibiotics reduces both the maximum growth rate and the final bacterial density, while extending the time before growth begins. This indicates that antibiotics can exhibit significant inhibitory activity even at concentrations well below the MIC. However, exposure to these antibiotic concentrations also increases the mutation rate to antibiotic resistance. Our results show that the activation of the cellular stress pathway, the SOS response, is triggered by sub-inhibitory antibiotic levels. This ubiquitous SOS response plays a crucial role in DNA repair and survival under stress, but also leads to higher mutation rates. Another phenomenon observed with sub-inhibitory antibiotic exposure is changes in bacterial persistence levels. Persistence is a temporary phenotypic state where bacteria enter dormancy under antibiotic pressure, enabling survival. Our results suggest that exposure to sub-inhibitory concentrations of antibiotics leads to metabolic changes, indicated by higher intracellular ATP levels, which counteract dormancy and reduce the formation of persister cells. Taken together, these results suggest that within an individual treated with antibiotics, where sub-inhibitory concentrations of the antibiotic are present, bacterial populations are inhibited and the frequency of persistence declines. However, the rate of mutation to antibiotic resistance increases. Understanding these trade-offs between mutation rates and persistence levels due to sub-inhibitory antibiotic exposure is crucial for designing antibiotic therapy and preventing treatment failure.

#65

First-in-class inhibitors of mycobacterial cell wall synthesis

P.C.B. Halicki[1]; S. Geden[1]; J. Kim[2]; J.M. Schomaker[2]; K. Rohde[1]
[1] Burnett School of Biomedical Sciences, University of Central Florida
[2] Department of Chemistry, University of Wisconsin-Madison

Tuberculosis (TB) is an infectious disease caused by *Mycobacterium tuberculosis* (Mtb) that shows high levels of morbidity (~10 million new cases/year) and mortality (~1.7 million deaths/year). The current TB regimens, entailing multidrug cocktails for ≥4 months with significant side effects, leave much to be desired, with the first- and second-line drugs inhibiting a limited number of bacterial targets. Potent antimycobacterial agents with novel targets and mechanisms of action (MoA) are urgently needed to overcome these limitations and the emergence of multidrug-resistant strains. To address this need, we tested a panel of cyclic sulfamate compounds and identified novel chemotypes that exhibit potent and highly selective activity against both extracellular and intracellular Mtb. Most importantly, whole genome sequencing of spontaneous resistant mutants supports the hypothesis that these compounds kill Mtb by inhibiting KasA. The unchanged susceptibility of resistant mutants to front-line TB drugs is further evidence that this series acts via a novel MoA. KasA encodes a β-ketoacyl synthase whose role in elongation of acyl-AcpM chains is required for the biosynthesis of mycolic acids. Despite being well-validated, this is still an underexploited drug target in Mtb. In-depth knowledge of structure-activity relationship will guide future med-chem optimization and evaluation of *in vivo* efficacy of this chemical series that could contribute to new alternative treatment options for TB.

#66

Structure based development of cross-class carbapenemase inhibitors

L. Jacobs[1], P. Jaishankar[2], J. Jackson[3], L. Shaw[3], A. Renslo[2], Y. Chen[1]

[1]Department of Molecular Medicine, Morsani College of Medicine, University of South Florida, Tampa FL

[2]Department of Pharmaceutical Chemistry and Small Molecule Discovery Center, University of California, San Francisco, CA

[3]Department of Cell Biology, Microbiology, and Molecular Biology, University of South Florida, Tampa, FL

Many bacterial species produce β-lactamase enzymes as a mechanism of resistance against β-lactam antibiotics. The emergence of carbapenemases, b-lactamases capable of hydrolyzing highly effective carbapenem antibiotics, have become a serious public health risk, driving the need for new antibiotic therapies. Here, we describe the development of cross-class carbapenemase inhibitors against the serine b-lactamase, KPC-2, and metallo-b-lactamase, NDM-1. In previous studies, our group discovered a heteroaryl phosphonate scaffold by screening the ZINC database against KPC-2 using the molecular docking program DOCK 3.5. Through structure-based design and synthesis of these non-covalent compounds, we identified compound **1** which displayed a 20 nM K_i against KPC-2 and a 31 mM K_i against NDM-1. The goal of this current study is to advance cross-class inhibition, by improving activity against MBLs decreasing the gap of inhibition between the two enzymes. New compounds were synthesized to optimize various substitutions on the compound **1** scaffold, showing low mM to nM inhibition against both SBLs and MBLs. Two compounds, **46** and **47**, displayed a K_i of ~220 nM against KPC-2. Both compounds, while not as potent against KPC-2 as compound **1**, have provided insight into how certain substitutions can affect the binding pose within the KPC-2 active site and further improve the binding affinity of compound **5** against KPC-2. Another derivative, compound **73**, showed a 1 mM K_i against NDM-1, improving the inhibition of compound **1** by 30-fold. Complex crystal structures revealed a unique binding pose of compound **73**, with two copies of the compound within the NDM-1 active site. Even more encouragingly, compound **86** showed 350 nM inhibition for KPC-2 and 800 nM K_i against NDM-1 and is the first cross-class nanomolar inhibitor from our group. Promising in vitro activity was seen with carbapenemase producing bacterial strains in combination with imipenem. Compound **73** displayed a 16-fold improvement in the MIC of KPC-2 and an 8-fold improvement in the MIC of NDM-1 in comparison to imipenem alone. The activity and complex structures of these compounds offer valuable information for the future development and synthesis of these novel carbapenemase inhibitor chemotypes**.**

