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Unveiling Friday Nov. 9

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Directions and Campus map:
Campus Directions
Campus Map (PDF)

Accommodations:
Best Western Radford Inn
1501 Tyler Avenue, Radford.
(540) 639-3000
Virginia Branch 2018 Annual Meeting
Friday, November 9, 2018

12:00-5:00p  Registration, Center for the Sciences Lobby
1:00-1:15p   Welcome, Center for the Sciences M73
             
HOST: Dr. Justin Anderson, Ph.D. Radford University
Dr. Jake Fox Ph.D., Associate Professor of Anthropological Sciences,
President of the Faculty Senate Radford University

Virginia Branch Logo Reveal
Dr. Justin Anderson Ph.D., Radford University

For the oral presentation sessions below, numbers in parentheses indicate the page on which the
associated abstract can be found. Underlined presenters are being judged in the oral presentation
competition. Presenter names are linked to the abstract title. Abstract titles are linked to their respective
presentation sessions.

1:15-2:45p  Oral Presentations, Session I, Center for the Sciences M73
Moderated by Dr. David Freier Ph.D., University of Lynchburg

Alexandra Cumbie, Old Dominion University (10)
Role of avian hosts in spread and maintenance of Borrelia burgdorferi in
Ixodes spp. collected off birds in southeastern Virginia

Steven McBride, Virginia Polytechnic Institute and State University (10)
Volatile methanol and acetone additions increase labile soil carbon and inhibit
nitrification

Gary Camper, Virginia Polytechnic Institute and State University (11)
Characterization of a pilus based secretion system in the Gram-positive
anaerobe Clostridium perfringens

Holly Packard, Virginia Polytechnic Institute and State University (11)
Confirming the essential role of select transcription factors in the
phytopathogen Pantoea stewartii during in planta growth through reverse
genetics

Kellie King, Virginia Polytechnic Institute and State University (12)
Discovery of new regulatory small RNAs in Brucella abortus

James Budnick, Virginia Polytechnic Institute and State University (12)
GABA, from neurotransmitter to host-pathogen signaling molecule.

2:45-3:00p  BREAK, Center for the Sciences Atrium
3:00-4:30p  **Oral Presentations, Session II**, Center for the Sciences M73
Moderated by Dr. David Popham Ph.D., Virginia Polytechnic Institute and State University

*Anna Phan*, Old Dominion University (13)
*The Prevalence of Borrelia miyamotoi in Ixodid Ticks in Virginia*

*Stephen DeVilbiss*, Virginia Polytechnic Institute and State University (13)
*Effects of base cations on fecal indicator persistence and bacterial community structure*

*Sarah Shawver*, Virginia Polytechnic Institute and State University (14)
*Long-term effects of antibiotic contaminated manure on soil microbial communities and antibiotic resistance gene abundance*

*Ian Hines*, Virginia Polytechnic Institute and State University (15)
*Influence of host genetics on the epithelial-associated microbiomes in aquaculture-raised Nile tilapia (Oreochromis niloticus).*

*Timofey Arapov*, Virginia Polytechnic Institute and State University (15)
*Receptor quantification in Sinorhizobium meliloti – can tagging mislead?*

*Karl Compton*, Virginia Polytechnic Institute and State University (16)
*Sinorhizobium meliloti Chemoreceptor McpV Senses Short-Chain Carboxylates via Direct Binding*

4:30-4:45p  **National ASM Information**, Center for the Sciences M73
Presented by Ms. Rachel Korba, ASM Young Ambassador to Virginia, Masters Student, Clinical Lab Sciences, VCU.

