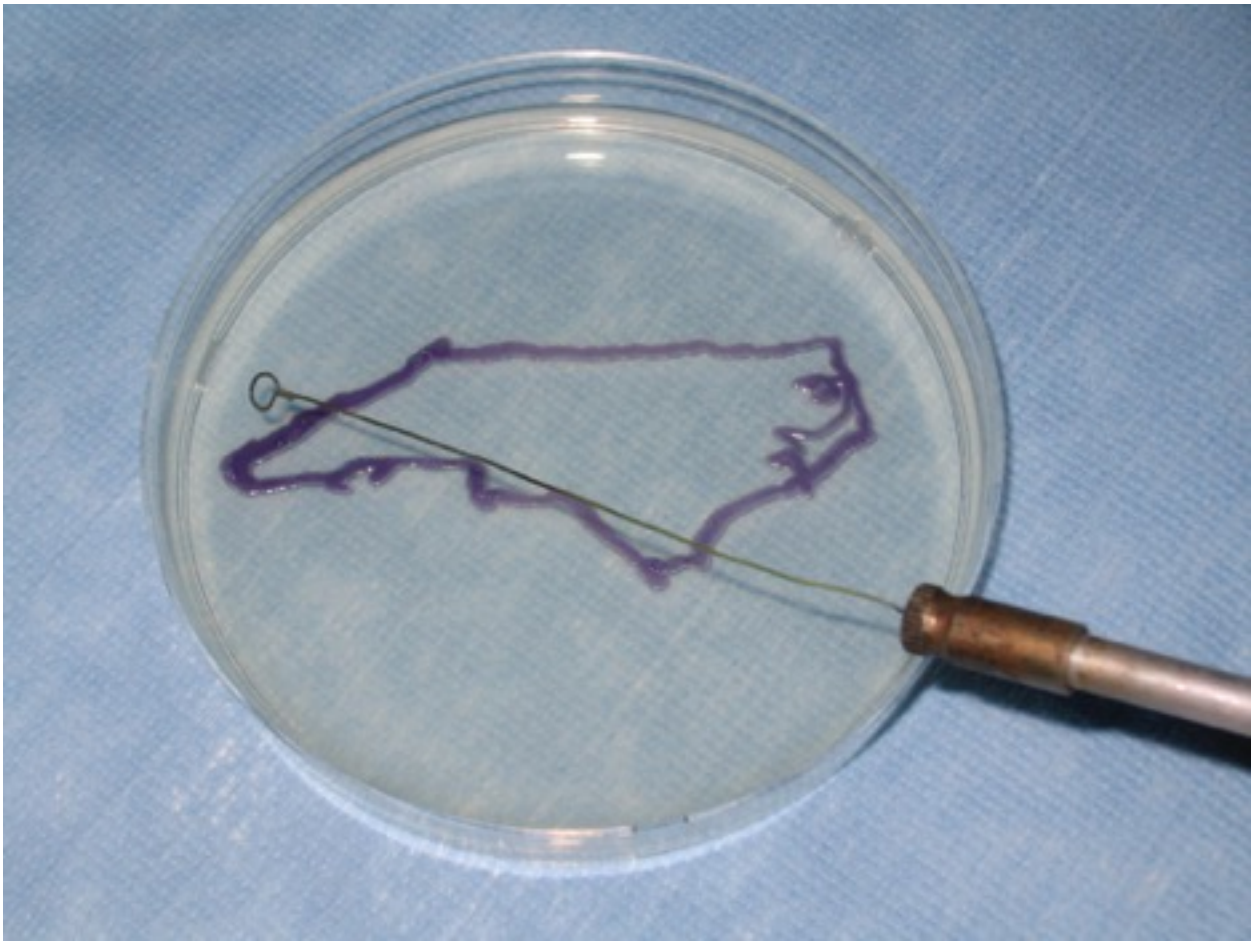
American Society for Microbiology | North Carolina Branch

North Carolina American Society for Microbiology

2016 Meeting



UNCW University of North Carolina Wilmington

October 1, 2016

Meeting Sponsors

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Schedule		
Preliminaries		
7:45	Registration Poster and talk set-up Breakfast/Coffee break Award Committees meeting/organization Vendors/Sponsors set-up	
8:45	Art Frampton	Welcome Introductory comments
Session 1 : Kevin Kiser, Chair		
9:00	Jenessa Winston	*Characterization of clinically relevant genetically tractable Clostridium difficile strain R20291 in a mouse model*
9:15	Brandon Anjuwon-Foster	*A Genetic Switch Controls the Production of Flagella and Toxins in Clostridium difficile*
9:30	Brian Moy	*The alternative sigma factor rpoN is an essential gene in Flavobacterium johnsoniae.*
9:45	Manita Guragain	*Dual Regulation of Essential Gene Expression and Biofilm Formation in Bordetella*
10:00	Richard Sobe	*Spermine negatively regulates Vibrio cholerae biofilm formation through the NspS/MbaA signaling system*
10:15	Kate Zulauf	*Export of phagosome maturation arrest effectors by the SecA2-dependent protein export system of Mycobacterium tuberculosis*
10:30	**Poster session 1** (Even-numbered posters should be attended) Coffee break Vendors/Sponsors	
Session 2 : Ryan Rhodes, Chair		
11:15	Robert Schilke	*CD40 increases oncolysis of GBM cells.*
11:30	Ryan Schuchman	*Identification of the pH sensitive step(s) in alphavirus replication*
11:45	Byron Hamilton	*What's an old book good for? Testing an Ayurvedic Cure for Acne*
12:00	Alexandra Barbour	*Microbial Characterization of Eastern Bluebird (Sialia Sialis) Nestling Fecal Sacs*
12:15	Zachary Johannesson	*Triclosan resistance in S. aureus isolated from noses and throats of nursing students*

Schedule		
12:30	Lunch Vendors/Sponsors Posters (unattended)	
Session 3 : Maryam Ahmed, Chair		
1:30	Suchawan Pornsukarom	*Assessing the impact of manure application in commercial swine farms on the transmission of antimicrobial resistant Salmonella in the environment*
1:45	Neveen Issa	*The Purification and Kinetic Analysis of Lactate Dehydrogenase Extracted from Beef Liver*
2:00	Jesse O'Campo	*Optimization of Xenorhabdus nematophilus Bacterial Growth Through Variation of Original Yoo Media Using a 5Liter Sartorius Stedim Bioreactor*
2:15	Elizabeth Gerdes	*Variation of Yoo Media for Optimization of Photorhabdus luminescens Phase I Bacterial Growth Using a 2 Liter Sartorius Stedim Bioreactor*
2:30	Ellen Johnson	*Identification of differentially regulated genes in Borrelia burgdorferi cells starved for N-acetylglucosamine*
2:45	Yawen Zhai	*Strategies to reduce bloating incidence in cucumber fermentation brined with calcium chloride*
3:00	Poster session 2 (Odd-numbered posters should be attended) Coffee break Vendors/Sponsors	
Plenary session : Wrennie Edwards and Art Frampton, Chairs		
4:00	Helen Lazear *NC Invitational Lecture*	*Zika Virus: New Pathogenic Phenotypes from an Old Virus*
4:30	Karl Klose *ASM Branch Lecture*	*Deadly Diarrhea: Vibrio cholerae in the time of cholera*
Postscript		
5:30	Art Frampton	Concluding remarks Awards
6:00	Reception	
8:00	**Adjournment**	

Abstracts (short talks)

1.1) **Jenessa A. Winston**, Rajani Thanissery, Stephanie A. Montgomery, and Casey M. Theriot

Characterization of clinically relevant genetically tractable Clostridium difficile strain R20291 in a mouse model

Clostridium difficile infection (CDI) is associated with increasing morbidity and mortality consequently posing an urgent threat to public health. Recurrence of CDI after successful treatment with antibiotics is high, thus necessitating discovery of novel therapeutics against this pathogen. To evaluate therapeutics against *C. difficile*, a mouse model approximating human disease with a clinically relevant strain is needed. We aimed to characterize the clinical course, including weight loss, bacterial load, toxin activity, and intestinal histopathology of mice challenged with clinically relevant genetically tractable *C. difficile* strain R20291. Five week old C57BL/6 WT JAX mice were pretreated with cefoperazone in their drinking water for 5 days, allowed a two-day wash out with regular water, and challenged with 105 spores of *C. difficile* R20291 on day 0. Mice were colonized with 107 colony-forming units of *C. difficile* per gram of feces on day 1. The most significant weight loss and clinical signs of disease in mice were seen between day 2 and 5. Mice remained persistently colonized with *C. difficile*, however weight gain and resolution of clinical signs were observed after day 7. *C. difficile* toxin activity, intestinal histopathology and alteration to the gut microbiota were defined throughout day 14 post challenge. This model was not uniformly lethal allowing for observation of a prolonged clinical course of infection concordant with human disease. Overall, this characterized *C. difficile* mouse model proves a valuable experimental platform to assess effects of novel therapeutics on amelioration of clinical disease and restoration of colonization resistance against *C. difficile*.

1.2) **Brandon R. Anjuwon-Foster** and Rita Tamayo

A Genetic Switch Controls the Production of Flagella and Toxins in Clostridium difficile

In the human intestinal pathogen *Clostridium difficile*, flagella promote adherence to intestinal epithelial cells. Flagellar gene expression also indirectly impacts production of the glucosylating toxins, which are essential to diarrheal disease development. Thus, factors that regulate the expression of the flgB operon will likely impact toxin production in addition to flagellar motility. Here, we report the identification a flagellar switch that controls the phase variable production of flagella and glucosylating toxins. The flagellar switch, located upstream of the flgB operon containing the early stage flagellar genes, is a 154 bp invertible sequence flanked by 21 bp inverted repeats. Bacteria with the sequence oriented according to the published genome expressed flagellar and toxin genes, produced flagella, and secreted the toxins (flg phase ON). Bacteria with the sequence in the inverse orientation were attenuated for flagellar and toxin gene expression, were aflagellate, and showed decreased toxin secretion (flg phase OFF). The orientation of the flagellar switch is heritable, but also reversible under distinct conditions for both phases. Lastly, we determined that the tyrosine recombinase RecV, which catalyzes inversion at the cwpV switch, is also responsible for inversion at the flagellar switch in both directions. Phase variable flagellar motility and toxin production suggests that these important virulence factors have both advantageous and detrimental effects during the course of infection.

1.3) **Brian E. Moy**, Ryan G. Rhodes

The alternative sigma factor rpoN is an essential gene in Flavobacterium johnsoniae.

Flavobacterium johnsoniae serves as a model organism for studying gliding motility, biochemistry and gene regulation in Bacteroidetes. Analysis of the *F. johnsoniae* genome revealed an RpoD homologue, an RpoN homologue and multiple extracytoplasmic function (ECF) sigma factors. The regulatory role of RpoN in Bacteroidetes is unknown, but has been well characterized in other organisms as being important for responding to environmental conditions and stressors. In this study we investigate the role of the alternative sigma factor rpoN (fjoh_0584) in *F. johnsoniae*. A reverse genetic approach was employed using a previously described allelic exchange system, in which regions upstream and downstream of rpoN were amplified via PCR and ligated into the suicide vector pRR51 to generate pBEM12. Following multiple conjugations with this deletion construct and analysis of 300 second recombinants a viable _rpoN mutant was not obtained, suggesting this sigma factor may be essential in *F. johnsoniae*. To provide additional evidence that deletion of rpoN is lethal in *F. johnsoniae* we generated a diploid rpoN strain using allelic exchange. The deletion construct (pBEM12) was transferred to the diploid rpoN strain, and 7 _rpoN mutants were confirmed from 50 second recombinants. Together, these results suggest that rpoN is essential in *F. johnsoniae*, which is a rare - but not unprecedented - finding when considering all organisms in which RpoN has been studied. To our knowledge this is the first study to evaluate the role of rpoN in a Bacteroidetes, and suggests this sigma factor plays an important role in basic cellular processes in *F. johnsoniae* and possibly other organisms in the phylum.

1.4) **Manita Guragain** and Rajendar K. Deora

Dual Regulation of Essential Gene Expression and Biofilm Formation in Bordetella

Bordetella are Gram-negative bacteria that colonize the respiratory tract of humans and animals. *B. bronchiseptica* causes respiratory disease and infections in animals and humans. *B. pertussis* is a strict human pathogen and causes whooping cough, a highly communicable disease. Despite intensive vaccination, whooping cough has experienced a marked resurgence worldwide. We have proposed that continued persistence of the bacterium in human populations is due to its ability to form biofilms. Biofilms are critical for colonization and survival of *Bordetella* in the mouse respiratory tract. We have recently identified a DNA binding regulator, BpsR that regulates biofilm development in *B. bronchiseptica*. Additionally, BpsR dually activates and represses the expression of a large number of *B. bronchiseptica* genes with roles in virulence, transcriptional regulation, metabolic and other cellular processes. Nothing is known about the role of BpsR in *B. pertussis*. Functional studies of BpsR in *B. pertussis* have been hampered by the failure to construct a mutant strain lacking the bpsR gene. This suggests that bpsR is an essential gene in *B. pertussis*. We hypothesize that by regulating essential metabolic pathways, pathogenesis and biofilm formation, BpsR controls the survival of *B. pertussis* in the respiratory tract. We are utilizing a tetracycline dependent conditional silencing system to examine its role in laboratory growth, gene expression and in vitro biofilm development of *B. pertussis*. These studies will be extended to an intranasal mouse model of *B. pertussis* infection and biofilm development to identify its role in colonization of and formation of biofilms on respiratory organ. The results of this study will not only provide insights into the poorly known regulatory pathways of *B. pertussis* but also lead to the development of targeted drugs to interfere with BpsR expression and activity.