#67

Mycobacterium abscessus BlaRI ortholog mediates regulation of energy metabolism but not β-lactamase expression

H.C. Davenport[1], L.E. Bonefont[1], C.T. Chaton[2], K.V. Korotkov[2], K.H. Rohde[1*]

[1]Division of Immunity and Pathogenesis, Burnett School of Biomedical Sciences, College of Medicine, University of Central Florida
[2]Department of Molecular and Cellular Biochemistry, University of Kentucky, Lexington, Kentucky, USA

Mycobacterium abscessus (*Mab*) is an opportunistic pathogen predominantly affecting immunocompromised individuals, such as those with cystic fibrosis. *Mab* is highly drug resistant, and understanding regulation of antibiotic resistance is critical to future antibiotic development. Regulatory mechanisms controlling *Mab*'s β-lactamase (Bla_{Mab}) that mediates resistance to β-lactams remain unknown. *S. aureus* encodes a prototypical protease-mediated two-component system BlaRI regulating the β-lactamase, BlaZ. BlaR1 binds extracellular β-lactams, activating an intracellular peptidase domain which cleaves BlaI to derepress *blaZ*. *Mtb* encodes homologs of BlaRI, that regulate the *Mtb* β-lactamase, *blaC*, but also additional genes related to respiration. We identified orthologs of $blaRI_{Mtb}$ in Mab and hypothesized that they regulate the β-lactamase bla_{Mab}. Surprisingly, neither deletion of $blaRI_{Mab}$ nor overexpression of only $blaI_{Mab}$ altered bla_{Mab} expression or β-lactam susceptibility. $BlaI_{Mab}$ did not bind upstream of the bla_{Mab} gene and no binding motif could be located. However, $BlaI_{Mab}$ did bind to conserved motifs upstream of several Mab genes involved in respiration, yielding a putative regulon that partially overlapped with $BlaI_{Mtb}$. Prompted by evidence that respiration inhibitors including clofazimine (CFZ) upregulate the BlaI regulon in *Mtb*, we found that CFZ triggers induction of $blaIR_{Mab}$ and its downstream regulon. Highlighting an important role for $BlaRI_{Mab}$ in adapting to disruptions in energy metabolism, constitutive repression of the $BlaI_{Mab}$ regulon rendered *Mab* highly susceptible to CFZ. We also demonstrated the BlaR-dependent inactivation of $BlaI_{Mab}$ through cleavage assays in the presence or absence of CFZ, although the exact mechanism by which CFZ triggers $BlaR_{Mab}$ signaling still remains unknown. In addition to our unexpected findings that $BlaIR_{Mab}$ does not regulate □-lactam resistance, this work highlights the novel role for mycobacterial BlaRI-type regulators in regulating electron transport and respiration.

#68

Unraveling Sars-CoV-2 M^{pro} Evolution: Insights Into Inhibitor Resistance Mechanisms

N. Kohaal[1], E. Lewandowski[1], Y. Hu[2], X. Zhang[1], A. Brown[1], J. Wang[2], Y. Chen[1]
1. Molecular Medicine, University of South Florida Morsani College of Medicine, Tampa, FL, United States
2. Medicinal Chemistry, Rutgers The State University of New Jersey, New Jersey, NJ, United States

Since its onset in late 2019, the global impact of the COVID-19 pandemic, triggered by the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), has been substantial across health, economic, and social dimensions. The main protease M^{pro}, plays a crucial role in viral replication and maturation, making it an ideal target for developing antiviral drugs to combat SARS-CoV-2. Protease inhibitors such as nirmatrelvir, which function by binding to the SARS-CoV-2 M^{pro} to inhibit viral replication, have been investigated and proven to be effective. Based on previous experience with HIV-1 resistance to protease inhibitors, there is concern regarding the potential selection of nirmatrelvir-resistant SARS-CoV-2 strains following widespread use and/or overdosing of nirmatrelvir/ritonavir, as several studies have identified resistance mutations, such as T21I, L50F, P252L and T304I against this therapeutic drug. In vitro viral passage and in vivo animal models have additionally uncovered a triple mutant, L50F/E166A/L167F, which not only confers resistance to nirmatrelvir but also maintains wild-type replication fitness. While the mechanism of resistance of E166A/L167F in the catalytic pocket are well understood, the function of L50F remains unclear. In this study, we used X-ray crystallography and a novel gel-based assay to elucidate the contribution of distant residue L50F in substrate binding. Despite the minimal impact on substrate hydrolysis in biochemical analysis, L50F rescues viral fitness. L50F is involved in distal protein-protein interactions which help place the substrate at the enzyme catalytic center Ultimately, this information can be used to guide the development of future therapeutics capable of combating resistance, lessening the global impact of the COVID-19 pandemic.

