AMERICAN SOCIETY FOR MICROBIOLOGY
SOUTH CAROLINA BRANCH

UNIVERSITY OF South Carolina
School of Medicine Greenville

SC ASM 2021 FALL MEETING

Celebrating Student Achievements in Microbiology

9:00 - 9:10 AM	Welcome address **Dr. Steven Fiester,** President SC-ASM, UofSC School of Medicine Greenville **Dr. Desmond Kelly**, Associate Dean for Research, UofSCSOMG
9:10 - 9:15 AM	Introduction **Dr. Ginny Webb,** Vice-President SC-ASM, UofSC Upstate
9:15 - 10:15 AM	*Understanding interactions in microbial populations* **Dr. Steve Diggle,** ASM Distinguished Lecturer, Georgia Institute of Technology
10:15 - 10:30 AM	Morning Break
10:30 - 11:30 AM	*Expanding biological research at SRNL: new programs in biomanufacturing and biomaterials* **Dr. Vahid Majidi,** Director Savannah River National Laboratory ▪ Introduced by Clemson University Microbiology Club
11:30 - 12:30 PM	Lunch Break
12:30 - 2:15 PM	Concurrent Sessions **Session A: Undergraduate, Medical Student, and Postdoctoral Fellow Research Presentations** ▪ See page 2 for presenter information **Session B: Graduate Student Research Presentations** ▪ See page 2 for presenter information
2:15 - 2:30 PM	Afternoon Break
2:30 - 3:00 PM	*Stressing about stress granules: How SARS coronavirus interferes with cellular responses to viral infection* **Dr. Anita Nag**, USC Upstate ▪ Introduced by Upstate SC Chapter of ASM
3:00 - 3:30 PM	*Microbiome changes within the context of relapsing malaria infection in a longitudinal study of rhesus macaques* **Dr. Regina Joyce Cordy**, Wake Forest University ▪ Introduced by UofSCSOMG Student Chapter of ASM
3:30 - 3:45 PM	Awards
3:45 - 4:15 PM	Concurrent Sessions **Session C: Microbiology Careers Panel** – Moderated by Susan Morrison, Ph.D. ▪ Dr. Regina Joyce Cordy ▪ Dr. Steve Diggle ▪ Dr. Vahid Majidi ▪ Dr. Anita Nag **Session D: SC ASM Business meeting**
4:15 - 4:20 PM	Concluding Remarks
4:20 - 4:45 PM	Executive Committee Meeting (Branch officers only)

Student, Trainee, and Postdoctoral Fellow Research Presentations

Concurrent Sessions: 12:30-2:15 PM

Session A

Undergraduate Student Research Presentations ~10 minutes

- Tropheryma whipplei *manifesting in the CNS: case report and comprehensive literature review*
  - **Mary Hunter Hyche,** Furman University
- *Investigating interactions between bacteriophage Cain and its host's proteome*
  - **Dallas Nivens**, Winthrop University
- *Applications of R and Shiny for interactive visualizations of host-pathogen interactions in dual RNA-seq data*
  - **Andrea Rojas,** Furman University
- *Effects of the Coronavirus Disease 2019 (COVID-19) on blood culture contamination rates*
  - **Brianna Sacchetti**, UofSC Upstate

Medical Student Research Presentations ~10 minutes

- *A Comparison of* H. pylori *Detection Methods*
  - **Kathleen Hill,** UofSC School of Medicine Greenville
- *Examination of Drug Resistant Acinetobacter baumannii response to phagocytosis by monocyte-derived macrophages*
  - **Elias Wheibe**, UofSC School of Medicine Greenville

Postdoctoral Fellow Presentation 20 minutes

- *Community-level SARS-CoV-2 sequence diversity revealed by wastewater sampling*
  - **Candice Swift**, UofSC