1.5) **Richard C. Sobe** and Ece Karatan

Spermine negatively regulates Vibrio cholerae biofilm formation through the NspS/MbaA signaling system

The bacterial pathogen *Vibrio cholerae* adopts planktonic or biofilm lifestyles required for maximum fitness in specific environments during its infectious cycle. Aquatic environments and passage through the gastric acid barrier are best suited with the biofilm-associated state while virulence factor-producing *V. cholerae* in the host intestine generally assume a planktonic lifestyle. A ubiquitous class of molecules known as polyamines has been reported to drive pathogens towards these distinct lifestyles. We have previously shown the self-produced polyamine, norspermidine, to induce a biofilm formation by *V. cholerae* in a manner dependent on the periplasmic binding protein, NspS, and the transmembrane phosphodiestase, MbaA. Moreover, several aquatic organisms with which *V. cholerae* is known to associate, including bivalves, copepods, and aquatic plants also produce norspermidine at high levels. However, a physiologically relevant polyamine signal for maintaining the planktonic lifestyle in the human intestine has not been described. Here we report spermine as a negative regulator of biofilm formation at levels as low as 10 μM while those found in the intestine reach up to 190 μM. Spermine-induced biofilm phenotypes are abolished in nspS or mbaA mutants. Finally, NspS-spermine binding assays result in a significant shift in protein stability indicating a probable ligand for this protein. Altogether, this study implies the impacts of spermine on *V. cholerae* physiology may occur in a cyclic di-GMP-dependent manner which results in alterations of and biofilm formation via the NspS-MbaA signaling pathway. We propose that norspermidine acts as a positive signal for biofilm formation in aquatic environments while spermine maintains the planktonic phenotype necessary for maximal virulence factor production in the host instestine.

1.6) **K. Zulauf**, J.T. Sullivan, and M. Braunstein

Export of phagosome maturation arrest effectors by the SecA2-dependent protein export system of Mycobacterium tuberculosis

Mycobacterium tuberculosis(Mtb) grows and replicates within the phagosome of macrophages. The ability to replicate in macrophages is critical for virulence and Mtb creates a suitable niche for growth by arresting phagosome maturation. Recently, we showed that the SecA2 protein export system is required for phagosome maturation arrest and consequently growth of Mtb in macrophages. In order to understand the mechanism(s) by which the SecA2 system inhibits phagosome maturation, we are working to identify proteins exported by SecA2 that contribute to this process. While the process of phagosome maturation arrest by Mtb remains to be fully understood, the Mtb secreted phosphatase SapM is known to be involved. SapM dephosphorylates PI3P present on phagosomal membranes preventing the recruitment of early endosome antigen-1(EEA1) and subsequent phagosome maturation. The _secA2 mutant of Mtb has a significant reduction SapM secretion and is defective for inhibiting EEA1 recruitment when compared to a wild-type strain. To determine if SapM secretion can explain the role of the SecA2 pathway in phagosome maturation arrest and growth in macrophages, we built a _secA2 strain with increased SapM secretion(_secA2+SapM). We then asked if the increased levels of SapM secretion in _secA2+SapM could counteract the phenotypes of a _secA2 mutant in macrophages. When SapM secretion was increased in _secA2+SapM, EEA1 recruitment to the phagosome was reduced to wild-type levels indicating that SecA2 secretion of SapM is required for Mtb to inhibit EEA1 recruitment. When we examined growth and phagosome acidification in macrophages infected with _secA2+SapM, we saw increased SapM secretion partially rescued the phagosome maturation arrest and the replication defects of the _secA2 mutant. These results indicate that SecA2 secretion of SapM contributes to phagosome maturation arrest and growth of Mtb in macrophages but the partial restoration of defects suggests additional SecA2 effectors are required for inhibition of phagosome maturation by Mtb.

2.1) **Maria C. White**, Robert M. Schilke, and Arthur R. Frampton Jr.

CD40 increases oncolysis of GBM cells.

Histone deacetylase (HDAC) inhibitors greatly enhance the ability of viruses to infect and kill multiple forms of cancer, including gliomas. We found that pre-treatment of glioma cells with VPA for 24 hours results in a significant modulation of multiple host genes which aids in the sensitization of EHV-1 infection. In the current study, we investigated the mechanism by which VPA was increasing EHV-1-mediated oncolysis of these glioma cells. Among the multiple genes modulated by VPA, there was significant upregulation of the costimulatory protein Cluster of Differentiation 40 (CD40). A glioma cell line that overexpressed CD40 showed a significant increase in infection compared to the normal glioma cells. In contrast, a glioma cell line that has CD40 removed from the cell line showed a significant decrease in total infection compared to normal glioma cells. Overall, this data suggests that overexpression of CD40 due to VPA treatment plays a major role in the synergistic oncolysis that was observed in this study.

2.2) **Schuchman R.**, Presentor; R. Vancini; A. Piper; D. Breuer; M. Ribeiro; D. Ferreira; J. Magliocca; V. Emmerich; R. Hernandez; and D. Brown

Identification of the pH sensitive step(s) in alphavirus replication

Using the presence or absence of virus RNA synthesis as a reporter indicating that virus entry has or has not occurred, previous studies have shown that inhibitors of endosome acidification can block virus RNA synthesis and thus have blocked entry. Using direct observation, we have shown that entry of alphaviruses occurs at the cell surface by penetration of the plasma membrane in the absence of endocytosis and exposure to low pH. To further characterize the role of pH in the viral lifecycle, we used BHK cells treated with Bafilomycin A1 (BAF) to produce a BAF-resistant Sindbis Virus mutant (BRSV). We have used this mutant to determine the role of the vacuolar ATPase (V-ATPase) in the replication of alpha viruses by examining the production of mature virus and virus RNA in the presence and absence of BAF compared to wild type. In the presence of BAF, RT-qPCR showed that BRSV was more efficient in establishing infection compared to SVHR. BRSV produced mature virus and virus RNA significantly greater than the SVHR in BAF-treated cells. Treatment of cells with BAF 1 hour after infection with SVHR or BRSV showed that RNA synthesis occurred at near normal levels but with a deficit in production of mature viruses. Electron microscopy showed that wild type and BRSV nucleocapsids accumulated at the surface of internal vesicles. BRSV capsids but not wild type were capable of maturing at the internal membranes. Repetition of these experiments in the presence of ammonium chloride (a non specific inhibitor of endosome acidification) yielded a decrease in RNA production and mature virus production for both SVHR and BRSV. Sequencing of BRSV revealed one mutation in E1 and two in E2. RT-qPCR and titration of infections with combinations of the individual mutations showed that only the E2 double mutant could bypass the BAF effect. These results further show that the pH-sensitive step lies in viral maturation and egress, not entry. Additionally, this indicates that E2 is a multifunctional protein with a role in virus assembly, entry, and RNA production.

2.3) **Byron Hamilton**, Melanie Lee-Brown

What's an old book good for? Testing an Ayurvedic Cure for Acne

Ancient remedies made from natural products are proving to be potential antimicrobials to fight bacteria that have become resistant to mainstream antibiotics. The Sushruta Samhita is one of the oldest Indian texts describing the use of natural products for medicinal purposes. A treatment for acne described in Sushruta Samhita, titled Yuv‡na-Pidak‡, is a mixture of four plants: *Coriandrum sativum* (coriander), *Saussurea lappa* (costus), *Acorus calamus* (sweet flag), and *Symplocos racemosa* (lodhra). Acne is most commonly caused by *Propionibaterium acnes*, a Gram positive, rod-shaped, aerotolerant bacterium, that readily acquires resistance to antibiotic treatments. This study has devised a novel method for processing, plating, and testing raw plant material in Kirby Bauer-like (KBL) assays. This KBL assay is being utilized to determine the sensitivity of *Propionibaterium* acnes to whole plant derivatives described in Yuv‡na-Pidak‡.

2.4) **A. T. Barbour**, J. Tutterow, C. Stracey, M. Lee-Brown

Microbial Characterization of Eastern Bluebird (Sialia Sialis) Nestling Fecal Sacs

A cross-discipline collaboration between Microbiologists and Ornithologists at Guilford College have recently investigated whether microbial communities can influence animal behavior. Like many songbird species, nestling Eastern bluebirds (*Sialia sialis*) produce mucous membrane enclosed fecal sacs. Two different nest sanitation behaviors have been observed: 1) the parents carry the fecal sac away from the nest or 2) the parents ingest the fecal sac. Many published reports hypothesize the benefits of ingesting fecal sacs, and why this behavior may change from ingestion to removal as the nestling ages. This study is novel in that it explores the microbiota of the nestling fecal sacs to help explain the switch in nest sanitation behavior. We hypothesize that the nutritional value and decreased predator detection favors ingesting the fecal sacs, over the risk of ingesting dangerous microbes. As the nestling ages and the diversity and abundance of microbes increases, the cost outweighs the benefit and the behavior changes to removal. Utilizing dilution plating, 16S rDNA analysis and Illumina sequencing, we can determine the relative abundance and diversity of microbes within fecal sacs from nestlings across a variety of ages, and nest locations. The long-range focus of this study is to determine if there is a correlation between parental nest sanitation behaviors and changes in the microbial populations of nestling fecal sacs.

2.5) **Zachary P. Johannesson**, Kevin B. Kiser

Triclosan resistance in S. aureus isolated from noses and throats of nursing students

Triclosan is an antimicrobial agent that, up until the recent ban by the FDA, was a common component of antibacterial consumer products, such as soaps and detergents. Triclosan works by inhibiting bacterial fatty-acid synthesis by binding to the bacterial enoyl-acyl carrier protein reductase, encoded by the fabI gene. Previous research looking at clinical isolates of Staphylococcus aureus indicated that mutations in fabI could lead to the development of triclosan resistance. In addition to causing infections, S. aureus is carried in the noses of approximately 30% of the population. This is of concern in healthcare settings where 80% of nosocomial infections are caused by nasal carriers. For this research we tested S. aureus isolates collected from the noses and throats of UNCW nursing students for triclosan resistance and looked for fabI mutations in those with the highest resistance. S. aureus isolates were first screened for susceptibility to triclosan on tryptic soy agar that contained increasing concentrations of triclosan (TSAT). Of the 331 isolates tested, nine were able to grow on TSAT plates with concentrations up to 1 µg/mL. Minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) were determined for each of these isolates with use of a 96-well microdilution test. MICs ranged from 2 µg/mL to 0.25 µg/mL concentrations, whereas all had MBCs of 8 µg/mL. This study reveals that S. aureus isolated from carriers can develop triclosan resistance similar to clinical isolates.

3.1) **S. Pornsukarom** and S. Thakur

Assessing the impact of manure application in commercial swine farms on the transmission of antimicrobial resistant Salmonella in the environment

Land application of swine manure in commercial hog farms is an integral part of their waste management system which recycles the nutrients back to the soil. However, manure application can lead to the dissemination of bacterial pathogens in the environment and pose a serious public health threat. The aim of this study was to determine the dissemination of antimicrobial resistant *Salmonella* in the environment due to manure application in commercial swine farms in North Carolina (n=6) and Iowa (n=7), two leading pork producing states in the US. We collected manure and soil samples twice on day 0 (before and after manure application) from four distinct plots of lands (5 soil samples/plot) located at 20 feet away from each other in the field. Subsequent soil samples were collected again on days 7, 14, 21 from the same plots.

A total of 1,300 soil samples (NC=600; IA=700) and 130 manure samples (NC=60;IA=70) were collected and analyzed in this study. The overall *Salmonella* prevalence was 13.22% (189/1,430), represented by 10.69% and 38.46% prevalence in soil and manure, respectively. The prevalence in NC (25.45%) was significantly higher than in

IA (2.73%) ($P<0.001$) and a consistent decrease in *Salmonella* prevalence was detected from Day 0-Day 21 in all the farms that tested positive. *Salmonella* serotypes detected in NC were not detected in IA, thereby highlighting serotype association based on manure storage and soil application method used in the two regions. Antimicrobial susceptibility testing was done by the broth microdilution method to a panel of 15 antimicrobial drugs. A high frequency of isolates (58.73%) were multidrug resistant (resistance to three or more class of antimicrobials) and the most frequent resistance was detected against streptomycin (88.36%), sulfisoxazole (67.2%), and tetracycline (57.67%). Genotypic characterization by pulse field gel electrophoresis revealed clonally related *Salmonella* in both manure and soil at multiple time points in the positive farms. Our study highlights the potential role of swine manure application in the dissemination and persistence of antimicrobial resistant *Salmonella* in the environment.