#69

Assessing the therapeutic potential of R-pyocins: Biocompatibility, thermostability, and serum stability against multi-drug-resistant *Pseudomonas aeruginosa*

I. Estrada[1], I.H. Sadab[1], S.P. Diggle[1]

[1]Department of Biological Sciences, Georgia Institute of Technology, Atlanta, GA, USA

The alarming rise of multi-drug-resistant (MDR) and extensively drug-resistant (XDR) *Pseudomonas aeruginosa* (*Pa*) strains underscores the urgent need for alternative therapeutic strategies. R-pyocins, proteinaceous antimicrobials produced by *Pa*, have emerged as promising candidates due to their potent bactericidal activity. However, their potential cytotoxicity to human cells and stability under physiological conditions require thorough investigation. In this study, we assessed the cytotoxicity of nine purified R-pyocins on human ling epithelial (A459), macrophage (J774), and fibroblast (3T3) cell lines using WST-1 assays. Remarkably, none of the tested R-pyocins exhibited significant cytotoxicity at concentrations up to 100 µg/mL, indicating their potential biocompatibility. Thermostability studies, conducted by exposing R-pyocins to temperatures ranging from 37°C to 90°C for 1 hour, revealed that all nine R-pyocins maintained their structural integrity up to 60°C. Lastly, the bactericidal activity of the R-pyocins against *Pa* strains was investigated in the presence of up to 75% fetal bovine serum, which did not impact their efficacy, suggesting that these antimicrobial proteins could remain active within the complex biological environment of the human body. These findings support the safety and stability of R-pyocins as potential therapeutic agents against MDR/XDR *Pa* infections. However, further *in vivo* studies are essential to validate their efficacy, biocompatibility, and pharmacokinetics in clinically relevant animal models before progressing to human trials. The development and optimization of R-pyocins as novel antimicrobials could provide a much-needed alternative to conventional antibiotics, addressing the growing global threat posed by antibiotic-resistant *Pa* strains and paving the way for innovative targeted therapies.

Molecular Biology of Microbes

#70

Phosphoregulation of FtsZ in *B. subtilis*

A. King[1], P. Horigian[1], J. Carroll[1], P. Eswara[1]
[1]Department of Molecular Biosciences, University of South Florida, Tampa, FL USA

Bacterial cell division is a highly regulated process in which multiple proteins, collectively called the divisome, are recruited to the septum and work together to divide the parental cell into two identical daughter cells. A main component of which is FtsZ: a key tubulin-like protein which polymerizes, via the hydrolysis of GTP. As such, its regulation is pivotal in correct Z-ring positioning and hence division. A way in which this regulation could occur is through post-translational modification such as phosphorylation. Using *B. subtilis* phosphoproteomic data we identified three key sites in the C-terminal linker (CTL) of FtsZ which were found to be phosphorylated. To study the significance of phosphorylation, we employed a mutagenesis-based approach either mimicking phosphorylation or dephosphorylation. We hypothesized that these alterations in charge separation of the CTL will cause functional changes in FtsZ, allowing for non-native intra CTL interactions and non-native interactions between the CTL and GTPase domain. To this end, each mutant protein was purified and analyzed via a GTPase assay. Variability was shown throughout the mutants made implying that changes in charge do play a role in the functionality of the protein. To further investigate the relevance of these mutants in vivo we plan to use a CRISPR-based approach targeting the native copy of *ftsZ* in the chromosome.

Establishing A Marine Microbial Natural Product Drug Discovery Program at Mote Marine Laboratory

K.T. Francis[1], Ph.D.
[1]Mote Marine Laboratory, Sarasota, FL USA

Marine ecosystems are some of the most biologically diverse on planet Earth. This biodiversity translates to incredible chemical diversity. The intense competition for resources across marine ecosystems like coral reefs and deep sea communities promotes the evolution of secondary metabolites, or marine natural products. Today, natural products and their derivatives make up 53% of all approved small-molecule drugs and 65% of approved small-molecule cancer drugs (Newman and Cragg 2020). Mote Marine Laboratory, a non-profit research institution founded in 1955, has a rich history of marine biomedical research. Discoveries from Mote scientists include antimicrobial activities in coral mucus-associated bacteria, antimicrobial activities in skate and ray epidermal mucus-associated bacteria, cytotoxic activity observed from bonnethead shark epigonal tissue against human cancer cell lines, and more. The new molecular microbiology program at Mote aims to expand the capacity of marine biomedical discovery by developing a marine microbial natural product drug discovery research program. This poster will describe the progress towards the specific aims of the program: 1) maintain and expand a diverse microbial isolate library, 2) create a pre-fractionated library of microbial extracts, 3) screen for various biological activity including antimicrobial, algicidal, and anti-tumor, and 4) identify compounds through bioassay guided fractionation and structure elucidation. We plan to leverage Mote's history of marine biomedical success and unparalleled access to sampling and facilities to identify novel active compounds with unlimited potential in many therapeutic applications.

#72

Evaluating *MAB_4287-4288* as a novel second BlaRI-type system in *Mycobacterium abscessus*

L. Bonefont[1] & K. Rohde[1]

[1]University of Central Florida College of Medicine, Burnett School of Biomedical Sciences, Orlando, Florida