Q and A for all presenters ~15 minutes

Session B

Graduate Student Research Presentations ~10 minutes

- *Abundance and Diversity of the Roseobacter Clade from Delaware And Chesapeake Bay Water Samples*
  - **Mir Alvee Ahmed**, Clemson University
- *Swe1 homologs in* Cryptococcus neoformans*: Roles in stress response, virulence, and the G2/M Checkpoint*
  - **Rodney J Colón Reyes**, Clemson University
- *Transcriptomic analysis reveals that municipal wastewater effluent enhances* Vibrio vulnificus *growth and virulence potential*
  - **Karlen E. Correa Velez**, UofSC
- *Genetic and Functional Diversity of Clade A Lucinid Endosymbionts from the Bahamas and Florida*
  - **Nichole M. Giani**, Clemson University
- *Generation and analysis of a fluorescent reporter system for virus gene family expression in lepidopteran host insects*
  - **Daniel Howard**, Clemson University
- *SARS-CoV-2 in wastewater bioaerosols, workers and across treatment processes*
  - **Mirza Isanovic**, UofSC
- Nigella sativa *as an antibiotic alternative in mitigating necrotic enteritis in broiler production*
  - **Vishal Manjunatha**, Clemson University
- *Dormitory SARS-CoV-2 wastewater surveillance as a COVID-19 response in a university campus setting*
  - **Sarah Sellers**, UofSC
- *Determining Importance of Rac2 in the Macrophage Response to Aspergillus fumigatus Infection*
  - **Chris Tanner**, Clemson University

Q and A for all presenters ~15 minutes

Undergraduate Student Research Presentation Abstracts

Tropheryma whipplei manifesting in the CNS: case report and comprehensive literature review

[1]Hyche MH, [2]Schammel CMG, [3]Devane M, [4]Fiester S, [5]Call M, [5]Kent P, [2,4]Knight J
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Tropheryma whipplei is a Gram-positive bacterium found in wastewater that can be transmitted to humans through feces and saliva. Rare infections manifest as Whipple disease (WD), with 18-30 new cases reported worldwide per year. As this bacterium typically targets the small intestine, gastrointestinal symptoms such as abdominal pain, diarrhea and weight loss are the typical presentation; other manifestations occur as swelling in the joints. Treatment involves a two- to four-week course of parenteral antibiotics followed by a year or more of oral antibiotic therapy. Short-term prognosis is typically good, but relapses, which have been reported as the most severe complication of infection, can occur even up to a few years after the initial infection, with a 2-33% recurrence after five years. Relapses typically manifest with central nervous system (CNS) symptoms including dementia, gait issues, memory and concentration problems and a variety of behavior/personality disorders and are noted in 20–40% of cases during the course of WD. Treatment for CNS infection involves antibiotics that penetrate the blood brain barrier, such as ceftriaxone, doxycycline, trimethoprim-sulfamethoxazole (TMP-SMX). Treatment can result in resolution of some CNS symptoms if the infection is caught early; however, prognosis is typically poor with a mean survival of four years.
Here we present a case of primary CNS T. whipplei, highlighting radiologic and histologic characteristics. Additionally, we present a comprehensive review of both the GI and CNS manifestation of this rare infection as reported in the literature with the goal of developing a diagnostic algorithm for early diagnosis and treatment to optimize patient outcomes.

Investigating interactions between bacteriophage Cain and its host's proteome

Dallas Nivens[1], Laela Walker[1], Bethany Wise[1] and Victoria J. Frost[1]
[1]Department of Biology, Winthrop University, Rock Hill, SC, USA

Bacteriophage and their host cells have been co-evolving for more than three billion years. This dynamic yet enduring relationship offers a wealth of opportunities for investigating the unknown molecular interactions occurring at the phage–bacterial interface. In our research, we are examining each individual gene product of bacteriophage Cain, a temperate phage in the K6 subcluster, and how it interacts with its host's proteome (*Mycobacterium smegmatis*). Previously, SEA-GENES students at Winthrop used phenotypic assays to identify several of Cain's gene products that effected the growth of *M. smegmatis*. To investigate this further, a Protein-Protein Interaction (PPI) assay has been developed (by Dr. D. Heller at the Howard Hughes Medical Institution). This assay specifically reveals which *M. smegmatis* protein interacts with the phage protein, as they are co-expressed in our system (known as a Bacterial Two-Hybrid, or B2H, assay). Cain gp*55*, a gene without a known function, was the first gene to be cloned into the B2H expression plasmid. With the assistance of inducible promoters and reporter genes, evidence has shown that Cain gp*55* interacts with NusA; a protein known to be an important cellular transcription regulator. The interaction occurring between these two proteins gives us insight into the possible function of Cain gp*55*, as well as other homologous genes found in similar phages. For example, one of these orthologs, Waterfoul gp*47*, also demonstrates the ability to interact with NusA in *M. smegmatis*. Furthermore, both Cain gp*55* and Waterfoul gp*47* interact with NusA in the pathogen, *M. tuberculosis*. This highlights the possible consistent function of these genes across other species of Mycobacteria. Knowledge of the intricacies of this system offers a detailed insight into what fuels the co-evolution of the two microbial adversaries. Significantly, such investigations also provide opportunities for discoveries that may have medical, biotechnological and/or industrial applications.