3.2) **Neveen Issa**, Bhumi Patak, Taylor Khadedra, Devang Upadhyay, Sivanadane Mandjiny and Leonard Holmes

The Purification and Kinetic Analysis of Lactate Dehydrogenase Extracted from Beef Liver

Lactate Dehydrogenase (LDH) is found in tissues such as blood and muscle. LDH enzyme reduces pyruvate to lactate with oxidation of NADH to NAD+. In the present research, extraction of LDH enzyme from beef liver and its purification were conducted. After extraction, purification of LDH was performed using dialysis and anion exchange chromatography. Biuret assay was conducted to determine protein concentration of purified samples. The characterization of LDH was performed using enzyme kinetic assays. The enzyme activity was measured to be 1.6 U/mL. The optimum pH for LDH activity was found to be from 6 to 7.3. Michaelis- Menten constants (Km) for NADH and sodium pyruvate were determined. In results, from Lineweaver- Burk, Eadie- Hofstee and Hanes plots, the Km for NADH was 2.57 mM, 2.68 mM, and 2.98 mM respectively. Whereas, Km for sodium pyruvate was 6.15 mM, 6.11 mM and 6.05 mM respectively. The specific activity of purified LDH was measured at 140 Units/mg at a protein concentration of 0.2 mg/mL.

3.3) **Jesse O'Campo**, Devang Upadhyay, Sivanadane Mandjiny and Leonard Holmes

Optimization of Xenorhabdus nematophilus Bacterial Growth Through Variation of Original Yoo Media Using a 5Liter Sartorius Stedim Bioreactor

Xenorhabdus nematophilus is a gram_negative bacterium in the family Enterobacteriaceae. This microbe can be described as entomopathogenic. *X. nematophilus* is not found free living in the soil environment. It exists in a symbiotic relationship with insect_parasitizing nematode *Steinernema carpocapsae*. The interaction is specific to each species, and both are found ubiquitously in soil environments. Their ecological significance is particularly apparent in agriculture, as a form of biological control of pest insect species. What this study focuses on is the media variation of original Yoo media with soytone, yeast extract and peptone concentrations and its effects on the growth kinetics of *X. nematophilus* under 5 L Sartorius Stedim Biostat¨ Fermentation System. Bacterial growth rate can be measured by inoculating the bacteria into a media and monitoring the changes in the bacterial density over time. The highest specific growth rate 1.44 h-1 and the lowest doubling time 0.5 hr were determined with the media composition of 2.5 % soytone, 0.5 % yeast extract and 1.0 % peptone of original Yoo media.

3.4) **Elizabeth Gerdes**, Devang Upadhyay, Sivanadane Mandjiny and Leonard Holmes

Variation of Yoo Media for Optimization of Photorhabdus luminescens Phase I Bacterial Growth Using a 2 Liter Sartorius Stedim Bioreactor

Photorhabdus luminescens is a bioluminescent, entomopathogenic bacterium which is part of a symbiotic relationship with beneficial nematode *Heterorhabditis bacteriophora*. In the present study, original Yoo media was varied with soytone, yeast extract and peptone concentrations to obtain the optimum *P. luminescens* Phase I bacterial growth. P. luminescens was inoculated into varied Yoo media and grown in a 2L Sartorius Stedim bioreactor for 24 hours. After incubation, the luminosity and the optical density were measured and recorded. The growth data was collected and entered into a Microsoft Excel¨ spreadsheet to allow a scatterplot to be created. From the scatterplot, the specific growth rate and the doubling time were calculated to determine which media composition produced the highest bacterial yield. This study showed that the media composition of 2.5 % soytone, 0.5 % yeast extract and 1.0 % peptone with original Yoo media had the highest specific growth rate 0.91 h-1 and the lowest doubling time 0.8 hr. The bacterium, *P. luminescens* Phase I bacterial growth was best under those conditions.

3.5) **Ellen Johnson** and Ryan G. Rhodes

Identification of differentially regulated genes in Borrelia burgdorferi cells starved for N-acetylglucosamine

Borrelia burgdorferi, the causative agent of Lyme disease, is a limited genome organism lacking the ability to biosynthesize many essential nutrients, including N-acetylglucosamine (GlcNAc). This amino sugar is required for cell wall synthesis and is a component of the complex growth medium when cells are cultured in vitro. When cultured without free GlcNAc, *B. burgdorferi* cells exhibit biphasic growth. During the first exponential phase, cells utilize the GlcNAc present in complex medium components (e.g. yeastolate and rabbit serum). When this source of GlcNAc is exhausted, the spirochetes are unable to generate new wall material and undergo a death phase. However, some cells survive, growing in a second exponential phase to high cell density. In order to identify genes important for second exponential phase growth in the absence of GlcNAc, RNA seq was used to compare transcript levels in starved and unstarved cells. One hundred and fifty-two genes were differentially regulated during GlcNAc starvation. Of those, five genes involved in carbohydrate utilization were upregulated Ð

bbb04 - 06 (chbA, chbB and chbC), bb0644 (nanE), and bb0629. Real-time PCR is currently being used to confirm differential expression; however, upregulation of the chitobiose specific transporter genes (chbA, chbB, and chbC) during GlcNAc starvation was expected and is supported by previous studies. The GlcNAc-6-phosphate 2-epimerase encoded by nanE catalyzes the reversible conversion of N-acetylmannosamine-6-phosphate (ManNAc-6-P) to GlcNAc-6-P. Thus, we predict in the absence of GlcNAc, *B. burgdorferi* cells utilize the upregulated PTS transporter, BB0629, to import ManNAc, which is then converted to GlcNAc-6-P by NanE. Deletion of nanE and bb0629 via allelic exchange is being pursued to test this hypothesis. Efforts centered on obtaining a comprehensive understanding of carbohydrate metabolism and basic physiology of *B. burgdorferi* are important in improving Lyme disease diagnosis and development of new treatment options.

3.6) **Yawen Zhai** and Ilenys M. PŽrez-D'az

Strategies to reduce bloating incidence in cucumber fermentation brined with calcium chloride

Bloating defect in fermented cucumbers is defined as the accumulation of carbon dioxide in concentrations high enough to form hollow cavities in the endocarp and seed cavity. Elevated bloater incidence in cucumbers fermented with 6% NaCl results in significant economic losses for the pickling industry. Air purging is the main intervention in current commercial operations to minimize bloating defect. This study focused on the evaluation of strategies to minimize the incidence of bloaters in cucumber fermentations brined with 100mM CaCl2 and 6mM potassium sorbate, a newly developed technology able to reduce the environmental footprint. The following interventions were considered: utilization of a *Lactobacillus plantarum* starter culture unable to produce CO2 from malic acid decarboxylation (FS965), cover brine acidification, and cover brine reformulation with enhanced buffering capacity. It was learned that *L. plantarum* FS965 leaded a slower fermentation and resulted in the formation of more acetic acid, incomplete utilization of glucose and fructose, and retarded disappearance of malic acid. However, utilization of a *L. plantarum* starter culture able to decarboxylate malic acid resulted in a faster and complete fermentation, and in a reduction of CO2 production and bloating incidence as compared to the natural fermentation. Acidification with acetic acid aided in the reduction of the total CO2 detected in cover brines and the headspace by 10-20%. While increasing buffering capacity, adding Ca(OH)2, resulted in consistently complete fermentations with higher final pH (3.5), bloating incidence remained insignificantly altered. Approximately 30-40% CO2 were detected in the headspace 4 days after acidifying cucumbers brined with CaCl2, suggesting that the majority of the gas influencing bloating derives from the tissue respiration. We are currently evaluating strategies to inhibit tissue respiration and nitrogen purging as alternatives to reduce the incidence of bloaters in fermented cucumbers brined with CaCl2.

NC Invitational Speaker
Helen Lazear
Asst. Professor, UNC-Chapel Hill

Arthropod-borne viruses (e.g. West Nile, dengue, Zika, chikungunya, and La Crosse viruses) face unusual biological and evolutionary constraints due to their cycling between arthropod vectors (e.g. mosquitoes, ticks) and vertebrate hosts (e.g. humans, birds, horses). Many arboviruses pose a significant threat to human and animal health, and this impact is expected to grow as climate change, urbanization, and global trade alter the interactions between humans and arthropod vectors, making arbovirus infections prime examples of emerging and re-emerging infectious diseases.
Our goal is to understand the innate immune mechanisms that restrict arbovirus pathogenesis. The interferon system is a critical early antiviral response in vertebrates and involves many interferon subtypes signaling through shared receptors to induce antiviral gene expression programs. Our research aims to distinguish the unique properties of different interferons and the mechanisms by which they control viral infections.

ASM Branch Lectureship Program Plenary Speaker
Karl Klose
Professor, Universty of Texas-San Antonio

Dr. Klose's lab is interested in bacterial pathogenesis -- how bacteria cause disease. Dr. Klose has worked most extensively with Vibrio cholerae, the bacterium that causes cholera, and is also researching *Francisella tularensis,* the bacterium that causes tularemia, or rabbit fever.
Cholera is found only where there are widespread problems with sanitation, so improving water and food supplies would eliminate the disease. Since that is unlikely to occur, a safe, cheap, effective vaccine is needed that would protect people. To design such a vaccine, the lab is addressing questions such as: How does *V. cholerae* know that it is in a human body and that is the place to express genes necessary for its survival and disease potential? What are the genetic factors responsible for *V. cholerae* to cause disease? How does this organism persist in aquatic environments, which lead to human infection?
Very little is known about *F. tularensis* or about tularemia. It is a highly virulent organism and can easily be aerosolized, so it is classified by the Centers for Disease Control (CDC) as a Category A select agent with the highest potential to be used as a biological weapon. The lab is working to identify genetic factors responsible for *F. tularensis* to cause disease and to develop suitable vaccine candidates to protect against tularemia infection.

Dr. Klose's plenary lecture is supported by the **ASM Branch Lectureship Program**. The ASMBL program, formerly known as the Waksman Foundation for Microbiology Lectures Program, allows ASM branches to secure outstanding lecturers for their scientific meetings. The program has been operating for over 40 years, and lecturers continue to enhance scientific meetings at the local level.

Poster presentations

1	**Salam Ibrahim**	*Impact of hydrocolloids on the production of acid whey in Greek yogurt*
2	**Tahl Zimmerman**	*Aspirin could impact the growth, functionality, and protein expression profile of Lactobacillus rhamnosus (ATCC 53103)*
3	**Hotaf Makki**	*Characterizing the Toxicity of Hemolysin from Heamophilus ducryi*
4	**Jada Isenhower**	*Identifying the role of putrescine in Vibrio cholerae biofilm formation.*
5	**Caitlin Wotanis**	*Interplay between polyamine synthesis, signaling, and transport on regulating biofilm formation in Vibrio cholerae*
6	**Nicholas Loekman**	*Antibiotic resistance profiles of S. aureus isolates collected from the nose and throats of nursing students*
7	**Kirsten Woolpert**	*Antibiotic-Producing Bacteria Discovered at Local Anne McCrary Park*
8	**Rajani Thanissery**	*INHIBITION OF SPORE GERMINATION AND GROWTH BY MICROBIAL DERIVED SECONDARY BILE ACIDS IN CLOSTRIDIUM DIFFICILE STRAINS THAT VARY BY RIBOTYPE.*
9	**Cody Postich**	*Actinomycete Isolation and Antibiotic Discovery of Freshwater and Anthill Sediments*
10	**Amanda Hyre**	*A Protective Role for Copper During Urinary Tract Infection*
11	**Kevin Zhou**	*Bactericidal Activity of Copper Against Uropathogenic Bacteria*
12	**Shelby Gantt**	*Indifferent to pollution: Stable microbial communities in the sponge Crambe crambe from inside and outside a Mediterranean harbor*
13	**Zhao Wang**	*Microbial responses to climate change associated stressors: pH and temperature*
14	**Daniel Williams**	*Coordinated transcriptional increases in cell wall synthesis genes in Neisseria gonorrhoeae lacking the lytic transglycosylase, ltgA*
15	**Mariah Tugel**	*Investigation of a pyrophosphatase proton pump in Flavobacterium johnsoniae gliding motility*
16	**Mallorie Iozzo**	*System for Rapid Metagenomic Analyses of Wastewater*

17	**Sydney McCune**	*Deletion and Characterization of fjoh_0470, an RpoN activator in Flavobacterium johnsoniae*
18	**Robert Gordon**	*THE USE OF SOIL EXTRACTION AGAR TO CULTIVATE ANTIBIOTIC-PRODUCING BACTERIA UNDER ENVIRONMENT-LIKE CONDITIONS*
19	**Ihasia Parker**	*Effect of Sugar Concentration on the Sugar Utilization Rate by the Bacterium Lactobacillus lactis*
20	**Ashraf Alsaidi**	*Heterorhabditis bacteriophora: FarmerÕs Friendly, Alternative Chemical Pesticides*
21	**Kristen Adams**	*Effects of Marine Media on the Growth of Antibiotic Producing Soil Bacteria from Masonboro Sound*
22	**Walter J. Sandoval Espinola**	*Active expression of the Mn-catalase from Lactobacillus plantarum into Clostridium beijerinckii*
23	**Hui Xu**	*The Mechanism of Chemotaxis and Motility in Borrelia burgdorferi*
24	**Priyanka Theophilus**	*Signaling the way forward: A study on the function of Borrelia burgdorferi chemotaxis receptors*
25	**Li Zhao**	*The Potential dissemination of Shiga-toxigenic Escherichia coli (STEC) and E. coli O157:H7 in the sustainable farms environment*
26	**Marty Roop**	*The pyruvate kinase PykM is a critical virulence determinant for Brucella abortus 2308 in mice, but its activity is not essential for glucose catabolism*
27	**Somer Jones**	*Deletion and Characterization of fjoh_0638, a Gene Encoding an RpoN Enhancer Binding Protein in Flavobacterium johnsoniae*
28	**Megan Polzin**	*The effects of oncolytic vesicular stomatitis virus on tumor-associated macrophages*
29	**John Farrow**	*Two distinct promoters control the transcription of pqsR in Pseudomonas aeruginosa*
30	**Lauren Scheetz**	*Investigation of the Type II Secretion System in F. johnsoniae*
31	**Michael Opata**	*Identification of two T cell subsets that protect and survive long-term in malaria infection*
32	**Rasi Fitria**	*Effect of starter cultures in completion of cucumber fermentation brined with 1.1 % calcium chloride (CaCl2)*