Pathogens must respond to myriad host and environmental pressures. To respond to these pressures, a diverse set of signal transduction mechanisms are utilized. One archetype of bacterial signal transduction mechanisms is two component systems (TCS). Canonically, TCS involve a phosphotransfer between a sensor histidine kinase and a response transcriptional regulator. An alternative prototypical protease-mediated TCS is the BlaRI system in *Staphylococcus aureus*. In a process that induces β-lactam resistance, BlaR senses extracellular β-lactams, leading to autoproteolysis that activates peptidase activity. Activated BlaR1 cleaves dimerized DNA-bound BlaI, de-repressing *blaRI* and *blaZ* (β-lactamase). Many mycobacteria have BlaRI-type systems (renamed BlaIR to reflect inverted genomic organization). Mycobacterial BlaIR systems are characterized by a BlaR lacking an extracellular β-lactamase binding domain that includes a conserved zinc metalloprotease domain and a BlaI that binds an inverted repeat operator motif. In mycobacteria, these established systems are *Rv1846c-1845c* (BlaIR$_{Mtb}$) in *Mycobacterium tuberculosis* (*Mtb*) and *MAB_2415c-2414c* (BlaIR1) in *Mycobacterium abscessus* (*Mab*), which regulate β-lactamase expression (*Mtb* only) and respiration (both *Mtb* and *Mab*). We have identified a second BlaRI-type system in *Mab*, *MAB_4287-4288* (BlaIR2). Using RT-PCR and EMSA, we established that BlaIR2 is encoded within a five gene operon, a unique characteristic in BlaRI-type systems, and is auto-regulatory. The BlaI2 binding motif, hypothesized to be unique from BlaI1, is being identified through EMSA. Additionally, mutation of the Zn metalloprotease domain, which should ablate peptidase activity, led to altered expression of the *blaIR2* operon, providing evidence for a protease-mediated de-repression mechanism of BlaI2-bound promoters. Describing a novel second BlaRI-type systems in *Mab* points to the possibility of a distinct network of BlaRI-type systems within an organism, compared to the prototype in *S. aureus*. Demystifying the complex regulatory network in the poorly characterized *Mab* is critical to responding to this extremely drug resistant emerging pathogen.

#73

Elucidating the role of YfhS in *Bacillus subtilis*

C. Gaucher[1], L. Suits[1], P. Eswara[1]

[1]Department of Molecular Biosciences, University of South Florida, Tampa, FL USA

With rates of antibiotic-resistant infections rising, it has become more vital than ever to understand the mechanisms behind bacterial physiology. Using Bacillus subtilis as a model organism, we aim to decipher the function of YfhS, a protein of unknown function. Our experiments have found that deletion of the yfhS gene causes a small colony phenotype. A suppressor screen was conducted to identify mutations that restored the colony size similar to wild type (WT) control. After whole genome sequencing, we noticed that multiple mutations mapped to ndh, a NADH dehydrogenase. NADH helps facilitate glucose oxidation, converting it into adenosine triphosphate (ATP) before becoming an energy source for various metabolic processes in the cell. We generated a strain in which yfhS was placed under an inducible promoter to complement ΔyfhS strain. While induction of ndh alone did not have any effect on colony morphology or cell viability, overexpression of yfhS in a ΔyfhS strain background was lethal. Experiments to understand the Ndh-mediated toxicity in cells lacking YfhS are currently underway.

#74

Title:
MraZ and DnaA coordinate DNA damage response in *Bacillus subtilis* to arrest cell division.

Author List:
K. Ruiz-Houston, K. Resendes, Dr. M. White, A. Hough, A. Wojtkowiak, and Dr. P. Eswara

Affiliations:
Department of Molecular Biosciences; University of South Florida, Tampa, FL

In cases of DNA damage, it is critical that bacterial cells arrest cell division to prevent passing down harmful mutations to their progeny. This occurs through the SOS-response, which operates via a RecA-dependent or independent pathway. In the model organism *Bacillus subtilis*, the mechanism of the RecA-independent response is largely unknown and thus the focus of this research. Through the RecA independent pathway, it has been shown that the *mra* operon is repressed as a mechanism to transcriptionally regulate cell division. The *mra* operon is part of the division and cell wall (dcw) cluster, a group of genes involved in cell division and cell wall synthesis well conserved among Gram-positive and Gram-negative bacteria. Our previous research has suggested that MraZ, a transcriptional repressor within the dcw cluster, and DnaA coordinate to repress the *mra* operon during DNA damage. In our current studies, we have shown that MraZ and DnaA directly interact through a bacterial-two hybrid assay. Furthermore, we show that this interaction requires the C-terminal 12 amino acids of MraZ. Using this data as well as AlphaFold model, we intend to elucidate the exact residues through which MraZ and DnaA interact. We speculate that this interaction between MraZ and DnaA is likely present across diverse bacterial species as these two proteins are highly conserved.

#75

Characterizing the function of the cell wall hydrolase EssH in type 7 secretion system of *Staphylococcus aureus* USA300

R. Agyen[1*], M. Bobrovskyy[1]
[1]Department of Molecular Biosciences, University of South Florida, 4202 E. Fowler Ave, ISA6055, Tampa, FL 33620
*Presenting the poster

ABSTRACT
The *Staphylococcus aureus* type 7 secretion system (T7SS) exports several effector proteins that are pivotal for bacterial virulence. Earlier work revealed that S. *aureus* mutants lacking T7SS, or its core components showed diminished virulence and persistence in mouse infection model. T7SS core components EsaA, EssA, EssB and EssC assemble into a translocon within the cell envelope to facilitate secretion of small WXG substrates and LXG polymorphic toxins. Recently, secretion of T7SS effectors was shown to rely on the activity of the cell wall hydrolase EssH, that itself is secreted via the general Sec pathway, however, the mechanism whereby it supports T7 secretion remains unclear. Here, we determine that EssH is selectively stabilized in the *S. aureus* culture supernatant under the conditions that induce T7-dependent secretion and identify the protease responsible for modulating its stability under these conditions. However, we find that secreted EssH is not sufficient to support T7SS activity, suggesting that EssH may act in a T7SS-specific manner prior to its release into the extracellular milieu. We identified the N-terminal region of EssH to be required for T7 secretion and investigate its potential role as specificity-determinant. Current work is ongoing to determine the role of EssH domains and their functions in supporting effector secretion via the T7SS in *S. aureus*.