Investigating the Effects of Type II Heat-Labile Enterotoxins on Human Respiratory Immune Responses

Andrea R. Rojas[1], Margaret C. Stroud[1], Mary-Peyton Knapp[2], Adam Okinaga[3], Terry D. Connell[4], Steven E. Fiester[2], Sergio Arce[2], Jennifer T. Grier[2]

[1] Furman University, [2]University of South Carolina School of Medicine Greenville,, [3]University of South Carolina [4]University at Buffalo Jacobs School of Medicine

Heat-Labile Enterotoxins (HLTs) are bacterial secreted proteins with unique immune properties. Type II HLTs are produced by certain strains of E. coli and have been found to act as mucosal and systemic adjuvants. The goal of this study is to determine the potential of three Type II HLTs, LT-IIa, LT-IIb, and LT-IIc, to function as adjuvants to boost respiratory specific immune responses. The human lung epithelial cell line, A549, was exposed to HLTs in the presence or absence of lipopolysaccharide (LPS), a known immunostimulant. The impact of enterotoxin exposure on cell structure and viability was measured by flow cytometry. Changes to immune gene expression were observed via quantitative reverse transcriptase polymerase chain reaction (qRT-PCR) to detect mRNA or enzyme linked immunosorbent assay (ELISA) to measure cytokine secretion. The addition of HLTs resulted in no significant changes to cell complexity or viability with or without LPS. Analysis of mRNA expression found that the immune response triggered by HLTs significantly differed from that induced by LPS. Secretion of IL-1β was decreased in the presence of HLTs and modulation of IL-6 secretion was also observed. Based on these results, we can conclude that Type II HLTs, can drive an altered immune response, on both an mRNA and protein level, without significant cellular cytotoxicity. Further study is needed to determine the mechanisms by which the HLTs may modulate immune signaling in the respiratory tract, particularly in the presence of other immune adjuvants. This study demonstrates that HLTs trigger immune responses that differ from standard adjuvants which may have therapeutic potential in treatments for targeted delivery to the respiratory tract.

Effects of the Coronavirus Disease 2019 (COVID-19) on blood culture contamination rates

Brianna Sacchetti[1], Justin Travis, Ph.D. [2], Ginny Webb, Ph.D. [1]

[1]USC Upstate Division of Natural Sciences and Engineering, [2]USC Upstate Department of Psychology

Hospital systems worldwide are continuously confronted with challenges caused by the coronavirus pandemic, ranging from needs for additional staffing to shortages of testing equipment. Blood cultures are necessary for the diagnosis of bloodstream infections, but little research has been completed regarding the relationship between the COVID pandemic and blood culture collection and contamination rates. This study compares patterns of blood culture collection and contamination rates separated by different staff types, hospital units, and collection techniques from before and during the COVID pandemic in one hospital. The relationship between community COVID rates and blood culture contamination rates was also analyzed. We found a significant increase of cultures collected monthly by nursing staff and those collected from a peripheral site after the onset of the pandemic, with a decrease in draws collected by phlebotomy staff. Contamination rates elevated from 2.1% during the pre-COVID period to 2.5% in all non-emergent departments during the pandemic. Nurse draws also saw an increase of contamination from 2.0% to 2.4%, for both peripheral (2.1% to 2.5%) and indwelling line draws (1.1% to 1.7%) after the start of the pandemic, but no changes in contamination were seen for phlebotomists. Adult acute and both adult and pediatric emergency departments saw increases in blood culture collection and contamination increased in adult acute, adult emergency, and pediatric intensive care units by 23%, 75%, and 59% respectively. A moderate positive correlation was found between state and county COVID incidence rates and monthly hospital blood culture contamination rates. Based on these results, the COVID pandemic has had an effect on blood culture collection and contamination in this hospital system, so it is suggested for medical facilities to implement repeated staff training on current blood collection techniques and to emphasize the importance of aseptic technique to decrease the incidence of contamination.