4:45-6:00p  **ASMDL speaker**, Center for the Sciences M73
Introduced by Dr. Nazir Barekzi, Norfolk State University

**ASM BRANCH LECTURESHIP SPEAKER**

*Harry L.T. Mobley, Ph.D.*
*Frederick G. Novy Professor and Chair*
*University of Michigan Medical School*

*Measuring Bacterial Gene Expression and Growth Rates During Human Infection*
6:00-6:20p  Branch Photograph, TBD

6:20 -8:00p  Buffet Dinner and Posters, TBD

8:30 – 11:00  Virginia Branch ASM Evening Social
Montgomery Room, Best Western

Featuring wine selections contributed by:

![Am Rhein's WINE CELLARS]

“Local Wine with a German Influence”
Virginia Branch 2018 Annual Meeting
Saturday, November 10, 2018

8:30-8:45a  Coffee and snacks, Center for the Sciences Lobby

8:45-10:15a  Oral Presentations, Session III, Center for the Sciences M73
Moderated by Dr. Ann Stevens, Ph.D., Virginia Polytechnic Institute and State University

Zach Bement, Old Dominion University (16)
Prevalence of Babesia microti in Ixodid Ticks in Virginia

Cameron Sayer, Virginia Polytechnic Institute and State University (17)
TnSeq identification of genes with uncharacterized roles in Bacillus subtilis spore germination

Bidisha Barat, Virginia Polytechnic Institute and State University (17)
Tn seq of uncharacterized genes involved in Bacillus subtilis spore germination

Ariana Umana, Virginia Polytechnic Institute and State University (18)
Exploring the role of MORN2 domain proteins in Fusobacterium nucleatum pathogenesis

Blake Sanders, Virginia Polytechnic Institute and State University (19)
FusoPortal: An online database of Hybrid MinION sequenced, assembled, and functionally annotated Fusobacterium genomes

Angie Saadat, Virginia Polytechnic Institute and State University (19)
Analysis of TpeL secretion in C. perfringens strain HN13

10:15-10:30a  BREAK and refreshments, Center for the Sciences Lobby

10:30 – 12:00  Concurrent Sessions:

Scholarship of Teaching and Learning Speaker
Center for the Sciences M70
An in Depth Look into the implementation of course-based undergraduate research experiences (CUREs)

Student Career Development
Center for the Sciences M65
Career Opportunity Panel: A panel presentation for early career graduates
10:30-12:00a  CONCURRENT SESSION: Scholarship of Teaching and Learning
Speaker, Center for the Sciences M70
Introduced by Nazir Barekzi, Norfolk State University

CUREs: An in Depth Look into the implementation of course-based undergraduate research experiences (CUREs)

Room: Center for the Sciences M70

CURE Presentations by:
Jim Herrick, James Madison University, A CURE for Salmonella: Engaging Students in Pathogen Microbiology and Bioinformatics

Lynn Lewis, University of Mary Washington, Transforming the Biology Major Through Course Based Research.

Nazir Barekzi, Norfolk State University, SEC3URE: Spartans Engaged in Community-focused Collaborative Course-based Undergraduate Research Experiences
10:30-12:00p  CONCURRENT SESSION: Student Career Development
Center for the Sciences M65
Introduced by Dale Beach, Longwood University

Career Opportunity Panel:
A panel presentation for early career graduates

Panelists:
Ms. Heaven Cerritos, Laboratory Technician, Indoor Biotechnologies Inc
Ms. Rachel Korba, ASM Young Ambassador from the ASM, Masters Student, Clinical Lab Sciences, VCU
Ms. Cassandra Isley, Vice President for Strategy and Development, Virginia BIO
Dr. Lauren Turner Ph.D., Lead Scientist, Division of Consolidated Laboratory Services (DCLS)
Dr. Jared Heffron Ph.D., Senior Scientist, Novozymes
Dr. Harry Mobley Ph.D., Frederick G. Novy Professor and Chair, University of Michigan School of Medicine
Ms. Amy White, Dean School of STEM, Virginia Western Community College

12:00-12:10p  Closing Remarks, Center for the Sciences M73
Dr. Justin Anderson Ph.D., Radford University

12:10-1:30p  Lunch and Business Meeting, Center for the Sciences M73

1:30p  Adjourn  Return to the real world
Role of avian hosts in spread and maintenance of *Borrelia burgdorferi* in *Ixodes* spp. collected off birds in southeastern Virginia ®

Alexandra Cumbie*, Erin Heller, Eric Walters, Holly Gaff, and Wayne Hynes
Old Dominion University, Norfolk, VA