#76

Exploring evolution of the epidemic *S. aureus* clone USA300

E.A. Felton[1,2], S.J. Kennedy[1], J. K. Jackson[1,2], E. Cella[3], T. Azarian[3], L.N. Shaw[1,2]
[1]Department of Molecular Biosciences, University of South Florida, Tampa, FL, [2] Center for Antimicrobial Resistance, University of South Florida, Tampa, Florida, USA, [3]University of Central Florida, Orlando, FL.

Staphylococcus aureus is an adaptable and dangerous human pathogen causing infection in almost every bodily niche. The USA300 lineage has become the predominant *S. aureus* clone in the United States since its emergence around the turn of the century. This makes USA300 strains unique in that we are able to observe the evolution of a new, expanding clone in real-time – all during the genomics era. Consequently, this study explores the epidemiology of USA300, and its genomic adaptation during this 25 year span. Herein, we use whole genome sequencing (WGS) to explore USA300 evolution, comparing a historic collection of isolates (circa 2005) with those isolated in the last 5 years. WGS was performed using the Hackflex library preparation; a method of optimization for Illumina DNA sequencing of microbial genomes. Isolation source distribution reveals a trend away from skin and soft tissue infections (SSTI) over time, with a continued adaptation to other niches. For example, 84.1% of historic isolates were SSTI related whilst only 12.2% of contemporary isolates were. Bioinformatic analysis identified changes exclusive to more recent isolates relating to the composition of mobile genetic elements, recombination events associated with the PVL toxin and the abundance of mutations within virulence genes. More recent strains had nSNPs in all major regulators of virulence (except *codY*), as well as variants in 9/10 secreted proteases, alpha toxin, *lukSF-PV*, and most MSCRAMM genes. This work provides deeper understanding of the molecular events that have led to the success of the USA300 lineage.

#77

Title: Defining the role and sensing mechanism of NnaR in *Mycobacterium abscessus* nitrogen assimilation

V.M. Cruz-López[1], B.S. Simcox[1], K.H. Rohde[1]
[1]Burnett School of Biomedical Sciences, College of Medicine, UCF, Orlando, FL, USA

Mycobacterium abscessus (*Mab*) is an opportunistic pathogen that predominantly affects individuals suffering from pre-existing immunosuppression and pulmonary diseases, such as Cystic Fibrosis (CF). Treatment options for *Mab* infections are limited due to its inherent antibiotic resistance. Survival within macrophage phagosomes, granuloma lesions and mucus-burdened CF airways requires adaptation via transcriptional remodeling to offset the effects of hypoxia, as well as increased levels of nitrate, nitrite, and other reactive nitrogen (N) species. In a recent study of hypoxia-induced transcriptional changes in *Mab,* we noted a five gene operon that included four genes with predicted roles in nitrate/nitrite transport (*narK3*), nitrite assimilation (*nirBD*), siroheme co-factor synthesis (*sirB*) and a putative nitrate/nitrite assimilation regulator NnaR (MAB_3520c). Subsequent studies showed that the operon is induced by nitrate/nitrite in a NnaR-dependent manner. NnaR is also required for N assimilation from nitrate and nitrite. *nnaR* encodes a peculiar orphan response regulator (ORR) with a *C*-terminal OmpR-type DNA-binding domain (DBD), fused to an *N*-terminal uroporphyrinogen III synthase HemD domain that is highly conserved in Actinomycetes. HemD is responsible for cyclization of the linear tetrapyrrole hydroxymethylbilane (HMB), to form uroporphyrinogen III (UroP III), which is a common biosynthetic precursor for siroheme, a cofactor required for nitrite reductases. In the context of $NnaR_{Mab}$, we hypothesize that the HemD domain enables sensing of changes in the levels of its substate or product. Preliminary data from a growth kinetics study performed on a $Mab_{nnaR\Delta hemD}$ strain suggests that this hypothesis may be correct. Grown in minimal media with varied N sources, strains lacking the HemD domain ($Mab_{\Delta nnaR}$ and $Mab_{nnaR\Delta hemD}$) display growth deficiencies. The mechanism of cue detection and signal transduction of the HemD-ORR is one of the key aims of this project.

#78

The distinct role of GdpP in regulating the localization of Penicillin-Binding-Proteins in *S. aureus*

Y. Jia, R. Zhang, S.J. Scaffidi, W. Yu*
Department of Molecular Biosciences, College of Arts and Sciences, University of South Florida, Tampa, Florida 33612, United States of America

Mutations in the *gdpP* gene have been frequently associated with *Staphylococcus aureus* β-lactam resistance independent of *mecA*. GdpP is a phosphodiesterase that plays a critical regulatory role in *S. aureus* by degrading the secondary messenger c-di-AMP, which is known to regulate cell size, stress response, and antibiotic resistance. This study examines the effects of the *gdpP* mutant on cell wall architecture, Penicillin-Binding Proteins (PBPs) localization, and β-lactam antibiotics resistance. We show that deletion of the *gdpP* leads to reduced cell size and altered surface protein anchoring. Staphylococcus Protein A (SpA), a key virulence determinant of *S. aureus*, exhibited altered cross-wall localization in the *gdpP* mutant. Our findings also indicate that the absence of GdpP disrupts PBPs localization. Four PBPs in *S. aureus* are major enzymes that participate in peptidoglycan synthesis. In non-dividing *gdpP* mutants, all PBPs mis-localized as foci to the cell periphery, indicating a specific role of GdpP in regulating PBP distribution. Van-FL staining and FDAA labelling revealed that the cell cycle was disrupted in the *gdpP* mutant. A decrease in cell division rates and an accumulation of Phase 1 cells were identified in *gdpP* mutants. Our HPLC analysis of cell wall crosslinking in *gdpP* mutant revealed no significant difference compared with the WT, indicating the enhanced β-lactam antibiotic resistance was not due to the alteration of cell wall biosynthesis. Overall, our findings highlight the critical role of GdpP in regulating surface proteins anchoring, PBPs localization, and maintaining the normal cell cycle. The increased β-lactam antibiotic resistance in *gdpP* mutant was likely due to altered PBPs site and reduced dividing events. This study not only expands our understanding of bacterial stress responses, but also underscores the complexity of c-di-AMP signaling in bacterial pathophysiology.