Medical Student Research Presentations Abstracts

A Comparison of *H. pylori* Detection Methods

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[1]University of South Carolina School of Medicine Greenville; [2]Kenyon College; [3]Clemson University; [4]Department of Epidemiology, Arnold School of Public Health, University of South Carolina; [5]Pathology Associates, Department of Pathology, Greenville SC; [6]Clinical Microbiology – Greenville Health System; [7]Clinical Chemistry – Greenville Health System

Background: Helicobacter pylori *(H. pylori*) infects over 50% of the world's population and can lead to gastric cancer if left untreated. An estimated 26,000 gastric cancer cases will occur in the United States in 2021 with 40% of cases becoming the primary cause of death. Invasive and non-invasive techniques are used to diagnose *H. pylori* infection, however, there is controversy regarding what technique should be considered the "gold standard" for diagnosis. **Objective**: To evaluate the efficacy of *H pylori* invasive detection methods: stained biopsy and Rapid Urease test [RUT]). **Methods:** 200 patients (100 H. pylori + and 100 H. pylori -) from a single institution that underwent gastric biopsies were retrospectively evaluated for *H. pylori* status. Demographics and clinicopathologic data were collected including diagnostic tests performed, treatment, and outcomes. **Results:** When histology was identified as positive, RUT was also positive (92.5%), likewise, when histology was negative, RUT was negative (93.9%, Table 3). Disparate results occurred in 7% of samples with n=2 (6.1%) of histology positive when RUT was negative and n=3 (7.5%) histology negative but the RUT test was positive ($p<0.001$). Of those that were *H. pylori* positive, 60% had a post-treatment test completed. Gastric cancer developed in 3 patients (1.5%), all of which were *H. pylori* positive. **Conclusions**: This study found that histology and RU testing yield similar results, therefore, there is no efficacious reason to run both tests. Since histology has a greater sensitivity (>95%), it should be considered the "gold standard" as the literature suggests.

Examination of Drug Resistant *Acinetobacter baumannii* response to phagocytosis by monocyte-derived macrophages

Elias Wheibe[1], Kyleigh Connolly[1], Steven E. Fiester[1,2], and Jennifer T. Grier[1]
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Acinetobacter baumannii is a nosocomial opportunistic pathogen that is usually associated with mucosal infections of various types. Of late, *A. baumannii* has been flagged by the CDC as an urgent threat due to its multidrug-resistant properties, making it a major concern within the public health community. It has been difficult to understand how this bacterium is able to gain a foothold and cause severe infections. We sought to study how necrotizing fasciitis-associated strains of *A. baumannii* interact with human macrophages to assess their capabilities and potential virulence factors regarding evasion of the immune response. Thus, extensively drug resistant *A. baumannii* was isolated at two timepoints (strains NFAb-1 and NFAb-2) from a patient over the course of a lethal necrotizing fasciitis infection that led to sepsis. The intracellular survival of these two strains was compared with each other, as well as to the type strains, ATCC 19606 and ATCC 17978, within a human macrophage-differentiated monocyte cell line, THP-1. Survival studies were complemented with quantitative reverse transcriptase-polymerase chain reaction (qRT-PCR) to investigate expression of potential bacterial virulence factors, Catalase E (KatE), Phospholipase C (PLC), Outer membrane protein A (ompA), and Pilus A (PilA), that could contribute to immune evasion. The intracellular survival assay demonstrated enhanced survival and bacterial growth of NFAb-1 and NFAb-2 within macrophages compared to type strains. Examining gene expression, KatE, PLC, and OmpA didn't show any significant differences, while the PilA gene had much higher expression in NFAb-1 and NFAb-2. Taken together, this data establishes that these necrotizing fasciitis-causing *A. baumannii* strains are more likely to survive a macrophage-mediated immune response and expression of pilus-associated genes may play a role. Our studies demonstrate a need to further investigate *A. baumannii* mechanisms of virulence to understand its ability to evade the immune response in hopes of understanding and combating infections.