33	**James Evans**	*Aiding and abetting: characterizing the diversity, host-specificity, and potential function of microbial symbionts in introduced North Carolina ascidians*
34	**Patrick Erwin**	*Diversity and structure of the gut microbiome in pygmy (Kogia breviceps) and dwarf (K. sima) sperm whales*
35	**James Seward**	*Peatland Microbes*
36	**Cody Moorman**	*GMCSF-MOG abrogates experimental autoimmune encephalomyelitis (EAE) through the induction of MOG-specific regulatory T cells.*
37	**Tony Perez**	*Culturing peat microbes*
38	**Rebecca Anthouard**	*PlrSR is a two-component system required for virulence of Bordetella bronchiseptica*
39	**Hunter Whittington**	*Fungicide Application to Soils, Biodegradation, and Responses of the Soil Microbial Community*
40	**Dakota Goad**	*Vesicular Stomatitis Virus as an Oncolytic Agent Against Cancer Cell Metastasis*
41	**Redife Ucar**	*Profiling of the ability of selected lactic acid bacteria to utilize carbohydrates found to be present in cucumber fermentations, including starter culture candidates of Lactobacillus plantarum and Lactobacillus pentosus.*

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1. *Salam A. Ibrahim*, Rabin Gyawali, Tahl Zimmerman, Shannon Walston, Temitayo Obanla, Nwadiuto Nwamaioha, and Reza Tahergorabi.

Impact of hydrocolloids on the production of acid whey in Greek yogurt

Greek yogurt has become popular in the U.S. markets and now accounts for more than a third of total yogurt sales. The popularity of Greek yogurt has resulted in a concomitant increase in production of an unwanted byproduct known as acid whey. In our current research, we are investigating the impact of food ingredients such as hydrocolloids on acid whey production of Greek yogurt. Non fat milk was supplemented with gums and proteins. Gum Arabic (GA), Inulin (IN), and Pectin (PE) at 0.01, and 0.05 % (w/v), and whey protein concentrate (WPC) and whey protein isolate (WPI) at 0.5 and 1.0% (w/v) were mixed slowly into milk at 50¡C with agitation. Milk without supplementation served as a control sample. The yogurt mixes were heated at 90¡C for 10 min, inoculated with 3.0% of starter culture, and incubated at 40°C for 4 h (pH 4.6), then refrigerated overnight at 4°C. The next day, each sample was centrifuged (1300 g, 10 min) and acid whey production was measured by calculating the water holding capacity (WHC). An analysis of variance of the data was carried using a completely randomized design. The Tukey test was used to determine statistically different groups. Our results showed that fortification of gum pectin and whey proteins significantly reduced the acid whey production compared to the control sample ($P<0.001$). The highest WHC was 39.71 ± 0.51, 50.23 ± 0.23, and 48.86 ± 0.24% in yogurts with pectin 0.05 %, WPC 1.0%, and WPI 1.0%, respectively compared with the control (34.95 ± 0.97%). Our results demonstrate that hydrocolloids can reduce acid whey and could be industrially applicable for the production of Greek yogurt.

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2. T**ahl Zimmerman**, Temitayo Obanla, Sarah Adjei-Fremah, Rabin Gyawali, Mulumebet Worku, Reza Tahergorabi, and Salam A. Ibrahim

Aspirin could impact the growth, functionality, and protein expression profile of Lactobacillus rhamnosus (ATCC 53103)

Many prescription drugs, such as antibiotics, have been shown to affect the activity and composition of the bacteria of the gut microflora. Little is known, however, about the effects of commonly used over-the-counter medications, such as aspirin. The purpose of this study was to determine the long-term effects of aspirin on the growth and functionality of the beneficial bacterium *Lactobacillus rhamnosus* (ATCC 53103), normally found in the gastrointestinal tract. One colony of *L. rhamnosus* was isolated and propagated three times in MRS broth and incubated at 37¡C. The final culture was then harvested and washed with 0.1% peptone water. Cells were transferred into 9mL MRS broth containing approximately 6 mg/mL aspirin, vortexed, and incubated for 4 h at 37¡C. The culture was harvested, transferred into MRS broth and incubated at 37¡C. The exposure protocol was repeated 5 times in a single week. The aspirin-exposed cells were then plated onto MRS agar containing 6 mg/mL aspirin. One colony of *L.rhamnosus* that had been exposed to aspirin was isolated from the plate and activated in MRS broth. The overall procedure was repeated once a week for 12 consecutive weeks. The effects of aspirin on *L .rhamnosus* were monitored using bacterial cell counts, measurements of _-galactosidase activity, and changes in the protein expression profile. *L. rhamnosus* survived long-term exposure to a sub inhibitory concentration of aspirin, however, inhibitory concentrations blocked cell growth. The _ Ðgalactosidase activity of *L. rhamnosus* was completely inhibited upon being. There was 54% more protein expressed in aspirin exposed cells than in unexposed cells. Regular intake of medical drugs such as aspirin can affect the growth and functionality of beneficial gut microflora, including *Lactobacillus rhamnosus*.

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3. **Hotaf Makki**, Misty D. Thomas, C.D. White

Characterizing the Toxicity of Hemolysin from Heamophilus ducryi

The genital ulcerative disease chancroid is caused by the bacterium *Haemophilus ducreyi*. There have been several outbreaks of chancroid in the United States, and it is endemic to Asia, Africa, and Latin America. The ulcers that chancroid produces are soft, painful, and bloody, and may become chronic if not treated. Nonsexual transmission also occurs and is caused by new strains of *H. ducreyi*, first discovered in Samoa when children presented with chronic lower extremity ulcerations. *H. ducreyi* produces and secretes the hemolysin virulence factor, which causes lysis of red blood cells by damaging the cell membrane. Hemolysin consists of two genes: hhdA and hhdB. The aim of this study was to detect the presence and determine the variability and toxicity of hemolyin in Class I, Class II, and Samoa strains. Strains 35000HP, HMC112, and SB5755 were subjected to Polymerase Chain Reaction (PCR) and we determined that all posses hhdA and hhdB. Whole genome sequencing showed there are several nucleotide mutations in the hhdA gene, also changing the predicted protein sequence. However, hhdB gene was identical in both classes of *H. ducreyi*. The cytotoxic effect of Class I, Class II and Samoa strains was tested on triple negative breast cancer cells (TNBC) to determine their ability to cause cell death. TNBC cell death was induced by all three types of *H. ducreyi* using bacterial supernatants. 35000HP demonstrated 80- 85% death of TNBC cells after 24 hours of exposure, HMC112 Class II strains caused death to 35-40 % of TNBC cells after 24 hours of exposure and SB5755 resulted in 25% cell death. Therefore, this method, which targets TNBC cells may be a rapid, cost effective means to detect the presence and toxicity of hemolysin in these strains.

4. **Jada M. Isenhower**, Ece Karatan

Identifying the role of putrescine in Vibrio cholerae biofilm formation.

Biofilms are formed when bacteria congregate, often in association with a surface. Formation of a biofilm is thought to allow *Vibrio cholerae*, the causative agent of the disease cholera, to survive harsh environmental conditions. *V. cholerae* grown in a biofilm exhibit a hyper-infectious phenotype, making biofilms a concern for human health. Polyamines norspermidine and spermidine affect biofilm formation in *V. cholerae*. However, the role of putrescine, a polyamine synthesized by *V. cholerae*, has not been extensively studied. The objective of this study was to better understand the role of putrescine in *V. cholerae* biofilm formation.

Putrescine is synthesized through two pathways: decarboxylation of ornithine by ornithine decarboxylase (ODC) encoded by the speC gene, and conversion of agmatine to putrescine by agmatine ureohydrolase encoded by the speB gene. To understand the effect putrescine biosynthesis has on *V. cholerae* biofilm formation, we chemically or genetically inhibited these two pathways. In order to correlate the effects of polyamines levels on a biofilm, cellular polyamine levels were quantified by High Performance Liquid Chromatography (HPLC).

Inhibition of ODC by DFMO caused an increase in biofilms; HPLC analysis indicated that DFMO caused a significant increase in cadaverine and a decrease in putrescine. Cadaverine has been previously shown to be involved in acid tolerance responses, and the increase in cadaverine may be in response to acid stress. _speB mutant biofilms showed no significant difference from wild type; HPLC analysis indicated a slight decrease in putrescine. Together, this indicates that ODC and not agmatine ureohydrolase may be the primary putrescine synthesis pathway in *V. cholerae*. Our results suggest that intracellular putrescine may act as an inhibitory signal for biofilms and when the ability to synthesize putrescine is limited, cadaverine levels may increase and act as a positive signal for biofilm formation as a protection mechanism for *V. cholerae*.

5. **Caitlin Wotanis**, Elizabeth Villa, Blake Sanders, Jada Isenhower, Richard Sobe, Alex Mozina, and Ece Karatan

Interplay between polyamine synthesis, signaling, and transport on regulating biofilm formation in Vibrio cholerae

Vibrio cholerae, an intestinal pathogen responsible for the diarrheal disease cholera, persists in aquatic environments in biofilms, aggregates of cells encased within a self-produced matrix. Biofilms can provide protection from environmental stressors and have been indicated in *V. cholerae* pathogenesis. Polyamines, small, cationic hydrocarbon molecules that are synthesized by virtually all cells, have been shown to play a role biofilm formation. The polyamine norspermidine is synthesized by the enzyme NspC and has been shown to enhance *V. cholerae* biofilm formation. Spermidine, which is not produced by *V. cholerae*, is abundant in the human intestine and inhibits biofilm formation. Norspermidine and spermidine act as extracellular signals to enhance and inhibit biofilms, respectively, by interaction with the NspS-MbaA signaling complex. In addition, *V. cholerae* is also capable of uptake of norspermidine and spermidine through the PotABCD1 transporter.

In this work, we aimed to determine the relative contributions of norspermidine synthesis, norspermidine and spermidine signaling and transport have on regulation of biofilm formation. To do this, we constructed various double mutants in polyamine synthesis, signaling, and transport pathways and assessed the resulting biofilm phenotypes. Our work indicates that without endogenous spermidine, the _potA, _potB, and _potC, _potD1 mutants form large biofilms. In the absence of nspC, biofilm formation is low. Furthermore, _nspC_potA, _nspC_potB, and _nspC_potC double mutants display an intermediate biofilm phenotype, while _nspC_potD1 biofilm formation is low. However, biofilms are restored in these double mutants with added norspermidine. Additionally, we show that when nspS is deleted in any background, biofilm formation is completely diminished, even in the presence of endogenous and exogenous norspermidine. This work establishes NspS as the dominant mediator of biofilm formation over the PotABCD1 transport system and NspC synthesis of norspermidine.

6. **Nicholas C. Loekman**, Zack Johannesson, Ashton L. Honeycutt, Matthew C. Mason, Aaron T. Kesinger, Caroline Jones, Kevin B. Kiser

Antibiotic resistance profiles of S. aureus isolates collected from the nose and throats of nursing students

Staphylococcus aureus (*S. aureus*) is an opportunistic bacterial pathogen that is carried harmlessly in the nose of 30% of the population as well as throat in unknown amounts. Studies have shown that MRSA is carried by 6.25% of healthcare workers, possibly contributing to the spread in hospitals where 86% of MRSA infections, which have a 20-50% mortality rate are contracted. As students enter nursing school, many are exposed to a clinical setting for the first time. To test whether or not this exposure leads to increased rates of MRSA carriage, the nares and pharynx of nursing students were swabbed and any S. aureus found was subjected to antibiotic susceptibility testing. Over seven semesters, 47.4% of those tested were carriers of *S. aureus* in their nares and/or pharynx, with only 2.29% carrying MRSA, indicating the increased clinical exposure in nursing school does not lead to increased carriage rates.

7. **Kirsten M. Woolpert** and Kevin B. Kiser

Antibiotic-Producing Bacteria Discovered at Local Anne McCrary Park

The discovery of new antibiotics is becoming a growing need in the world as bacteria evolve to be immune against our medicines. With the vast amount of soil covering the Earth, more antibiotic compounds are bound to be in our soils, waiting to be discovered. With this research project, my goal was to find the type of soils that are the most diverse in their microbial activity. In doing so, I found a microbe that was producing an antibiotic that showed resistance against four of the six ESKAPE pathogens, including *S. aureus*, *K. pneumoniae*, *A. calcoaceticus*, and *P. aeruginosa*. After sequencing the 16S rRNA, I discovered that this bacterial species is *Pseudomonas soli*. Though it's known to produce an antibiotic compound, not much research has been done to determine what the compound is and it's effectiveness, demonstrating a need for continued study.