ScrA, a novel regulator of adhesion, is controlled by a coordinated and complex network of regulation in *Staphylococcus aureus*

S.M. Walker[1], M.-E. Jobson[1], L.N. Shaw[1]
[1]Department of Molecular Biosciences, College of Arts and Sciences, University of South Florida, Tampa, FL

Staphylococcus aureus is a gram-positive human pathogen that can cause myriad diseases in the human host, such as bacteremia, osteomyelitis, and endocarditis. Its ability to colonize and infect the host can be attributed to a complex web of regulation controlling diverse virulence factors. One such regulator, ScrA, has previously been characterized to play a role in bacterial clumping through its link to the SaeRS two-component system. Although downstream targets of ScrA have been identified, the regulatory factors controlling *scrA* expression have no yet been identified. Herein, we set out to identify novel regulators of *scrA* using a high-throughput screen for transcriptional regulation using a transposon library. In so doing, we found 7 activators and 5 repressors of *scrA* transcription. Of these, ArlRS was found to be an activator during stationary phase, which may suggest that ScrA plays a role in modulating factors that help *S. aureus* adapt to growing population densities. Environmental-response regulator CodY was also found to activate *scrA*, while the regulator of heat shock, HrcA, was found to be a repressor. This suggests that ScrA abundance may be influenced by environmental conditions, facilitating survival during times of heat stress and nutrient availability, to increase production of virulence factors. Finally, we found that NorG dynamically modulates *scrA* promoter activity, potentially linking ScrA to quinolone and beta-lactam resistance. Although the precise mechanisms of regulation need further exploration, our study reveals that the transcriptional control of *scrA* is a complex process with global effects in diverse bacterial responses.

#80

Signal peptidase SpsB is a key determinant of staphylococcal surface protein septal trafficking, LtaS localization and cell cycle

R. Zhang[1], Y. Jia[1], S.J. Scaffidi[1], J.J. Madsen[2], W. Yu[1*]

[1]Department of Molecular Biosciences, College of Arts and Sciences, University of South Florida, Tampa, Florida 33612, United States of America

[2]Department of Molecular Medicine, Morsani College of Medicine; Center for Global Health and Infectious Diseases Research, Global and Planetary Health, College of Public Health, University of South Florida, Tampa, Florida 33612, United States of America

In *Staphylococcus aureus*, many surface proteins have YSIRK/G-S signal peptide (SP_{YSIRK+}) (which helped proteins to deposit septum and cleaved by SpsB), and LPXTG motif sorting signal (which helped proteins to anchor to septal peptidoglycan by SrtA). SpsB is a type I signal peptidase in the general secretory (Sec) pathway. Previous study, we identified that LtaS-mediated lipoteichoic acid (LTA) synthesis affects septal localization of YSIRK+ proteins in *S. aureus*. The LtaS is also cleaved by SpsB. We use SpA as a reporter to study the mechanism of SpsB on $SP_{SpA(YSIRK+)}$ and $SP_{SplE(YSIRK-)}$. The SpA with $SP_{SpA(YSIRK+)}$ shows delocalization and increased protein level in cytoplasm, cell membrane and cell wall. The SpA with $SP_{SplE(YSIRK-)}$ also shows increased protein level in cytoplasm, cell membrane and cell wall, but no delocalization. We also found that the SpA with $SP_{SpA(YSIRK+)}$ level is increased in cytoplasm, cell membrane and cell wall in the spsB double mutation than in the SrtA signal mutant, and SpA cannot be anchored the cell wall in the double mutantion strain. The SpA with $SP_{SplE(YSIRK-)}$ also shows the same phenotype. For deeper understanding the mechanism of SpsB, we mutate 37^{th} amino acid A to P. The 36th and 37th amino acid is a cleavage site of signal peptidase, an enzyme cleavage signal peptide to translocate proteins across cell membrane. The A37P mutation increases the SpA targeting the side wall and SpA level in cytoplasm. Interestingly, depletion of *spsB* led to aberrant cell morphology, cell cycle arrest and daughter cell separation defects. We also found that SpsB is predominantly localized at the septum of dividing staphylococcal cells. Furthermore, SpsB spatially regulates LtaS as *spsB* depletion enriched LtaS at the septum. Collectively, this study provides novel molecular insights into YSIRK+ protein septal trafficking and identifies SpsB as a novel and key regulator orchestrating protein secretion, cell cycle and cell envelope biogenesis.