The role that birds play in the maintenance and the movement of ticks and tick-borne pathogens is an important area of research. The home ranges of resident bird species, and the migratory nature of others, can influence vector-borne disease ecology in a region. Of particular interest is the interaction of birds, both resident and migratory, with *Ixodes* spp. which are the primary vector species of *Borrelia burgdorferi*, the causative agent of Lyme disease. In this study, *Ixodes* spp. were removed from birds captured at various locations in southeastern Virginia. All ticks were pulverized, and their DNA extracted and tested for *Borrelia burgdorferi*. Nearly 1000 ticks were removed from birds, 288 were identified as *Ixodes* spp., with 24 positive for infection with *Borrelia burgdorferi* sensu stricto. The majority (23/24) of positive ticks came from resident bird species. These results indicate that birds may play a role in movement of infected ticks within a region, but do not play a large role as a reservoir host compared with rodent species.

Volatile methanol and acetone additions increase labile soil carbon and inhibit nitrification ®

Steven McBride*, Ernest Osburn, John Barrett, Michael Strickland
Virginia Polytechnic Institute and State University, Blacksburg, VA

A new framework has emerged in soil biogeochemistry which posits that labile C compounds are directly assimilated by microorganism before stabilizing on soil colloids. However, this model primarily focuses on dissolved inputs and overlooks another source of low molecular weight C, volatile organic compounds (VOCs). In this study we determined the effects of two VOCs (methanol, and acetone) on soil respiratory dynamics during a 28 d lab incubation. Following the incubation, we quantified soil C and N pools, microbial biomass, and nitrifier abundance to determine the effect of volatile compounds on soil C and N dynamics. We found that VOC addition resulted in a respiration spike 4.1- to 5.5-fold greater than the control, though respiration returned to baseline within one week. Also, VOC additions resulted in a 1.6-1.7 fold increase in labile C, suggesting that VOCs can enter soil C pools following assimilation by soil microorganisms. We also found that soil N pools and nitrifying microorganisms were altered with VOC addition, with soils exposed to VOCs having ~2.25-fold less total extractable nitrogen, and decreased NO3- concentration and nitrifier abundance relative to untreated soils. In soils
amended with VOCs, NO3- levels increased ~34-220 fold once VOC additions were stopped, suggesting an inhibitory effect of methanol and acetone on nitrifying microorganisms. Overall, our results show that VOCs increase soil respiration, contribute to soil labile C pools, and inhibit nitrification, suggesting soil C and N dynamics are different in soil microsites with high VOC concentrations.

**Characterization of a pilus based secretion system in the Gram-positive anaerobe *Clostridium perfringens***

Gary J. Camper*, Stephen B. Melville  
Virginia Polytechnic Institute and State University, Blacksburg, VA

*Clostridium perfringens* is a gram positive obligate anaerobe that is the cause of both mild and severe disease in humans and animals. Many of these diseases require secreted toxins in order to cause the typical disease state. Mechanisms by which these proteins are secreted are poorly understood, especially in Gram-positive bacteria. It has been found that a Von Willebrand Factor A domain containing protein, CPE0517 (VWA), is secreted outside of the cell via a type IV pili system, involving multiple T4P genes. A VWA-sfGFP fluorescent fusion protein is capable of localizing to the cell membrane, as expected of a secreted protein, but of the fusion protein was not detected in the supernatant. Overexpression of the VWA operon, consisting of a SipW leader peptidase, two other proteins of unknown function and VWA leads to the formation of a biofilm-like matrix in the liquid medium, which appears to be dependent on genes involved in type IV pili formation. Confirmation of these phenotypes is being performed via complementation in cis utilizing an allele replacement genetic system.