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8. **Rajani Thanissery** and Casey M. Theriot

INHIBITION OF SPORE GERMINATION AND GROWTH BY MICROBIAL DERIVED SECONDARY BILE ACIDS IN CLOSTRIDIUM DIFFICILE STRAINS THAT VARY BY RIBOTYPE.

The changing epidemiology of *Clostridium difficile* infection over the past decades presents a significant challenge in the management of *C. difficile* associated diseases. The gastrointestinal tract microbiota provides colonization resistance against C. difficile and growing evidence suggests that microbial derived secondary bile acids (SBA) play a role. We hypothesized that spore germination and growth of *C. difficile* strains that vary by age and ribotype will vary in their sensitivity to SBA. *C. difficile* strains R20291 and CD196 (027), M68 and CF5 (017), 630 (012), BI-9 (001) and M120 (078) were used to measure taurocholate (TCA) mediated spore germination and growth inhibition with microbial derived SBA (_MCA, HDCA, UDCA, LCA, iLCA, DCA, iDCA) found in the mouse and human large intestine. Many SBA were able to inhibit TCA-mediated spore germination and cell growth in a dose dependent manner, but the level of inhibition varied by strain and ribotype. Interestingly, the historic strain CD196 (027) was more sensitive to germination inhibition by SBA at lower concentrations when compared to the more recent epidemic strain R20291 (027). C. difficile M120 (078) a highly divergent strain resulted in minimal inhibition of both germination and cell growth by most SBA. This data suggest a differential response of SBA to both spore germination inhibition and growth inhibition between strains elucidating their role in disease pathogenesis. Future studies will investigate how secondary bile acids alter other steps of the *C. difficile* life cycle including toxin production and sporulation.

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9. **Cody A Postich** and Dr. Kevin Kiser

Actinomycete Isolation and Antibiotic Discovery of Freshwater and Anthill Sediments

Antibiotics lose potency against deadly pathogens because developed antibiotic resistance rises from overuse on infections and diseases. Microbiology experiments incorporate bacterial research from sediments in aquatic environments. These studies benefit the medical field by obtaining greater knowledge about the growing strength ESKAPE pathogens. The goals of antibiotic discovery consist of discovering unique bacteria species that produces antibiotic properties against ESKAPE pathogens and incorporating those properties into manufacturing antibiotics. The research design starts by taking sediment samples from four locations, two in the anthill topsoil and two from the banks of freshwater environments. These environments were chosen because of soil properties from moisture content to aeration capability. Four isolates, two from each environment, resisted many of the ESKAPE pathogens using cross-streak tests. The promising bacteria had unique characteristics and their scientific names identified through microscopy, PCR and BLAST results. Future research involves isolating and characterizing antibiotics from the isolates.

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10. **Amanda Hyre** and Sargurunathan Subashchandrabose

A Protective Role for Copper During Urinary Tract Infection

Uropathogenic *Escherichia coli* (UPEC) is one of the most common bacterial infections in humans. We have previously identified that urine copper content in patients presenting with UTI is higher than in healthy individuals. We have also identified that in a mouse model, animals receiving oral copper supplementation had lower pathogen burden than those not supplemented with copper, indicating a protective role for copper during urinary tract infection (UTI). Additionally, RNA-seq identified an upregulation of genes specifically related to copper homeostasis in UPEC during human UTI. Taken together, these data suggest that copper plays a bactericidal role in host innate immune response during UTI. However, the mechanism by which copper is mobilized to the urinary bladder during UTI is yet unknown and is the major focus of this work. We hypothesized that ceruloplasmin, the predominant copper binding protein in mammals, is the source of urinary copper during infection. We found that ceruloplasmin is present in human UTI urine samples but not in healthy human urine, and present data indicating that ceruloplasmin is the source of urinary copper in a majority of UTI patients.

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11. **K. Zhou**, S. Subashchandrabose

Bactericidal Activity of Copper Against Uropathogenic Bacteria

Urinary tract infections (UTI) are one of the most common type of bacterial infection in people. The most common caused of UTI is uropathogenic *Escherichia coli* (UPEC). Other bacterial pathogens that cause UTI include *Klebsiella pneumoniae*, *Proteus mirabilis*, *Providencia stuartii*, *Acinetobacter baumanii*, and *Staphylococcus aureus*. Antibiotics have been used for the last seven decades in the treatment of UTI. However, antibiotics are becoming less effective against UTI because of increasing antibiotic resistance in UPEC and other uropathogens. Recently, we identified that during clinical UTI in humans, copper is mobilized to urine as a host effector to mitigate UPEC colonization. Here, we investigated the bactericidal activity of copper against UPEC and other common uropathogens. Bacteria in exponential phase of growth were harvested and exposed to copper in vitro at a physiologically relevant concentration. Our results demonstrate that copper is a highly effective bactericide with >99% killing in the treatment group as compared to the control group. We also observed differences in susceptibility to copper between different uropathogens. This is not surprising considering the differences in genomic content observed in these diverse uropathogens. In summary, our results demonstrate that copper is an effective bactericide in vitro. Future studies include probing the effect of copper on uropathogen colonization in the mouse model of UTI.

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12. **Shelby Gantt**, Susanna L—pez-Legentil and Patrick M. Erwin

Indifferent to pollution: Stable microbial communities in the sponge Crambe crambe from inside and outside a Mediterranean harbor

Marine sponges have been shown to harbor diverse microbial symbiont communities that play key roles in host functioning, yet little is known about how anthropogenic disturbances, such as heavy metal pollution, impact sponge-microbe interactions. The Mediterranean sponge *Crambe crambe* is known to accumulate heavy metals in polluted environments including harbors. In this study, we investigated whether the microbiome of *C. crambe* differed between sponges inhabiting a polluted harbor in Blanes (Spain) and a nearby (<1 km) natural environment. Triplicate sponge and ambient seawater samples were collected from each site and the microbial composition of each sample was determined by 16S rRNA gene sequence analysis (Illumina Hi-Seq platform). No significant differences in the diversity or structure of microbial communities in *C. crambe* were detected between habitats, while a significant difference in community structure was observed in ambient seawater inside and outside of the polluted harbor. The microbiome of *C. crambe* was clearly differentiated from free-living seawater microbes and dominated by Proteobacteria, specifically a single betaproteobacterium that accounted for >86% of all sequence reads. These results indicate that sponge microbiomes exhibit greater stability and pollution tolerance than their free-living microbial counterparts, potentially mitigating the effects of pollutants on coastal marine communities.

13. **Zhao Wang**, Sara K. Blinbry, Tiffany C. Williams, Dana E. Hunt, Zackary I. Johnson

Microbial responses to climate change associated stressors: pH and temperature

Climate change is predicted to decrease pH and increase temperature in the surface ocean. As microbes play a fundamental role in marine nutrient and energy cycling, their responses to pH and temperature changes can provide insight into the potential effects of climate change in the ocean. In this study, we manipulated coastal (Beaufort Inlet, Beaufort NC) samples to identify potential microbial community composition and activity responses to pH (-0.3 units) and temperature change (+ 3 °C) predicted to occur in the next 100 years, for each factor singly and in combination. In general, microbial processes seemed similar for both control and climate change-manipulated mesocosms. However, when examining the microbial community via 16S rRNA gene sequencing, there were notable differences in community composition between control and manipulated mesocosms, with temperature having the largest effect on the microbial community that dominated over pH responses. We are continuing this work on offshore (shelf break) samples which we anticipate will show larger community responses to pH and temperature manipulations since this environment exhibits much lower variability in these environmental variables.

14. **Candra O. Broadie**, Hatajai A. Lassiter, Antonio T. Baines, Sherrice V. Allen, Joseph P. Dillard, Robert A. Nicholas, and Daniel Williams

Coordinated transcriptional increases in cell wall synthesis genes in Neisseria gonorrhoeae lacking the lytic transglycosylase, ltgA

Lytic transglycosylase A in *Neisseria gonorrhoeae* cleaves the _-1,4-glycosidic bond between peptidoglycan (PG) monomers to liberate 1,6-anhydro PG fragments that are either recycled or released as cytotoxic fragments. To gain further insight into the effect of LtgA on cellular processes in *Neisseria gonorrhoeae*, we performed a proteomic analysis comparing wild type and an isogenic ltgA null mutant strain. Proteins were separated by two-dimensional gel electrophoresis and identified by MALDI-TOF mass spectrometry, which revealed several proteins that were increased in their level of expression upon loss of LtgA. The most notable changes corresponded to enzymes related to aminosugar and pyrimidine metabolism. Quantitative real-time RT-PCR of mRNA from an ltgA null strain confirmed increased transcription of genes encoding enzymes involved in UDP-N-acetylglucosamine (UDP-GlcNAc) synthesis, a major precursor in peptidoglycan (PG) and lipooligosaccharide (LOS) synthesis, during normal growth conditions and following exposure to penicillin. We also found that the ltgA mutant strains were more susceptible to _-lactam antibiotics, vancomycin, and the human cathelicidin antibacterial peptide, LL-37, than their corresponding wild-type parental strains. Our results suggest that increased expression of enzymes responsible for production UDP-GlcNAc is an adaptive response due to inactivation of ltgA and/or exposure to penicillin.

15. **Mariah Tugel** and Ryan Rhodes

Investigation of a pyrophosphatase proton pump in Flavobacterium johnsoniae gliding motility

Flavobacterium johnsoniae is an aerobic, gram-negative bacterium found in soil and freshwater. *F. johnsoniae* does not use flagella or pili to move. Instead, it uses cell surface adhesins for gliding motility. These adhesins are delivered to the surface by the Type IX secretion system and are propelled along the surface by an unidentified motor. While it is known that inner membrane proteins are involved, the mechanics underlying gliding motility are not fully understood. Evidence suggests the proton motive force (PMF) powers gliding, but the motor proteins have not been identified. We hypothesize that a proton-translocating pyrophosphatase channel (fjoh_1479) may contribute to gliding by moving protons across the inner membrane and adding to the PMF. To test this, an allelic exchange system is being used to generate an unmarked deletion in the pyrophosphatase channel. A DNA fragment downstream of fjoh_1479 was PCR amplified with engineered SalI and SphI restriction sites and cloned into the suicide vector pRR51. This ligation mix was transformed into E. coli cells and transformants were isolated on LB containing ampicillin. Ten transformants were screened by colony PCR, and a positive clone was selected for plasmid extraction and confirmation restriction digest. The confirmed plasmid was designated pMT01. The DNA fragment upstream of fjoh_1479 was PCR amplified with engineered BamHI and SalI restriction sites, and resulted in multiple fragments. Gel extraction was used to isolate the correct 2.1 kbp product. Currently, this DNA fragment is being cloned into pMT01 to generate pMT02, the deletion construct. Following confirmation of the deletion construct, pMT02 will be transferred to F. johnsoniae by triparental conjugation to generate an unmarked deletion in fjoh_1479. Gliding motility of the mutant will be compared to wild-type cells to determine the role of the pyrophosphatase pump in this process.

16. **Gretchen Branstetter***, Bonnie Chen*, Mallorie Iozzo, Jeffrey Muday* and James Curran* & *Wake Forest University

System for Rapid Metagenomic Analyses of Wastewater

Wastewater treatment plants (WWTP) are essential modern infrastructure that use complex microbial systems to render organic wastes safe for discharge. Metagenomic analyses (MG) have great potential to facilitate WWTP operations (1,2), but cost and expertise are generally beyond the scope of WWTP facilities. We present a system that can streamline MG. In under 30 minutes, samples can be prepared for submission to readily available sequencing facilities. Gene and identification can be performed by free, online services. Then, problem genera can be identified using a Python program that will run on a laptop.

17. **Sydney A. McCune**, and Ryan G. Rhodes

Deletion and Characterization of fjoh_0470, an RpoN activator in Flavobacterium johnsoniae

Flavobacterium johnsoniae has two cytoplasmic sigma factors (RpoN and RpoD), which are required for gene expression. These factors direct RNA polymerase to gene promoter regions for transcription of genes into mRNA. In other organisms RpoN is involved in responding to internal and external stimuli, and requires an enhancer binding protein (EBP) to stabilize and bind to the promoter in order to recruit RNA polymerase. Analysis of the *F. johnsoniae* genome revealed six putative RpoN EBPs. This research is focused on fjoh_0470, which encodes an EBP that may be involved in the regulation of an ABC transporter operon that is in close proximity on the genome (fjoh_0471-0473) and has a predicted RpoN promoter. To evaluate the function of this EBP we are using an allelic exchange system to generate an unmarked deletion in fjoh_0470. Primers were designed with engineered restriction sites. Regions flanking the gene were amplified by PCR, cloned into pRR51, a suicide vector, and confirmed via colony PCR and restriction digest. The deletion construct (pSAM02) was introduced to *F. johnsoniae* via tri-parental conjugation. Cells were plated on a medium containing erythromycin to select for integration of pSAM02. One colony was isolated and inoculated in liquid medium without antibiotic to allow for a second recombination event. Cells were then plated on a medium containing streptomycin to select for the second recombination. Colony PCR was performed on ten second recombinants with primers flanking the deletion site to identify clones carrying the unmarked deletion of fjoh_0470. PCR identified three potential mutants, but further characterization did not confirm the mutation. Currently, we are conducting additional tri-parental conjugations to isolate the mutant. Upon successful isolation we will compare phenotypes of the mutant and wild-type cells for motility and metabolism, as well as evaluate the expression of the nearby ABC transporter.