Post-doctoral Research Presentation Abstract

Community-level SARS-CoV-2 sequence diversity revealed by wastewater sampling

Candice L. Swift[1], Mirza Isanovic[1], Karlen E. Correa Velez[1], R. Sean Norman[1]
[1]Department of Environmental Health Sciences, University of South Carolina, USA

Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), the causative agent of the ongoing COVID-19 pandemic, is shed in the feces of symptomatic and asymptomatic patients and is thus detectable in the influent to wastewater treatment plants. Wastewater surveillance of SARS-CoV-2 offers several advantages compared to clinical sampling alone, including the ability to independently monitor infection trends apart from clinical data, detection of asymptomatic cases, and earlier detection of positive cases and associated infection trends. Furthermore, wastewater samples are a composite of many individuals, which facilitates community-level health monitoring. Here, we sequenced SARS-CoV-2 amplified from raw wastewater collected from two South Carolina wastewater treatment plants during July 2020 and January 2021, which spanned two peaks in SARS-CoV-2 transmission in the state. In the wastewater collected during January 2021, we detected single nucleotide substitutions resulting in four signature mutations in the surface glycoprotein (spike protein) that are associated with variants of concern (VOCs): S477N (B.1.526, Iota), T478K (B.1.617.2, Delta), D614G (present in all VOC as of May 2021), and H655Y (P.1, Gamma). Notably, the N501Y mutation, which is a signature mutation in the Alpha, Beta, and Gamma VOCs, was identified in samples from July 2020, although these VOCs were not known to be prevalent in South Carolina during this time. Comparisons of mutations detected in this work to sequence repositories such as NCBI Virus and GISAID demonstrated that several of the mutations were reported in data collected from other states but were not captured in the deposited clinical data from South Carolina. This work affirms the value of wastewater surveillance as a public health tool to capture the sequence diversity of SARS-CoV-2.

Graduate Student Research Presentation Abstracts

Abundance and Diversity of the Roseobacter Clade from Delaware And Chesapeake Bay Water Samples

Alvee Ahmed[1], Elio M. Ortiz[1], Barbara J. Campbell[1]
[1] Department of Biological Sciences, Clemson University, Clemson, SC, USA

The Roseobacter clade is a major group in marine bacterioplankton communities. Roseobacters are found as free living, particle associated or commensals of marine plankton and animals. We focused on the temporal and spatial abundance and functional potentials of Roseobacters in two important estuaries of the US, the Chesapeake and Delaware Bays. We binned 28 high-quality (>90% complete, <5% contamination) Roseobacter metagenome assembled genomes (MAGs) from 36 metagenomes sequenced from Chesapeake and Delaware bay water samples. A phylogenomic tree prepared using Anvi'o-identified 70 single copy marker genes showed most of the Roseobacter MAGs were only distantly related to 62 previously reported Roseobacer genomes/MAGs. Based on average amino acid identity (AAI) values calculated by MiGA, only a few MAGs were similar to one of two known Roseobacter genomes- *Planktomarina temperata* RCA23 and *Rhodobacterales bacterium* HIMB11. Such Roseobacter MAGs were abundant in medium and high salinity samples in both bays whereas MAGs related to *P. temperata* RCA23 were abundant in spring and *R. bacterium* HIMB11 related MAGs were abundant in summer and fall. Peak to Trough Ratio (PTR) indicated the latter group of MAGs were actively replicating in the estuarine environments during summer months. Metatranscriptomic analyses showed that MAGs used in this study and closely related genomes/MAGs from previously reported studies vary in expression of some genes. Roseobacter MAGs from two bays are novel and they express genes of unique functional potentials which will help to understand the biogeochemical cycles in dynamic estuarine environments.

Swe1 homologs in *Cryptococcus neoformans*: Roles in stress response, virulence, and the G_2/M Checkpoint

Rodney J Colón Reyes[1*], Jin-Tae Choi[2*], Yong-Sun Bahn[2], Lukasz Kozubowski[1]
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Wee1 Serine/Threonine kinase is a conserved cell cycle regulator acting in the G_2/M checkpoint in fungi and animals. Here we report that the two Wee1 homologs, CnSwe1 and CnSwe102, present in the genome of the human fungal pathogen *Cryptococcus neoformans*, are important components of the G_2/M checkpoint. Single deletion strains *swe1*Δ and *swe102*Δ were generated and a strain that harbors *swe102*Δ deletion and expresses *SWE1* from a promoter suppressed by copper was also constructed. These mutant strains were subjected to various DNA damaging agents and stressors such as osmotic and ER stress. Strikingly, cells lacking Swe102 and expressing Swe1 at reduced levels were significantly more sensitive to hydroxy urea and MMS, agents that interfere with replication. CnSwe1 and CnSwe102 were tagged with a fluorescent tag, mCherry and used to complement the single deletions and to elucidate their subcellular localization. Both proteins exhibited an overall diffuse localization throughout the cell. Both deletion strains exhibited slower growth at 30 and 39˚C as compared to the WT. The *swe102*Δ strain was less virulent than the wild type in animal models. We are currently investigating if treatment with DNA damaging agents can cause the localization of CnSwe1 or CnSwe102 into distinctive puncta in *C. neoformans* and if CnSwe1 and/or CnSwe102 can complement the *S. cerevisiae swe1*Δ mutant.