**Confirming the essential role of select transcription factors in the phytopathogen *Pantoea stewartii* during in planta growth through reverse genetics**

Holly Packard*, Brandi J. Thomas, Chase M. Mullins, and Ann M. Stevens  
Virginia Polytechnic Institute and State University, Blacksburg, VA

Pantoea stewartii subsp. stewartii is a bacterial phytopathogen that causes Stewart’s wilt disease in corn. Genes involved in leaf water-soaking symptoms and xylem biofilm formation are known factors important to the pathogenesis of *P. stewartii*. However, much remains to be discovered about in planta specific requirements for the survival and virulence of wilt-disease causing bacteria like *P. stewartii*. Previous work in our lab utilized RNA-Seq and Tn-Seq approaches to analyze the wild-type *P. stewartii* transcriptome expressed in planta and the ability of a library of *P. stewartii* mutants to survive in the xylem, respectively. Bioinformatics analysis of these datasets has identified numerous genes of interest that are hypothesized to play an essential role in the growth of *P. stewartii* within the xylem. A number of annotated transcription factors were selected for further work due to their potential role for broader influence on regulons of bacterial genes required in planta, including nsrR (nitric oxide stress response), iscR (iron-cluster assembly), and gcvR (glycine cleavage system). Other annotated, but unnamed,
transcriptional regulators were also selected to elucidate their role in _P. stewartii_ during infection. Reverse genetics approaches are underway to generate deletion and complementation strains of each chosen gene. These strains will then be used for in planta assays to evaluate virulence and colonization capabilities of the mutants. Ultimately, this work will broaden our understanding of the regulatory networks being employed by the bacteria in planta and may reveal possible disease intervention strategies.

**Discovery of new regulatory small RNAs in *Brucella abortus***

Kellie A. King*, James A. Budnick, Kirsten A. Kohl, and Clayton C. Caswell
Virginia Polytechnic Institute and State University, Blacksburg, VA

Small RNAs (sRNAs) are a class of regulatory molecules that impact gene expression in bacteria. These sRNA are often located in intergenic regions, and they have been shown to play crucial roles in the virulence in bacteria, including *Brucella abortus*. This bacterium causes brucellosis, which is a zoonotic infection of cattle, bison, and elk, but can also be transferred to humans by coming in-contact with infected animals and animal products. *B. abortus* is an intracellular pathogen that resides in macrophages during infection. This is a specialized niche where *B. abortus* encounters various stressful conditions that influence gene expression during trafficking through the macrophage. In this study, *B. abortus* was stressed in various conditions that could be encountered in the macrophage, including low pH, oxidative stress, and nutrient limitation. Transcriptomic data reveals high levels of transcripts located in intergenic regions, which is characteristic of sRNAs. Northern blot analyses confirmed the presence of 12 new sRNAs. Genetic manipulation is being employed to delete these sRNA from the genome, and further phenotypic assays and infection models will be used to determine the contribution of these new sRNA to the biology and virulence of *B. abortus*.

**GABA, from neurotransmitter to host-pathogen signaling molecule.**

James A. Budnick*, Lauren M. Sheehan, Lin Kang, Joshua Pitzer, Pawel Michalak, Martin R. Roop, and Clayton C. Caswell
Virginia Polytechnic Institute and State University, Blacksburg, VA

Gamma-aminobutyric acid (GABA) is the most abundant inhibitory neurotransmitter of the central nervous system. In plants, GABA is an important part of the immune system and is excreted when the plant is wounded or under stress. Studies revealed that macrophages, primary immune cells, synthesize and catabolize GABA as a means of cell-cell communication. The transport of GABA has been shown in the bacteria Agrobacterium tumefaciens and Pseudomonas aeruginosa but questions still remain as to how these microbes utilize the amino acid.
Brucella abortus, a mammalian pathogen, contains orthologs of the known GABA ABC transporter found in A. tumefaciens. We hypothesize that GABA acts as a host signal that Brucella detects in order to adapt to the host environment.

To test this hypothesis, we deleted the orthologous periplasmic-binding protein of the putative GABA ABC-type transporter in B. abortus, and this strain was significantly attenuated in a mouse model of chronic Brucella infection. Moreover, using 3H-GABA, it was demonstrated that the deletion strain was unable to import GABA in vitro, and transport is not inhibited by the presence of other amino acids. Furthermore, experimental evidence suggests that GABA is not metabolized by B. abortus, but is serving as a signaling molecule, and the import of GABA induces transcriptional alterations consistent with a functional signaling response. Transcriptomic data comparing bacteria treated with and without GABA revealed putative virulence factors regulated by the presence of this non-proteinogenic amino acid. Our results lead us to hypothesize that Brucella relies on host derived GABA during infection.