#81

Identification of novel factors contributing to staphylococcal cell envelope integrity and surface protein septal trafficking

S.J. Scaffidi, R. Zhang, Y. Jia, S. Adikhanov,
M. Cubero, W. Yu
Department of Molecular Biosciences, University of South Florida, Tampa, Florida, USA

Abstract

The bacterial cell envelope provides integrity to the cell and mediates key interactions with the environment. Deeper understanding of how bacteria maintain cell envelope integrity is particularly important in pathogenic bacteria, as it represents an excellent antimicrobial target. Staphylococcal protein A (SpA), a highly abundant surface protein and key virulence factor of *Staphylococcus aureus*, is a model protein used to analyze surface protein trafficking through the cell envelope. Here we screened a transposon mutant library of *S. aureus*, using SpA immunofluorescence microscopy. The utility of SpA surface localization allowed us to identify novel factors implicated in regulating cell envelope homeostasis, including a previously uncharacterized gene *SAUSA300_2311*. Bioinformatic analysis revealed that *2311* is a putative inhibitor of its upstream gene product *2310*, a LytTR family transcriptional regulator. We named these two genes *salI*, *Staphylococcus aureus* LytTR Regulatory System (LRS) inhibitor, and *salT*, *Staphylococcus aureus* LRS transcriptional activator. The mutant of *salI* showed highly aberrant cell morphology, defects in cell wall biosynthesis and cell division defects. Both SpA localization and expression were severely altered in the *salI* mutant. RNA-seq analysis of the *salI* mutant revealed upregulated expression of *salT,* phage genes, cell wall modification genes, as well as several staphylococcal virulence genes. We confirmed the upregulation of *salT* was due to autoregulatory feedback activation of its native promoter, which previous literature indicated was critical for LRS. We generated a mutant that over-expressed *salT* further and through RNAseq identified teicoplanin resistance factor B (*trfB*), as being a key player in these mutant phenotypes. Overall, this study identifies and characterizes novel genes implicated in envelope homeostasis and virulence in *S. aureus*, including a novel regulatory pathway of SpA expression. These findings may elucidate novel targets for therapeutic intervention.

Ferric Uptake Regulator (Fur) plays a significant role in the survival of *Salmonella* Typhimurium on meat.

G. Bharathan[1], K.E.R. Chinchilla[2], H. Sheffield[1], M. Hayden[1], A. Madi[1], J. Buhr[3], and S. Srikumar[1].
1 Department of Poultry Science, College of Agriculture, Auburn University, Alabama, AL 36830
2 Zamorano University, Honduras, C.A.
3 US National Poultry Research Centre, Athens, Georgia

Food is an important source of *Salmonella* infections, and poultry products are one of the leading causes of disease outbreaks. The success of *Salmonella* Typhimurium as a pathogen lies in its ability to quickly adapt to the changing environment. This adaptability of the bacterium is strictly regulated by numerous DNA-binding transcriptional regulatory factors that respond to signals from the external environment. To identify the factors that are critical for the survival of *Salmonella* on meat, different deletion mutants of transcriptional regulatory factors of *Salmonella* Typhimurium ST 4/74 were grown on chicken meat pieces (breast fillets-1 gram piece each), incubated at 37°C and aliquots were plated onto XLD agar plates. The bacterial counts in CFU/mL were plotted against time to plot the growth curves. Of the 9 deletion mutants, ST4/74Δ*fur* (Ferric Uptake Regulator) was highly inhibited compared to the wild type. Fur protein, during iron-replete conditions, represses the siderophore production gene. On exhaustion of free iron in the environment, Fur protein is released, thereby activating the different siderophore genes, which allows the bacterium to chelate the iron (Fe^{3+}) bound to various proteins from the environment. Taking clues from the previous study in milk, inhibition of *fur* deletion mutant could be due to an intracytoplasmic iron accumulation, which could be toxic to the bacterium. To have a better understanding of iron regulation, we deleted the gene for enterochelin production, a major siderophore in *Salmonella* (*entC*). ST4/74Δ*entC* grew similar to the wild type, relaxing the inhibition from *fur* deletion. This led to the hypothesis that the deletion of the *entC* siderophore gene blocked the uptake of iron by the bacteria, thereby reducing the iron toxicity in the cytoplasm, which aided in a revival of the growth of the bacterium on the meat surface. Furthermore, the double mutant ST4/74Δ*fur*Δ*entC* also did not show a significant reduction in growth when compared to the wild type. Our data gives preliminary insights into how iron uptake by *Salmonella* Typhimurium in iron-rich meat is strictly regulated by *fur* and aids in the colonization of the food matrix.

#83

bb0556, an essential gene for *Borrelia burgdorferi* infectivity

S. George[1], C. Waldron[1], C. Thompson[1], Z. Ouyang[1]
[1]Department of Molecular Medicine, Morsani College of Medicine, University of South Florida, Tampa FL USA

The Lyme disease pathogen, *Borrelia burgdorferi* has many unique mechanisms for survival, adaptation and virulence. The genes *bb0554, bb0555* and *bb0556* are located in an operon and were previously proposed to form a xanthine dehydrogenase. Typically, these enzymes convert hypoxanthine to xanthine and xanthine to uric acid, a known scavenger of reactive oxygen species. Knockout of all three genes suggest the operon plays a role in *B. burgdorferi* response to reactive oxygen species. In contrast to previous studies, our work on a single gene knockout of *bb0556* did not show increased susceptibility to reactive oxygen species, reactive nitrogen species, or osmotic stress. However, the *bb0556* mutant remained non-infectious in the mammalian host using an *in vivo* luciferase imaging system. Bacterial burden was also diminished in the joint, heart, distal skin and injection skin tissues in the *bb0556* mutant compared to wild-type infected mice. These findings indicate the gene may play additional roles outside of a xanthine dehydrogenase complex that contribute to *B. burgdorferi* virulence.