Transcriptomic analysis reveals that municipal wastewater effluent enhances *Vibrio vulnificus* growth and virulence potential

Karlen E. Correa Velez[1,2] and R. Sean Norman[1,2]
[1]Department of Environmental Health Sciences, University of South Carolina; [2]NIEHS Center for Oceans and Human Health and Climate Change Interactions, University of South Carolina

Vibrio vulnificus is an opportunistic pathogen indigenous to estuarine and marine environments and associated with aquatic organisms. *V. vulnificus* is of utmost importance because it causes 95% of the seafood-related deaths in the US due to rapid progression of septicemia. Changes in environmental parameters associated with climate change and coastal population expansion are altering geographical constraints, resulting in increased *Vibrio* spread, exposure, and rates of infection. In addition, coastal population expansion is resulting in increased input of treated municipal sewage into areas that are also experiencing increased *Vibrio* proliferation. This study aimed to better understand the influence of treated sewage effluent on effluent-receiving microbial communities using *Vibrio* as a model of an opportunistic pathogen. Integrated transcriptomic approaches were used to analyze the changes in overall gene expression of *V. vulnificus* NBRC 15645 exposed to wastewater treatment plant (WWTP) effluent for a period of six hours using a modified seawater yeast extract media that contained 0%, 50%, and 100% filtered WWTP effluent. RNA-seq reads were mapped, annotated, and analyzed to identify differentially expressed genes using the Pathosystems Resource Integration Center analysis tool. The study revealed that *V. vulnificus* responds to wastewater effluent exposure by activating cyclic di-GMP-influenced biofilm development. Also, genes involved in crucial functions, such as nitrogen metabolism and bacterial attachment, were upregulated depending on the presence of treated municipal sewage. This altered gene expression increased *V. vulnificus* growth and proliferation and enhanced genes and pathways involved in bacterial survival during the early stages of infection in a host. These factors represent a potential public health risk due to exposure to environmental reservoirs of potentially *Vibrio* strains with enhanced virulence profiles in coastal areas.

Genetic and Functional Diversity of Clade A Lucinid Endosymbionts from the Bahamas and Florida

Nichole M. Giani[1], Jean Lim[2], Annette S. Engel[3], and Barbara J. Campbell[1]
[1]Department of Biological Sciences, Clemson University; [2]Rosenstiel School of Marine and Atmospheric Science, University of Miami; [3]Department of Earth and Planetary Sciences, University of Tennessee–Knoxville

Lucinidae clams are known to harbor environmentally acquired endosymbionts within their gills. Although there has been recent work on the genomic and functional diversity of lucinid endosymbionts, there are uncertainties regarding the extent of their diversity and differences in metabolisms from different locations. Location is hypothesized to affect the genetic and functional potential of lucinid endosymbiont. The analyses used in this study include species diversity, genetic (phylogenomic and 16S rRNA gene) and functional diversity (KEGG), phage presence and horizontal gene transfer. In this study, the genetic and functional differences in endosymbionts were examined for six lucinid host species collected from eight sites in Florida, USA, and San Salvador, The Bahamas. Forty-two metagenome assembled genomes (MAGs) were generated and clustered into three species and six subspecies. MAGs were closely related to thiotrophic, gammaproteobacterial clade A endosymbionts recognized as the genus *Ca*. Thiodiazotropha from predominately seagrass-dwelling lucinids, but separated into three species and six subspecies from different hosts based on phylogenomics, average nucleotide identity (ANI), and average amino acid identity (AAI) analyses. All subspecies had several genes related to systems involved in sulfide, nitrogen, and amino acid metabolism, defense, vitamins, and structure/motility. Most of the metabolic variability among species involved carbohydrate metabolism and membrane transport where the most abundant accessory genes were in energy metabolism and signaling/cellular processes. Prophage and horizontally transferred genes analyses were used to determine potential causes for the genetic and functional differences shown at different locations in the endosymbionts. Genes were found to be transferred within the same species regardless of the location while the location affected the types and amount of prophage components present. This research significantly expands the known diversity and representatives from three lucinid endosymbiont species and suggests key differences in genetic adaptations based off of different locations and processes found at these locations.