The Prevalence of Borrelia miyamotoi in Ixodid Ticks in Virginia

Anna Phan*, Dr. Holly Gaff, Dr. Wayne Hynes
Old Dominion University, Norfolk, VA

Borrelia miyamotoi is a Gram-negative spirochete bacterium that has recently been reported in Ixodes spp ticks in southeastern Virginia. This bacterium is transmitted by its primary vector Ixodes scapularis and can cause Lyme-like symptoms plus a relapsing fever. Another related tick, Ixodes affinis, which has not been reported to bite humans, may play a role in maintaining B. miyamotoi in its sylvatic cycle. Both I. scapularis and I. affinis are well established in southeastern Virginia and are known to share hosts as juveniles. In this study, we determined the prevalence of B. miyamotoi in these two tick species. Questing I. scapularis and I. affinis were collected from various field sites across Virginia. The presence of B. miyamotoi was detected by real-time PCR following extraction of tick DNA. Borrelia miyamotoi was detected in low prevalence within both I. scapularis and I. affinis, especially in recent years (2016 and 2017). Host sharing between these two tick species may affect the sylvatic cycle and natural reservoirs of the pathogen. Further research is needed with the active surveillance of B. miyamotoi in I. scapularis and I. affinis in southeastern Virginia and beyond to determine the dynamics of B. miyamotoi.

Effects of base cations on fecal indicator persistence and bacterial community structure

Stephen DeVilbiss*, Meredith K. Steele, and Brian D. Badgley
Virginia Polytechnic Institute and State University, Blacksburg, VA

Land use changes have caused an increase in salinity and associated base cation concentrations in many of North America’s freshwater drainage basins. Despite being recognized as a significant water quality stressor, much of the focus of freshwater salinization has been on the total amounts, rather than the types, of salt
inputs. Additionally, the compounded effects of multiple stressors as well as interactions between different water quality contaminants remain poorly understood. We have observed potential interactions between specific base cations and fecal indicators in controlled laboratory incubations. Specifically, divalent base cations increased the survival of Escherichia coli, a common fecal indicator bacterium, by up to 40% compared to controls with no salt additions. Further, individual base cations caused differential effects on freshwater bacterial community structure. These results indicate that different types of salt pollution, and not simply salt quantity, may exacerbate bacterial water quality impairments and alter bacterial community structure highlighting a need for increased research as to whether these effects extend to pathogen persistence or other aspects of public and ecosystem health. Moreover, a more nuanced approach to monitoring and managing salt inputs on water quality may be required than a focus simply on total dissolved solids or conductivity.

Long-term effects of antibiotic contaminated manure on soil microbial communities and antibiotic resistance gene abundance®

Sarah Shawver*, Carl Wepking, Mike Strickland, and Brian Badgley
Virginia Polytechnic Institute and State University, Blacksburg, VA

Antibiotic usage has increased since their discovery, and more recently, microbial resistance to antibiotics has been a growing concern. In agroecosystems, livestock treated with antibiotics have the potential to spread antibiotics, resistant bacteria, and antibiotic resistance genes (ARGs) to soil through manure. The resulting spread of ARGs and other changes in soil microbial communities could impact human health and ecosystem functioning. The objective of this study was to determine how long-term repeated additions of manure from cattle treated with antibiotics impact soil microbial communities and ARG prevalence.

Our study site was a grass field at Kentland Farm in Blacksburg, VA. We used a randomized block design with four treatments: no manure control, manure control, and manure from cows given either cephapirin or pirlimycin. Manure was land applied monthly from November 2014 to May 2017. Soil samples were collected in May each year. ARGs were quantified using qPCR. Microbial communities were analyzed with 16S and ITS amplicon sequencing.