#84

Elucidating the function and significance of MraW in *Bacillus subtilis*

E. Valdespino[1], M. White[1], P. Eswara[1]
[1]University of South Florida, Department of Molecular Biosciences, Tampa, FL. 33612

Cell division is a highly regulated process which is carefully orchestrated by a variety of proteins. The division and cell wall (*dcw*) cluster is highly conserved among prokaryotes and is responsible for regulating peptidoglycan synthesis and cell division. In *Bacillus subtilis*, the 5' region of *dcw* cluster consists of *mraZ-mraW-ftsL-pbpB*. While the roles of *mraZ, ftsL, and pbpB* have been previously defined, the role of *mraW* in the *dcw* cluster remains unclear. MraW is a 16S rRNA methyltransferase (N^4 cytosine C1402). The significance of methylating rRNA and its impact on protein synthesis and/or cell division remains unclear. Homologues of *mraW* are known to improve translation accuracy in *Escherichia coli* and contribute to oxidative stress response in *Staphylococcus aureus*. However, the exact molecular mechanisms are not clear. We have demonstrated that *mraW* overexpression is lethal in the presence of DNA damaging agents such as ciprofloxacin. Ongoing experiments aim to characterize the role of MraW in *B. subtilis* in the DNA damage response as well as translation fidelity.

Elucidating the Role of Transcriptional factors in the Multiplication of *Salmonella* Typhimurium in Chicken Egg yolk.

A. Madi, G. Bharathan, H. Sheffield, J. Buhr, and S. Srikumar

Department of Poultry Science, College of Agriculture, Auburn University, Alabama, AL36830

Chicken egg yolk is very nutritious, and therefore can support the multiplication of pathogenic microorganisms, particularly *Salmonella* Typhimurium. In contrast, egg whites are more antimicrobial in nature, suppressing *Salmonella* multiplication. Consequently, undercooked egg yolks and whites, as well as their products have often been associated with *Salmonella* outbreaks. Our study aims to understand how *Salmonella* adapts and survives in egg yolk, particularly when the pathogen is exposed to yolk associated stress and antimicrobial factors. We inoculated 10 *S.* Typhimurium mutants, each deleted for a gene encoding DNA binding transcription factors (TFs) in to pasteurized egg yolk and compared the growth kinetics to the isogenic wild type. Of these 10 mutants, particular strains *viz.* ST4/74Δ*fnr* (regulating anaerobic stress response), ST4/74Δ*hilD* (regulating SPI1 invasion) had aberrant growth kinetics compared with the wild type. While ST4/74Δ*fnr* was highly inhibited, ST4/74Δ*hilD* was hyper-replicating with respect to the wild type. The *fnr* gene encodes the FNR (fumarate and nitrate reduction) protein which plays a crucial role in the bacterial response to anaerobic stress. Inhibition of ST4/74Δ*fnr* shows that *Salmonella* is exposed to anaerobic conditions during survival in yolk. The *hilD* gene, regulates *Salmonella* virulence by modulating bacterial invasion to epithelial cells by regulating the type 3 secretion system producing *Salmonella* Pathogenicity Island 1 (SPI1). The hyper replication of the *hilD* mutant is intriguing – as this could show that the pathogen is trying to down regulate the expression of the *hilD* gene. We argue that the *hilD* downregulation could signal energy conservation by inhibiting the unnecessary activation of SPI1 during yolk survival. This observation, however, will need further validation studies. Overall, our findings provide early insights into the survival mechanisms used by *Salmonella* in egg yolk.

#86

Elucidating the role of LytH in surface protein trafficking

Scaffidi, S., Yu, W., Zhang, R., Cubero-Rojas, M.F.
Department of Molecular Biosciences, University of South Florida, Tampa, Florida, USA

The bacterial human pathogen *Staphylococcus aureus* possesses a myriad of virulence factors to cause disease. Staphylococcal protein A (SpA) is one of the most dominant cell wall anchored proteins utilized by *S. aureus* during host immune evasion. While the biochemical pathway of SpA secretion and presentation on the bacterial cell surface has been well studied, we aimed to identify factors capable of spatially or temporally regulating its trafficking. Here we performed a comprehensive genome-wide screening of 1,920 non-redundant transposon mutants of *S. aureus* USA300 using immunofluorescence (IF) microscopy, which allowed for the examination of *de novo* SpA on the cell surface. One of the major hits from this screen was a lytH mutant, which displayed a severe mislocalization of SpA. LytH, an autolysin-associated protein proposed to have peptidoglycan (PG) hydrolyzing activity, may play a role in cell wall dynamics, potentially influencing virulence. To further investigate this finding, we transduced the transposon insertion from the lytH mutant into *RN4220Δspa pCL55-Pitet-spa* and found that the SpA mislocalization was not due to *spa* transcriptional regulation. Immunofluorescence microscopy of membrane-bound SpA indicated that its trafficking was not disrupted in the *lytH* mutant, suggesting that LytH does not affect SpA synthesis or translocation. Using fluorescently labeled D-amino acids, we determined that SpA mislocalization is likely due to improper PG incorporation. Importantly, further microscopy of the cell wall and autolysis assays did not reveal signficant defects in morphology, cell division or autolysis in the transposon mutant. This indicates that the observed SpA mislocalization may be specific to interactions with the cell wall rather than broader cell division or autolysis processes. Future studies should explore the involvement of multiple PG hydrolases in this phenomenon to better understand the relationship between LytH, PG dynamics and protein localization. These insights could inform the development of new therapeutic strategies targeting *S. aureus* cell wall-related processes.