Generation and analysis of a fluorescent reporter system for virus gene family expression in lepidopteran host insects

Daniel Howard[1], Alexis Yoh[2], Matthew Turnbull [1,3]
[1]Departments of Biological Sciences, [2]Genetics and Biochemistry, and [3]Plant and Environmental Sciences, Clemson University

Parasitoid wasps are one of the most diverse groups of organisms on the planet. They play an important role in pest control as they often infest pest lepidopteran larvae (caterpillars). Some wasps in this group are associated with mutualistic viruses known as Polydnaviruses (PDVs). PDVs are integrated into the wasp genome, only replicate within wasp ovarian tissue, and are transmitted during parasitization of a lepidopteran host. PDV-encoded products suppress host immunity, allowing the wasp egg to reach maturity. No PDV replication is observed in lepidopteran hosts, but differing levels of PDV gene expression are observed in susceptible and non-susceptible hosts, leading researchers to postulate that PDV gene regulation plays an important role in the host range of the wasp. We hypothesize that the expression pattern of the Vinnexin (Vnx) genes from the PDV associated with *Campoletis sonorensis* (CsIV) will differ within and across susceptible and non-susceptible hosts. To test this, we are generating a series of baculovirus expression vectors (BEVs) encoding dual fluorescent reporters informing as to vector infection (mCherry) and putative promoter activity (Ft-N1 fluorescent timer). BEVs being synthesized drive Ft-N1 expression by forward or reverse upstream regions from three CsIV Vinnexin genes (vnxG, vnxQ1, vnxQ2) and the previously characterized promoter region of CsIV cys-motif gene WHv1.6. We report results of Ft-N1 kinetics in the Sf9 cell line. Initial BEV studies will characterize reporter kinetics in permissive Sf9 cells. Once verified, recombinant reporter-viruses will be used to infect lepidopteran larvae that are permissive, semi-permissive, and non-permissive to CsIV. We predict significant differences in timer expression between genes in sf9 cells as well as across larval species and tissues.

SARS-CoV-2 in wastewater bioaerosols, workers and across treatment processes

Mirza Isanovic[1], Karlen E. Correa Velez[1], and R. Sean Norman[1]
[1]Department of Environmental Health Sciences, Arnold School of Public Health, University of South Carolina, Columbia, SC, United States

Within urban and suburban sewersheds, SARS-CoV-2 released through urine and feces is transported through sewage systems into municipal wastewater treatment plants (WWTPs). Studies have shown that viral RNA is detectable in untreated wastewater but not in WWTP effluent. In this study we investigated the treatment steps between the influent and final treated effluent to identify the point at which viral RNA is no longer detectable. Additionally, we examined air surrounding high turbulence treatment steps to test for the presence of SARS-CoV-2 RNA in WWTP-generated bioaerosols. To examine potential worker exposure to SARS-CoV-2, WWTP workers were also tested for presence of SARS-CoV-2. The data show that despite the high viral concentration in the influent, SARS-CoV-2 RNA concentration decreased significantly in the main treatment steps and was not detected in the effluent. Additionally, SARS-CoV-2 RNA was not detected in any air samples and the worker rate of infection was not significantly different from the rate of infection in the surrounding community. Together, these results suggest that the WWTP workers are not at an increased risk of contracting COVID-19 and that the WWTP successfully reduces the amount of SARS-CoV-2 that enters effluent receiving waters providing a vital public health service to communities.