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18. **Robert Gordon**

THE USE OF SOIL EXTRACTION AGAR TO CULTIVATE ANTIBIOTIC-PRODUCING BACTERIA UNDER ENVIRONMENT-LIKE CONDITIONS

Due to the increasing prevalence of antibiotic resistant pathogens it is urgent that new antibiotics are discovered to combat new resistant strains. Even with modern techniques we are only able to cultivate around one percent of microbes present. An additional issue in identifying antibiotic producing bacteria is that the potential antibiotic producing colonies grown do not always produce their antibiotics under their present conditions. Agar has been prepared using water that has been filtered through the same soil as the sample being cultured in an attempt to leach the nutrients and any other abiotic factors present within the soil. This should allow the bacteria present in the sample to grow in nutritive conditions similar to that of their natural environment. A new species belonging to the genus *Paenibacillus* has been isolated using this method and appears to be producing a broad-spectrum antibiotic.

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19. **Ihasia Parker,** Devang Upadhyay, Sivanadane Mandjiny and Leonard Holmes

Effect of Sugar Concentration on the Sugar Utilization Rate by the Bacterium Lactobacillus lactis

Lactobacillus lactis is a well-known gram-positive bacterium that helps to keep our intestinal tract healthy and protect against harmful bacteria and fungi. By having the ability to live and flourish in the gastrointestinal tract, *Lactobacillus lactis* is the exemplary bacterium that shows resilience. In previous research, it has been shown that Lactobacillus lactis has important use as probiotic bacteria as well as for the production of vaccines, vitamins and treatment for digestive health, immune boosting and respiratory infections. Aside from the medical benefits, it is used commercially as a lactic starter for yogurts, cheese and fermented milk products. In this study, *Lactobacillus lactis* was incubated in tryptic soy media containing different sugars: glucose, fructose, maltose, cellobiose and lactose to analyze the carbohydrate utilization rate at different sugar concentrations. The importance of sugar utilization regarding *L. lactis* is to understand which specific carbohydrate at a particular concentration will provide optimum growth of this bacterium. This information will help us to produce *L. lactis* and gain more commercial advantage and knowledge for biomedical applications.

20. **Ashraf Alsaidi**, Jeison Valencia, Elizabeth Gerdes, Devang Upadhyay, Sivanadane Mandjiny and Leonard Holmes

Heterorhabditis bacteriophora: FarmerÕs Friendly, Alternative Chemical Pesticides

The entomopathogenic nematode, *Heterorhabditis bacteriophora*, is an environmentally safe alternative to chemical pesticides. It is half of a symbiotic relationship with the bacteria, *Photorhabdus luminescens* which lives in the nematode gut. *Heterorhabditis bacteriophora* has a wide range of susceptible insects making it a very effective alternative to current biological control practices. The nematode has been proven to be safe to humans, non-target insects, wildlife, fauna, and water. For this reason, as well as consumers increasing consciousness of health issues, *Heterorhabditis bacteriophora* should be considered as a viable alternative and researched more thoroughly.

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21. **Kristen L. Adams** and Kevin B. Kiser

Effects of Marine Media on the Growth of Antibiotic Producing Soil Bacteria from Masonboro Sound

The discovery of metabolites from marine resources has recently proven to be a new source for novel antibiotics used to treat bacteria infections. Marine ecosystems such as Masonboro Sound, generate an evolutionary pressure on microorganisms that allow for a difference metabolically and genetically from their terrestrial counterparts. Culturing *Streptomyces* on media similar to their natural environment such as Marine agar 1622 or a manipulated ISP-2 media could result in more diverse isolates. In this study, soil samples were taken from the Masonboro sound, diluted with sterile H2O and cultured on Marine 1622 and ISP-2 media. After culturing, isolates were tested for antibiotic producers by a TSB overlay inoculated with Staphylococcus epidermis. The marine media exhibited 3 zones of inhibition and the ISP-2 media produced two zones of inhibition. Further testing includes a cross streak test against the "ESKAPE" pathogens, as well as Gram staining and 16S rRNA sequencing to identify the species of the antibiotic producers.

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22. **Walter J. Sandoval-Espinola**, Sue Dagher and Jose M. Bruno-Barcena

Active expression of the Mn-catalase from Lactobacillus plantarum into Clostridium beijerinckii

Biobutanol generated by solventogenic *Clostridium* is once again being targeted as an alternative advanced-biofuel. However, many limitations hinder *Clostridium* proliferation, including intolerance to oxygen exposure and coping with oxidative stress. Members of the Clostridia group are considered strict anaerobes, mostly due to traditional cultivation methods arising from a one-size-fits-all approach, even with genetically unrelated species. Here, we report a genetic tool for stable heterologous gene expression in *C. beijerinckii* and a comparative study of the impact of oxygen on the growth of *C. beijerinckii* SA-1N and SA-1K; two new strains carrying replicative vectors. Singularly, SA-1K bears, and actively expresses, the well-characterized manganese catalase gene (MnKat+) from Lactobacillus plantarum. The growth kinetics under oxygen exposure demonstrate that this new strain carrying active MnKat (SA-1K) was able to grow at comparable rates to those of SA-1 under anaerobiosis, while building up greater biomass at the expense of solvent output. Surprisingly, mid-log SA-1N cultures (control lacks MnKat, MnKat-) were also able to tolerate continuous exposure to oxygen. We also evaluated the impact of continuous air flow stripping the fermentation gases (CO2 and H2) from the headspace on the yield parameters, by culturing of these new strains. Overall, this work provides evidence of the natural aerotolerance of *C. beijerinckii* whose fitness can be improved by the heterologous expression an active manganese catalase.

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23. **Hui Xu** and MD A. Motaleb

The Mechanism of Chemotaxis and Motility in Borrelia burgdorferi

The requirements for bacterial chemotaxis and motility range from dispensable to crucial for host colonization. Although more than 50% of all sequenced prokaryotic genomes possess at least one chemotaxis signaling system, many of those genomes contain multiple copies of a chemotaxis gene. However, the function of most of those additional genes are unknown. Most motile bacteria possess at least one CheY response regulator that is typically dedicated to the control of motility, and which is usually essential for virulence. Of the three *Borrelia burgdorferi* CheY proteins, only CheY3 controls motility and chemotaxis, and is also crucial for virulence and dissemination within the murine host. Thus, the CheY3 appears to be a typical chemotaxis response regulator. However, we observed a notable difference with CheY2. Our recent results suggest that cheY2 has varied effects on the natural infection cycle of *B. burgdorferi*. Mutants deficient in this gene exhibit normal motility and chemotaxis in vitro, but show reduced virulence in mice. While _cheY2 spirochetes survive normally in Ixodes ticks, mice fed upon by _cheY2-infected ticks did not develop a persistent infection. We propose that CheY2 serves as a regulator for a *B. burgdorferi* virulence determinant that is required for productive infection within vertebrate, but not tick hosts. Moreover, various studies in other bacteria have shown that phosphorylated CheY (CheY-P) binds to the flagellar motor switch protein FliM, and induces a clockwise rotation of the flagellar motor resulting in tumbling of the cell. Here, we used affinity blotting to provide a direct evidence that *B. burgdorferi* FliM binds to both CheY3-P and CheY2-P. In vitro phosphorylation assay demonstrated that CheY2-P half-life is remarkable shorter than CheY3-P. It is intriguing that CheY2-P binds to FliM, but fails to modulate bacterial motility. Our data suggest that CheY2, despite resembling a typical response regulator, functions distinctively than most other chemotaxis CheY proteins. The function of CheY1 remains elusive.

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24. **Priyanka A.S. Theophilus** (presenter), Ki Hwan Moon, and Md. A. Motaleb

Signaling the way forward: A study on the function of Borrelia burgdorferi chemotaxis receptors

Lyme disease is the most prevalent vector borne infection that affects approximately 300,000 people annually in the United States. The disease is highly debilitating and multisystemic affecting the joints, muscles, brain and heart. Currently, there are no vaccines for this disease. Lyme disease costs the U.S. health care system $1-2 billion annually. Motility and chemotaxis of *Borrelia burgdorferi*, the Lyme disease spirochete, contribute to its invasive behavior. After a tick bite, spirochetes are deposited in the skin and migrate to various tissues to produce multiple clinical manifestations. Moreover, when a tick feeds on an infected host, spirochetes are acquired by the tick. Thus, the question is, what drives *B. burgdorferi* to migrate from one host to the other or to migrate to the preferred host tissues? Does *B. burgdorferi* get attracted to specific chemical cues? *B. burgdorferi* uses its chemotaxis system to sense the environment. On sensing ligands, the chemotaxis receptors (Mcp) communicate with the flagellar motors in order to swim towards needed nutrients or away from toxic substances, which are important for the bacterial growth, survival and disease progression. During tick to mouse transition, we hypothesize that these Mcp proteins recognize specific ligands in ticks and during migration to the host, recognize a different set of ligands specific to the host tissue. To implement this hypothesis, we generated a mutant in *B. burgdorferi* mcp4 via homologous recombination. Making use of this mutant, our aim is to test if the spirochetes prefer colonizing a mouse over a tick model or vice versa.

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25. **Zhao L.** and Thakur S.

The Potential dissemination of Shiga-toxigenic Escherichia coli (STEC) and E. coli O157:H7 in the sustainable farms environment

The emergence and growth of the “Eat Local” movement has contributed directly to the growth of sustainable farms which promotes rearing livestock and growing fresh produce within the same agricultural system. The interface of food animals and fresh produce in agricultural production is an area in need of information that could potentially reduce the risk of pathogen transmission and fresh produce contamination. The objective of this study was to determine the role of food animals in the transmission and dissemination Shiga-toxigenic *Escherichia coli* (STEC) and E. coli O157:H7 to fresh produce and environment in sustainable farms in North Carolina (n=2) and Tennessee (n=3). We collected 1,493 samples (NC=629; TN=864) from sustainable farms including produce, feces, soil, litter, water and insect. The overall STEC prevalence was 16.81% (NC=5.25%; TN=25.23%) and E. coli O157:H7 (TN=1.27%). Antimicrobial susceptibility was conducted using broth microdilution (Sensititre¨) with a panel of 15 antimicrobials. The most frequent resistance was against streptomycin (97.68 %), ampicillin (81.39%), and tetracycline (41.86%). Polymerase chain reaction was performed to identify the resistant determining genes. The resistance coding genes, including blaTEM (ampicillin resistance: 83.78%) and addA1 (streptomycin resistance: 45.83%), were detected with the highest frequency. Genotypic characterization by pulse field gel electrophoresis revealed clonal relatedness among STEC isolated from animal and environmental sources based on spatial proximity. Our study highlights the potential role of food animals on STEC and *E. coli* O157:H7 dissemination to fresh produce and environment in the sustainable farms.

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26. **Joshua E. Pitzer**1, John E. Baumgartner1, Tonya Zeczycki2, James A. Budnick3, Clayton C. Caswell3, Daniel W. Martin1, and R. Martin Roop II1

Departments of 1Microbiology and Immunology and 2Biochemistry and Molecular Biology, Brody School of Medicine, East Carolina University, Greenville, NC; 3Department of Biomedical Sciences and Pathobiology, Virginia-Maryland Regional College of Veterinary Medicine, Virginia Tech, Blacksburg, VA

The pyruvate kinase PykM is a critical virulence determinant for Brucella abortus 2308 in mice, but its activity is not essential for glucose catabolism

The proton symporter MntH serves as the sole high affinity manganese (Mn) transporter in *Brucella*. The extreme attenuation displayed by a *B. abortus* mntH mutant in mice indicates that Mn is an especially important micronutrient for these bacteria during their residence in mammalian hosts. Glucose catabolism has recently been shown to be critical for the ability of *Brucella* strains to persist in alternatively activated macrophages, which in turn is required for the maintenance of chronic infections in mice. *Brucella* strains are thought to catabolize glucose exclusively via the pentose phosphate pathway, and the enzyme pyruvate kinase is required for maintaining carbon flow through this pathway. The brucellae produce a single pyruvate kinase, PykM, which is predicted to be a Mn-dependent enzyme. Biochemical characterization of the *Brucella* PykM confirmed that Mn serves as the most efficient metal co-factor for this enzyme, and phenotypic analysis of a mntH mutant demonstrated that high affinity Mn transport is required to support wild-type PykM activity in *B. abortus* 2308. A *B. abortus* pykM mutant also exhibits significant attenuation in mice, but retains its ability to utilize glucose in in vitro assays. These experimental findings indicate that PykM serves as a critical virulence determinant for *Brucella* strains, and support the proposition that high affinity Mn transport mediated by MntH plays an important role in supporting the activity of this enzyme. But they also indicate that *Brucella* strains can catabolize glucose via enzymatic pathways that do not rely upon the activity of pyruvate kinase.