In general, ARGs were less abundant in no manure controls compared to manure treatments, suggesting that manure, even without antibiotics, can select for increases in ARGs. Furthermore, our results suggest that environmental factors, including weather patterns, may influence the impact of antibiotics on soil microbial communities and ARGs prevalence. We detected shifts in overall microbial community structure among years and treatments. However, treatment effects differed between microbial and fungal communities. Manure additions altered fungal communities compared to no manure controls. However, in bacterial communities, the interactions of treatment and year were more complex.
Influence of host genetics on the epithelial-associated microbiomes in aquaculture-raised Nile tilapia (*Oreochromis niloticus*).  

Ian S. Hines*, Tim J. Bushman, Oscar Galagarza, Roderick V. Jensen, David D. Kuhn and Ann M. Stevens  
Virginia Polytechnic Institute and State University, Blacksburg, VA  

Aquaculture provides a sustainable alternative to wild-caught fisheries. An animal’s microbiome, is vital to its nutrition, growth, and overall health. Exogenous factors such as diet and the rearing environment are known to affect the microbiome. However, little is known about the role that host genetics plays on the microbiome structure. To investigate the potential influence of host genetics on the community of microorganisms present in the host, skin and intestinal epithelial tissues were harvested from three proprietary family lines of Nile tilapia (*Oreochromis niloticus*) that differ by one genetic trait. Prior work demonstrated that one of the genetically-related lines has a much higher growth rate than the other two, when age and environmental conditions are controlled. It was hypothesized that the animal’s microbiome was a contributing factor to the observed differences in growth. Therefore, the V4 region of the bacterial 16S rRNA gene was amplified using DNA separately extracted from the scales or midgut portions of the intestines. The resulting PCR products were gel purified and sequenced via Illumina MiSeq protocols. Preliminary QIIME bioinformatics analysis of the intestinal samples has revealed noticeable differences in the microbiome structure between the faster growing fish and the other family lines. The most abundant bacterial families in the more productive fish line, Mycoplasmataceae and Fusobacteriaceae, are virtually absent in the other two lines, which have higher levels of Enterobacteriaceae. Analysis of skin samples is on-going. Thus, fish lines differentiated only by the alteration of a single trait exhibited differences in the host-associated microbial communities.

Receptor quantification in *Sinorhizobium meliloti* – can tagging mislead?  

Timofey Arapov*, Birgit Scharf  
Virginia Polytechnic Institute and State University, Blacksburg, VA  

Bacterial Methyl accepting Chemotaxis Proteins (MCPs) are widely studied sensory proteins. Certain *Caulobacter crescentus* MCPs possess a motif in their C-terminal region near the terminus that acts as protease recognition site leading to rapid degradation during swarmer to stalked cell transition. Consequently, disruption of this motif increases cellular receptor quantities. A number of *Sinorhizobium meliloti* chemoreceptors also possess a potential degradation signal. Interestingly, our work shows evidence that the fusion of two popular peptide tags to the C-terminus of an MCP with a putative degradation signal increases its cellular quantity as much as 6-fold. We hypothesize that the addition of a tag reduces binding of the protease, which leads to greater MCP stability. These results suggest the use of peptide tags for detection can confound proper cellular protein quantification.
**Sinorhizobium meliloti Chemoreceptor McpV Senses Short-Chain Carboxylates via Direct Binding®**

K. Karl Compton*, and Birgit E. Scharf
Virginia Polytechnic Institute and State University, Blacksburg, VA

Alfalfa is an important forage crop that has very high protein yields due to the symbiotic association with the bacterium *Sinorhizobium meliloti*. Through a series of concerted plant-microbe interactions, the bacterium is ingested into a root organ called a nodule, where it is provided plant photosynthates to support nitrogen fixation activity, ultimately yielding fixed nitrogen to the plant. Localization of the bacterium to the plant root is the first step in initiating this symbiosis, and is mediated by chemotaxis, or the biased movement of the bacterium to the source of attractant compounds. An enormous variety of molecules are released by plant roots throughout their lifetime, which include amino acids, organic acids, sugars, polyphenols, and flavonoids, to name but a few. One purpose of this wide range of secreted molecules may be to recruit mutualistic microorganisms, such as *S. meliloti*. In this work, 1-4 carbon acids are shown to be chemoattractants for *S. meliloti*. In vitro experiments and structural homology modeling indicate Methyl-accepting Chemotaxis Protein V (McpV) is the receptor for certain small monocarboxylic acids. *mcpV* deletion mutants demonstrate the necessity of the gene for organic acid chemotaxis, and binding studies with the purified sensor domain of McpV show direct binding to most of the chemoattractants tested. Chemotaxis can be instrumental in firmly establishing nodulation in legumes and can guarantee the effective delivery of engineered bacteria optimized for symbiosis and crop yield.