***Nigella sativa* as an antibiotic alternative in mitigating necrotic enteritis in broiler production**

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As consumers continue to demand antibiotic-free animal products, the poultry industry is facing significant challenges of coccidiosis and necrotic enteritis (NE) which lead to high mortality and unacceptable growth without antibiotic treatment. The current study aims at using the natural product *Nigella sativa* (black cumin seed) in poultry feed to inhibit coccidiosis and prevent or lessen NE in broilers. After examining five commercial black cumin seed oil (BCSO) products, the brand with the highest anti-*Clostridium perfringens* (Cp) activity was selected for *in vivo* study. GC-MS analysis of the oil revealed the presence of major bioactive compounds including p-cymene, thymoquinone, carvacrol and thymol. An i*n vivo* study consisted of 320 Cobb 500 broiler chicks distributed randomly among five treatments, each replicated eight times with eight chicks per replicate. The animal trial compared two inclusion levels (2 and 5 ml/kg) of BCSO in feed as the treatments for chickens challenged with coccidia and Cp strain Cp#4. Broiler growth performance and disease outcomes were measured for the animal trial. BCSO levels of 2 and 5 ml/kg were effective in reducing NE lesion scores and mortality as compared with the positive control group, but they were less effective in reducing mortality as compared with bacitracin methylene disalicylate (BMD) antibiotic control. Additionally, broiler performance, esp. in weight gain, was improved with BCSO as supplements to a certain extent when there was a Cp infection in broiler chickens. Further, cecal samples on days 21 and 28 continued to decline in numbers of Cp spores with increasing concentrations of BCSO, and significant reduction ($p < 0.05$) of both spores and vegetative cells of Cp were noted on day 28 as compared with positive control. In conclusion, *Nigella sativa* has the potential as a natural alternative to commonly used antibiotic treatment in mitigating *C. perfringens* infection and mortality.

Dormitory SARS-CoV-2 wastewater surveillance as a COVID-19 response in a university campus setting

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The University of South Carolina, a large university in a small urban city, turned to several risk mitigation tools in response to the Coronavirus Disease 2019 (COVID-19) pandemic as students and staff returned to the campus in person for the fall 2020 semester. Part of the university's public health response was to use building-level wastewater surveillance of the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) to track COVID-19 hotspots as the disease spread throughout the campus community and to direct the university's pop-up clinical testing. This study aims to assess the effectiveness of the university's environmental monitoring approach in slowing the spread of COVID-19 in high-density facilities, such as on-campus student housing (dormitories). We collected 24-hour composite wastewater samples from 11 buildings across the University of South Carolina's campus, including dormitories and the designated isolation/quarantine building. The wastewater samples were then homogenized, centrifuged, and concentrated to 400 µL for RNA extraction. The RNA was amplified using the multiplex N1 and N2 primer/probe set in a droplet digital polymerase chain reaction (ddPCR). Finally, viral abundance was calculated following the data from the ddPCR and sent to the university's administrative staff to appoint the clinical testing team to buildings with high viral RNA shedding. Once the student was further identified and isolated, the contact tracing team could quarantine students considered close contact. We found a trend between the concentration of SARS-CoV-2 in the campus's wastewater and the number of COVID-19 cases identified in each housing location. However, further analysis is needed to strengthen this correlation and determine how extrinsic factors may contribute to the viral signal. Eventually, we aim to approximate the prevalence of COVID-19 cases without relying on individual testing to identify positive cases by using the viral load of SARS-CoV-2 in the community's wastewater.

Determining Importance of Rac2 in the Macrophage Response to Aspergillus fumigatus Infection

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Aspergillus fumigatus is a fungus found ubiquitously in the environment including in the air we breathe. Though not a threat to most people, immunodeficient or immunosuppressed individuals are at risk for developing severe infection, including the life-threatening condition of invasive aspergillosis. The leukocyte-specific GTPase protein Rac2 is associated with major roles in innate immune defense. Currently Rac2 has been demonstrated crucial for survival against a variety of infections. Here, we use rac2 mutant larval zebrafish lines and morpholino approaches to elucidate roles of Rac2 in mounting the host defense response against *A. fumigatus* infection. Zebrafish provide an optimal model for studying leukocyte behavior with in-vivo imaging techniques. Preliminary data demonstrates significantly impaired survival in zebrafish lacking Rac2 expression during *A. fumigatus* infection. Fluorescence microscopy imaging data suggests no deficiencies in macrophage migration and phagocytosis without rac2, suggesting its roles may lie further downstream. Rescue of rac2 expression in macrophages alone will be done to assess any exclusively attributable benefit to survival of infection. Previous studies demonstrate a lack in basic motility for rac2-/- neutrophils, hence the focus on macrophage contributions. Rac2's importance in macrophage migration, chemotaxis, phagocytosis, ROS and spore killing will be assessed to better understand the behavior of macrophage Rac2 in *A. fumigatus* infection, and its importance in host defense.