27. **Somer N. Jones**, Ryan G. Rhodes

Deletion and Characterization of fjoh_0638, a Gene Encoding an RpoN Enhancer Binding Protein in Flavobacterium johnsoniae

In the regulation of gene expression, sigma factors are important proteins that are necessary for binding and recruitment of the RNA polymerase to promoter regions to initiate transcription in bacteria. *Flavobacterium johnsoniae* has two cytoplasmic sigma factors, RpoD and RpoN, which are characteristically different. RpoD has been classified as the "housekeeping" sigma factor in *F. johnsoniae* while RpoN is predicted to respond to internal and external stimuli to regulate gene expression. Also, while RpoD is able to bind directly to the promoter region in order to recruit RNA polymerase, an enhancer binding protein (EBP) is necessary for RpoN to bind and recruit the RNA polymerase. This study aimed to disrupt fjoh_0638, one of six genes predicted to encode a protein with the common features of an EBP: a DNA binding domain, an RpoN interaction domain, and an ATPase domain. In addition, fjoh_0638 is unique as it may respond to extracellular signals as part of a two component system with the sensor kinase fjoh_0673. To disrupt this gene, primers designed with engineered restriction sites were used to amplify the regions upstream and downstream of fjoh_0638 by polymerase chain reaction. The two flanking regions were cloned into the suicide vector pRR51 to generate pSNJ02. The deletion construct, pSNJ02, was confirmed by restriction digest and DNA sequencing. pSNJ02 will be transferred to *F. johnsoniae* by triparental conjugation to generate an unmarked deletion in fjoh_0638 by allelic exchange. The deletion mutant will be evaluated for gliding motility, bacteriophage sensitivity, and metabolic phenotype. Additionally, RT-PCR will be performed to assess transcript levels of fjoh_0638 in wild-type cells under different growth conditions to characterize the expression of this EBP.

28. **Megan Polzin**, Maryam Ahmed, and Darren Seals

The effects of oncolytic vesicular stomatitis virus on tumor-associated macrophages

Vesicular stomatitis virus (VSV) is currently being developed as a therapeutic option for patients with aggressive forms of cancer due to its ability to preferentially infect and kill tumor cells, while sparing healthy cells. However, less is known about how VSV impacts other cells within the invasive tumor microenvironment. My project involves determining whether VSV can modulate M2 macrophages, a cell type known to facilitate and enhance cancer metastasis. We hypothesized that VSV will preferentially target and infect M2 macrophages, thereby inhibiting their stimulatory effects on cancer invasion. Utilizing the ability of the THP-1 macrophage cell line to differentiate into various macrophage subsets, we observed that monocytes were more susceptible to infection and killing by VSV than macrophages. Furthermore, both populations were more permissive to infection with a type I interferon (IFN)-inducing strain of VSV (rM51R-M virus) as compared to the wild-type (wt) strain of VSV (rwt virus). Infection of proinflammatory M1 macrophages and pro-tumorigenic M2 macrophages with rwt or rM51R-M viruses also decreased podosome formation, a major player in cancer cell intravasation and extravasation. This effect was most apparent in M2 macrophages and occurred mainly in response to rwt virus. These results indicate that macrophage populations are differentially susceptible to infection with VSV and that VSV has the capacity to modulate M2 macrophage subsets. Future studies will determine the replicative potential of VSV strains in M1 and M2 macrophages and the functional consequences of VSV in these cells.

29. **John M. Farrow**, III and Everett C. Pesci

Two distinct promoters control the transcription of pqsR in Pseudomonas aeruginosa

The ubiquitous Gram-negative bacterium *Pseudomonas aeruginosa* is an opportunistic pathogen that can cause serious infections in immunocompromised individuals. P. aeruginosa virulence is controlled partly by intercellular communication, and the transcription factor PqsR is a necessary component in the *P. aeruginosa* cell-to-cell signaling network. PqsR acts as the receptor for the Pseudomonas quinolone signal, and it controls the production of 2-alkyl-4-quinolone molecules which affect *P. aeruginosa* physiology and pathogenicity. Previous studies showed that the expression of pqsR is positively controlled by the quorum-sensing regulator LasR, but it was unclear how LasR is able to induce pqsR transcription. In this study we further investigated the control of pqsR, and discovered two separate promoter sites that contribute to pqsR expression. We found that LasR activates pqsR transcription at the distal promoter site, which has the most significant effects on pqsR expression. However, activation at this site can be negatively impacted by the regulator CysB. We also found that the proximal promoter site contributes to pqsR transcription, but initiation at this site is inhibited by a negative regulatory sequence element, and potentially by the H-NS family members MvaT and MvaU. We propose a model where positive and negative regulatory influences at each promoter site are integrated to modify the amount of pqsR expression. This arrangement could allow for information from both environmental signals and cell-to-cell communication to influence PqsR levels and quinolone production.

30. **Lauren Scheetz** and Ryan Rhodes

Investigation of the Type II Secretion System in F. johnsoniae

Flavobacterium johnsoniae is a member of the Bacteroidetes, and serves as a model organism for studying motility, biochemistry, and physiology in this phylum due to its short generation time and ease of genetic manipulation. Numerous protein secretion systems aid gram-negative bacteria in moving large proteins across the inner and outer membranes. Genome analysis suggests *F. johnsoniae* possesses five of the twelve known secretion pathways: the Sec and Tat pathways for secretion across the inner membrane, and the Type II, VIII and IX secretion systems (T2SS, T8SS, and T9SS) for movement across the outer membrane. In F. johnsoniae, proteins destined for secretion outside the cell presumably rely on the Sec and Tat pathways to cross the inner membrane and on the T2SS or T9SS for transport across the outer membrane. While the T9SS has been well characterized in gliding motility and chitin utilization in *F. johnsoniae*, little is known about the T2SS in this organism or other Bacteroidetes. Herein, we aim to characterize the T2SS in *F. johnsoniae* by deleting gspD (fjoh_0618), the main channel protein of the T2SS, using an allelic exchange system. To delete gspD, the deletion construct, pLES02 was transferred to *F. johnsoniae* by triparental conjugation. Ten second recombinants were screened by PCR resulting in the isolation of seven colonies carrying the putative deletion. Following confirmation, a deletion clone was compared to wild-type cells and a T9SS mutant (_gldNO) for the ability to secrete amylase and protease. Results of these agar plate assays indicate that enzymes involved in these processes may be primarily secreted through the T9SS, as the T9SS mutant did not hydrolyze these substrates, whereas the _gspD mutant exhibited zones of clearing similar to wild-type cells. We are currently evaluating lipase activity to determine if this enzyme might be secreted by the T2SS.

31. **Michael M. Opata**, Karis M. Norwood, Samad A. Ibitokou, Victor H. Carpio, Kyle D. Wilson, and Robin Stephens

Identification of two T cell subsets that protect and survive long-term in malaria infection

Malaria and other chronic infections generate effector and effector memory T cells, but the infection is not completely controlled. While the parasite is present, some level of protection is provided, but this decays as specific cytokine production disappears. This indicates that protection in chronic malaria is partly provided by continuously activated CD4 T cells. Current vaccination protocols also result in immunity that decays. In order to invent new vaccination strategies to combat this problem, we need to understand what T cell types are potentially both protective and long-lived. Therefore, we tested effector (Teff), and central (Tcm) and effector memory (Tem) T cell subsets for their ability to both protect and survive in vivo. Using transgenic T cells specific for the Merozoite Surface Protein-1 of Plasmodium chabaudi, and our previously defined effector and memory subsets, we sort-purified individual cell populations, and tested their ability to protect in combination with immune B cells. Strikingly, Teff reduce parasitemia by half, at all stages of effector maturation. When Teff subsets were rested in na•ve animals to allow generation of memory, the least mature Teff subset, TeffEarly, which we have shown generate the most memory cells, decreased parasitemia the most on infection of adoptive hosts. Interestingly, these earliest activated T cells survive as well as the memory subsets, of which only Tem Late (CD62LloCD27-) reduced parasite by 10%. Therefore, we have identified two long-lived T cell subsets that may represent a correlate of protection or a target for longer-lived vaccine-induced protection against malaria.

32. **Rasi Fitria** and Ilenys Muniz Perez-Diaz

Effect of starter cultures in completion of cucumber fermentation brined with 1.1 % calcium chloride (CaCl2)

Cucumber fermentations brined with 1.1 % CaCl2 and no NaCl was developed as an alternative for commercial processing with a reduce environmental footprint. The pilot tested NaCl free fermentation included the use of a *Lactobacillus plantarum* starter culture. It was theorize that its utilization would aid in achieving a consistently rapid and complete fermentation to assure quality and safety in the absence of NaCl, a preservative. This research was conducted to determine the function and necessity for a starter culture to achieve a complete lactic acid fermentation of cucumbers brined with CaCl2 in the laboratory. Additionally, this study compared the effect and performance of 5 lactic acid bacteria (LAB), which included the cucumber fermentation isolates *Lactobacillus pentosus* LA0445 and 1.8.6, *L. plantarum* 3.2.8, and the Spanish olive fermentation isolate *L. pentosus* IG. Fresh cucumbers were fermented in 1-gallon jars and were brined with or without 1.1 % CaCl2 and 0.1 % potassium sorbate. Cover brine samples were aseptically collected at 4 time intervals during the fermentation for biochemical and microbiological analyses including measurements of pH , sugars and organic acids concentrations, LAB, Enterobacteriacea, yeast and molds counts. This study showed that complete fermentations were achieved when brining with CaCl2, regardless of the inoculation with a starter culture, at a faster rate than those conducted without the salt. However, the addition of a starter culture helped with a faster reduction in pH and completion of the fermentation, proving our hypothesis. Furthermore, *L. pentosus* performed as well as *L. plantarum* in cucumber fermentations in 1-gallon jars and both species consistently induced complete conversion of sugar into organic acids. These results suggested that although a cost saving may be achievable by the exclusion of a starter culture, its inclusion is likely to yield faster, complete and thus microbiological stable fermentations.

33. **Evans, J.**1*, L—pez-Legentil, S.1, Shenkar, N.2, Erwin, P.1

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Aiding and abetting: characterizing the diversity, host-specificity, and potential function of microbial symbionts in introduced North Carolina ascidians

Several ascidian species have been introduced around the world, exhibiting remarkable success in crossing geographic borders and adapting to local environmental conditions. To examine the potential role of microbial symbionts in the success of these introductions, we determined the host-specificity of microbial communities inhabiting three ascidian species commonly found off the North Carolina coast and compared them with seawater samples. Replicate samples (n=5) of 2 worldwide introduced species: *Polyandrocarpa zorritensis* and *P. anguinea* were collected with replicate samples of ambient seawater (n=4) at the Wrightsville Beach Marina in September and October 2015, and one cryptogenic species: *Distaplia bermudensis* was collected at the Bridge Tender Marina (located 170 m away from the previous location) in June 2015. Microbial communities of ascidian hosts and ambient seawater were characterized by next-generation (Illumina) sequencing of 16S rRNA gene sequences. Ascidians hosted unique and diverse symbiont communities, consisting of 5,696 unique microbial OTUs (at 97% sequenced identity) from 47 bacterial and 3 archaeal phyla. Permutational multivariate analyses of variance revealed clear differentiation of ascidian symbionts compared to bacterioplankton in surrounding seawater and distinct microbial communities in each ascidian host species. Further, 103 universal core OTUs (present in all replicates of all 3 host ascidians) were identified, some of which have been previously described in the microbiome of marine invertebrates and have been linked to ammonia-oxidization, denitrification, pathogenesis, and heavy-metal processing, among other functions. These results suggest that the microbial symbionts in ascidians exhibit a high degree of host-specificity, forming intimate associations with their hosts that may contribute to their adaptation to new environments via increased tolerance thresholds and enhanced holobiont function.Several ascidian species have been introduced around the world, exhibiting remarkable success in crossing geographic borders and adapting to local environmental conditions. To examine the potential role of microbial symbionts in the success of these introductions, we determined the host-specificity of microbial communities inhabiting three ascidian species commonly found off the North Carolina coast and compared them with seawater samples. Replicate samples (n=5) of 2 worldwide introduced species: *Polyandrocarpa zorritensis* and *P. anguinea* were collected with replicate samples of ambient seawater (n=4) at the Wrightsville Beach Marina in September and October 2015, and one cryptogenic species: *Distaplia bermudensis* was collected at the Bridge Tender Marina (located 170 m away from the previous location) in June 2015. Microbial communities of ascidian hosts and ambient seawater were characterized by next-generation (Illumina) sequencing of 16S rRNA gene sequences. Ascidians hosted unique and diverse symbiont communities, consisting of 5,696 unique microbial OTUs (at 97% sequenced identity) from 47 bacterial and 3 archaeal phyla. Permutational multivariate analyses of variance revealed clear differentiation of ascidian symbionts compared to bacterioplankton in surrounding seawater and distinct microbial communities in each ascidian host species. Further, 103 universal core OTUs (present in all replicates of all 3 host ascidians) were identified, some of which have been previously described in the microbiome of marine invertebrates and have been linked to ammonia-oxidization, denitrification, pathogenesis, and heavy-metal processing, among other functions. These results suggest that the microbial symbionts in ascidians exhibit a high degree of host-specificity, forming intimate associations with their hosts that may contribute to their adaptation to new environments via increased tolerance thresholds and enhanced holobiont function.

34. **Erwin PM**, Rhodes RG, Kiser KB, Keenan-Bateman TF, McLellan WA, Pabst DA

Diversity and structure of the gut microbiome in pygmy (Kogia breviceps) and dwarf (K. sima) sperm whales

Mammals host diverse bacterial and archaeal symbionts (i.e., microbiomes) that play important roles in digestive and immune system functioning. The gut microbiomes of cetaceans (whales, dolphins and porpoises), however, remain largely unexplored, in part due to their protected status and difficulties associated with sample collection. In this study, we utilized stranded specimens to characterize the gut microbiomes of two closely-related, deep diving species with similar diets Ð the pygmy (*Kogia breviceps*, n = 9) and dwarf (*K. sima*, n = 4) sperm whales Ð using fecal samples collected during necropsy. Next-generation sequencing of 16S rRNA gene tags revealed diverse microbial communities in kogiid whales, comprised of 1,720 unique symbiont taxa (97% operational taxonomic units, OTUs) from 12 bacterial phyla and one archaeal lineage (Euryarchaeota). The gut microbiomes of *K. breviceps* and *K. sima* averaged 432 (±7 SE) and 416 (±18 SE) symbiont OTUs per host individual and were dominated by the bacterial phyla Firmicutes (43% and 57%, relative abundance) and Bacteroidetes (33% and 13%), respectively. Notably, the high diversity and phylum-level composition of kogiid microbiomes differed from previously reported microbiomes in other toothed whales (bottlenose dolphins and beluga whales), which exhibited low diversity (52 ±8 and 84 ±18 OTUs, respectively) communities dominated by Proteobacteria (60% in dolphins) and Actinobacteria (66% in beluga whales). Comparative analyses of community similarity at the OTU-level revealed distinct gut microbiomes in pygmy and dwarf sperm whales, driven largely by differences in the relative abundance of shared symbiont taxa, that were clearly differentiated from previously characterized microbiomes in other toothed and baleen whale species. These results provide first insights into the diversity, composition and structure of the gut microbiomes in pygmy and dwarf sperm whales, two elusive and poorly understood cetacean species, and indicate that host identity plays an important role in structuring cetacean microbiomes, even at fine-scale taxonomic levels.

35. **James Seward**, Michael Carson, Jamie Lamit, Joe Yavitt, Jim McLaughlin, Christopher Schadt, Nate Basiliko and Suzanna BrŠuer

Peatland Microbes

Peatland environments around the world serve as critical carbon sinks and are estimated to hold as much as one-third of the earthÕs total terrestrial carbon, while only making up a small percent of the world's land surface area (ca. 3-4%). Since the retreat of the glaciers at the end of the last ice age, global temperatures are now 3o to 5o C warmer on average than they were during those times. In southern Appalachian peatlands, we hypothesize that this temperature increase has caused peatland deterioration and has triggered a shift from carbon accumulation to carbon release. Analyses of changes occurring in our local southern Appalachian peatlands may help predict what may happen to northern peatlands as temperatures continue to increase. Herein, we aim to compare various peatland sites across a longitudinal gradient by analyzing the microbial communities that inhabit these peatland bogs and fens. Initial results from our research efforts in conjunction with the Department of Energys Global Peatland Microbiome Project have shown distinct differences in microbial communities from peatlands that have greater accumulation of carbon (bogs) to those that have greater decomposition (fens). It is our hope that a more detailed analysis may allow us to predict which hydrolytic and/or fermentative groups may be involved in catalyzing the shift from C-storing to C-releasing. Our end goal is to develop a new factor representing microbial decomposition pathways (Microbial Gene Types) that will become a key input to strengthen the predictive power of global climate and land models such as the CLM4.5 model.

36. **Cody D. Moorman**, Alan D. Curtis, II, and Mark D. Mannie

GMCSF-MOG abrogates experimental autoimmune encephalomyelitis (EAE) through the induction of MOG-specific regulatory T cells.

FoxP3+ CD25high regulatory T-cells (Tregs) play a crucial role in maintaining peripheral tolerance by suppressing auto-reactive T-cells. Developing a safe therapeutic approach to induce autoantigen-specific Tregs could provide an effective treatment for Multiple Sclerosis (MS), as well as for other autoimmune diseases. Previous studies have shown that the fusion protein GMCSF-MOG has tolerogenic activity that inhibits experimental autoimmune encephalomyelitis (EAE) in mice. In an effort to elucidate the mechanism by which GMCSF-MOG is inhibiting EAE, we investigated the ability of GMCSF-MOG to induce Tregs in vivo. In this study, we provide evidence that GMCSF-MOG can induce MOG-specific FOXP3+ Tregs. Treatment of 2D2-FIG mice with GMCSF-MOG resulted in an increase of FOXP3+ Tregs in the blood, with an additional increase observed after multiple immunizations. Furthermore, the depletion of GMCSF-MOG-induced Tregs by use of the anti-CD25 mAb PC61 restores susceptibility to EAE in C57BL/6 mice. In conclusion, subcutaneous immunization with GMCSF-MOG induces antigen-specific Tregs, which play a role in the inhibition of EAE in mice.

37. **Tony Perez**, James Seward, Aly Edwards and Suzanna BrŠuer

Culturing peat microbes

Peat-forming bogs serve as a host to a vast array of unique microbes and microbial communities. While peatland ecology is generally understudied, they account for many important environmental processes, such as serving as global carbon sinks. Herein, we aim to cultivate and isolate these microbes that are responsible for vital ecological and biogeographical processes such as hydrolysis, fermentation and methanogenesis of organic matter in peatlands. In an in-vitro setting, we have taken filtered samples from local southern Appalachian peat bogs in an attempt to grow and isolate these distinctive microbes with the addition of specific chemicals such as Birchwood xylan. This project will elucidate the physiology and ecological roles of these low-pH tolerant microorganisms from acidic peat bogs, and will enhance our understanding of the mechanisms that influence global carbon cycles.

38. **Rebecca Anthouard**, Ashley Bone, Peggy Cotter

PlrSR is a two-component system required for virulence of Bordetella bronchiseptica

Despite high vaccination rates, whooping cough has been on the rise in recent years. *Bordetella pertussis*, the causative agent of whooping cough, only infects humans. A closely related subspecies, *Bordetella bronchiseptica*, infects a broad range of mammals, including mice, allowing us to study Bordetella infections in the context of a natural host. *Bordetella* infection relies heavily on virulence factors, all of which are controlled by the two-component system BvgAS. Recently, the Julio lab identified another two-component system, PlrSR (highly homologous to NtrYX), that is required for *Bordetella* colonization. Additional work from our lab has shown that PlrS is required for two reasons: to activate BvgAS, and to allow bacteria to survive in the lower respiratory tract. Here, we used lacZ reporters to show that PlrS does not regulate bvgAS transcriptionally. Additionally, overexpression of PlrR or PlrR-D52E (a phosphomimetic) compensates for ÆplrS and restores BvgAS-dependent phenotypes, suggesting that PlrS likely affects BvgAS through PlrR. We also performed RNAseq comparing ÆbvgA to ÆbvgAÆplrS and found that PlrS affects secretion, motility, and metabolism. Future work is aimed at further characterizing the PlrSR regulon and the mechanism by which PlrSR regulate BvgAS activity.

39. **Hunter Whittington**, Andrea Azcarate-Peril, Mahatam Singh, and Jose Bruno-Barcena

Fungicide Application to Soils, Biodegradation, and Responses of the Soil Microbial Community

One of the more esoteric aspects of the use of agricultural chemicals is their transient effect on the soil microbial population, and conversely the impact that soil microbes have on these compounds. Surveying the vast soil microbial community has recently become more tangible with the decline in price of high-throughput sequencing. Furthermore, advancements in chemical analytics have allowed for detection of labeled compounds even in a chemically complex medium. In this study, we use a combination of next-generation sequencing and liquid chromatography followed by tandem mass spectrometry to examine both aspects of this chemical-microbe interaction in an in vitro soil system. After treating agricultural soils from four different geographic locations with just 2.4 ppm of the triazolopyrimidine fungicide ametoctradin, we found that all four of the major ametoctradin derivative metabolites were detected, with M650F03, one of the most difficult of the major metabolites to degrade, being detected most abundantly. Additionally, the soil microbial community responded with significant changes at the phylum level. The Proteobacteria increased in relative abundance by up to 25% and the Acidobacteria decreased in relative abundance by around 10%. Interestingly, many of the phyla observed had no significant response to treatment with ametoctradin. Overall, we have shown that soil microbial communities respond similarly to treatment with ametoctradin regardless of geographic location and that degradation of this compound appears to be microbially mediated.

40. **Dakota W. Goad**, G. Travis Tabor, Darren F. Seals and Maryam Ahmed

Vesicular Stomatitis Virus as an Oncolytic Agent Against Cancer Cell Metastasis

Vesicular stomatitis virus (VSV) is currently being investigated as a candidate oncolytic agent due to its capacity to kill cancer cells while exhibiting low virulence in vivo. My project involves investigating the impact of VSV on cancer cell invasion mechanisms. A main trigger for cancer metastasis is the formation of actin-rich structures known as invadopodia, which function to degrade the extracellular matrix. Studies have shown that VSV can manipulate the cytoskeletal structure of cancer cells to potentiate viral replication. We hypothesize that VSV will alter invadopodia structures and inhibit their proteolytic function due to the global ability of VSV to inhibit host gene expression in infected cells. Surprisingly, our results showed that at 6 hours post infection, the invadopodia structures on Src- transformed fibroblast cells were both higher in number per cell and in overall robustness. This enhancement was correlated with an increase in the expression of the invadopodia-associated proteins, Tks-5 and Dynamin 2. In contrast to its effects on invadopodia formation, VSV decreased matrix degradation and we observed an increase in the expression of cortactin, a protein involved the in the regulation and secretion of matrix metalloproteinases. These results suggest that the increase in invadopodia formation by VSV is separable from its ability to decrease proteolytic degradation of the extracellular matrix. The varying expression of critical proteins for invadopodia formation post infection may indicate that the replication cycle of oncolytic VSV interplays with factors associated with invadopodia formation and alters their proteolytic activity.

41. **Redife Ucar**

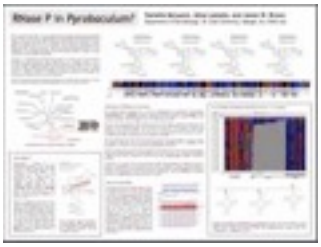
Profiling of the ability of selected lactic acid bacteria to utilize carbohydrates found to be present in cucumber fermentations, including starter culture candidates of *Lactobacillus plantarum* and *Lactobacillus pentosus*.

Carbohydrate utilization is the main energy generating metabolic activity in lactic acid bacteria (LAB). Significant variability in the ability to utilize various carbohydrate sources have been reported at the species level for this group of industrially relevant microbes. It is the objective of this exploratory research to evaluate the ability of a selected group of LAB including *Lactobacillus plantarum*, *L. pentosus*, *L. brevis*, *L. buchneri* and *Pediococcus pentosaceous*, isolated from commercial cucumber fermentations. Putative carbohydrate utilization pathways were compared using the publically available genome sequences. Carbohydrates found in cucumber fermentations including: glucose, fructose, xylose, trehalose, cellobiose, furfural, gentiobiose and lyxose, and citrulline were targeted in the analysis. Although, several putative genes associated with xylose synthesis are found in the genome sequences studied, catabolic pathways were not found. Conversion of cellobiose to _-D-glucose seems to be possible by *L. plantarum*, *L.pentosus*, *L.brevis*, and *L. buchneri*. According to the bioinformatic analysis, gentiobiose may serve as the substrate for an ABC (ATP-binding cassette) transporter associated with glucose metabolism on the glucose/mannose transporter system for the genome sequences studied. Putative genes encoding for enzymes associated with the lyxose and furfural metabolic pathways were absent. Genes encoding for the citrulline catabolic pathway were found in the *L. plantarum* and *L. pentosus* genome sequences. The *P. pentosaceous* and *L. brevis* genome sequences encode for arginine deiminase (arcA), which converts citrulline to arginine. *L. buchneri* seems to be able to use citrulline effectively both ways. The ability of the selected species to utilize the targeted carbohydrates in a cucumber juice medium was studied. Results suggest all the LAB tested, are able to derived energy for growth from all the substrates tested in a fermented cucumber juice medium at pH 4.7 at variable rates, possibly enabling their strategic utilization as starter cultures.

Presentation Awards

The Mary Poston Award was established to recognize the best paper given by a student at meetings of the NC Branch of the ASM. Mary Poston was a longtime employee of Duke University who contributed much to the NC Branch and she was held in high esteem both by her colleagues and by medical students. She contributed much to the NC Branch, including service as Branch Secretary-Treasurer from 1950 until her death in 1961. Many letters of appreciation have been written over the years by student recipients of the Mary Poston Award, commenting on the confidence the award gave them and on the importance of the competition for the award as part of their graduate training.

The Thoyd Melton Award was established to recognize an outstanding oral presentation by a graduate student. At the time of his premature death on Nov. 22, 2000, Thoyd Melton was Associate Vice Chancellor for Academic Affairs and Dean of graduate studies at N.C. A&T State University. Prior to this position, Dr. Melton was a member of NC State University's Department of Microbiology and an Associate Dean of the Graduate School. Dr. Melton was very active in research and particularly in graduate education. In 1999, he received the William A. Hinton Research Training Award from ASM. This award honors an individual who has made significant contributions toward fostering the research training of underrepresented minorities in microbiology.



The Best Poster award is open to anyone presenting a poster at the NC ASM meeting.

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The Paul Phibbs Award is awarded for the best presentation by an undergraduate student at NC ASM Branch meetings.

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