**Prevalence of Babesia microti in Ixodid Ticks in Virginia®**

Zach Bement*, Holly Gaff, Wayne Hynes
Old Dominion University, Norfolk, VA

Human babesiosis is a disease caused by an infection with the protozoan pathogen, *Babesia microti*. In the USA, *Ba. microti* is primarily transmitted through the bite of an infected *Ixodes scapularis* tick. *Ixodes scapularis*, as well as the related vector, *Ixodes affinis*, are well established in southeastern Virginia. Though *Ixodes affinis* are not reported to bite humans, it is possible that they play an important role in maintaining pathogens like *Ba. microti* in their sylvatic cycles. This study examines the prevalence of this pathogen within *Ixodid* ticks collected in southeastern Virginia. Questing *I. scapularis* and *I. affinis* were collected by flagging various field sites from 2010 to 2017. The prevalence of *Ba. microti* in the ticks was determined by screening extracted DNA from the collected ticks, using real-time PCR. Positive results were then confirmed by sequencing mitochondrial 18s rRNA. This study shows that *Ba. microti*-infected ticks are present in Virginia, where human babesiosis is considered a rare disease. In addition, *Ba. microti* was detected in *I. affinis*, where it has not previously been reported. The interactions between the two
tick species that share hosts sharing may be driving an increase in the natural reservoir of tick-borne pathogens like *Ba. microti*. Further research and active surveillance is needed to understand the contribution of *I. affinis* to the ecology of *Ba. microti*.

**TnSeq identification of genes with uncharacterized roles in Bacillus subtilis spore germination**

Cameron V. Sayer*, Bidisha Barat and David L. Popham  
Virginia Polytechnic Institute and State University, Blacksburg, VA

Bacillus cells faced with unfavorable environmental conditions undergo an asymmetric division process ultimately leading to the formation of the bacterial spore. Specialized cellular structures afford spores resistance to a variety of environment conditions including traditional decontamination techniques. The dormant spore can return to a vegetative growth state through a regulated process termed spore germination. In the present study we employed a high-throughput screen, transposon sequencing (TnSeq), to identify genes with previously uncharacterized roles in spore germination. Null mutants of identified genes exhibited delayed germination in several assays. Stage I of spore germination features the release of the major component of the spore core, Ca+2 dipicolinic acid (DPA), while stage II is characterized by the degradation of the modified peptidoglycan of the cortex. Assays measuring both DPA and hexosamines during germination indicated significantly slower release in several mutant strains suggesting delays in both stage I and II, respectively. Ultimately, multiple phenotypic factors suggest a failure to initiate spore germination through a GerA mediated response rather than a specific slowing of germination rate. Quantitative western blots of GerA revealed decreased abundance of the receptor in some mutant strains although the mechanisms leading to this decrease are not yet clear. Further work is focused on exploring functions of identified genes and potential relationship to the GerA receptor.

**TnSeq of uncharacterized genes involved in Bacillus subtilis spore germination**

Bidisha Barat*, Cameron V. Sayer, David L. Popham  
Virginia Polytechnic Institute and State University, Blacksburg, VA

Germination of dormant *Bacillus subtilis* spores with specific nutrient germinants starts at the inner membrane with the interaction of a germinant with Ger receptor proteins and progresses through core rehydration and cortex breakdown. Deficiencies in Ger receptors such as GerA, GerB, GerK, receptor-associated proteins such as GerD, Ca2+-dipicolinic acid channels, and lytic enzymes can potentially inhibit the germination process. In an effort to better understand the underlying mechanisms of germination, a high-throughput genetic screening method called transposon sequencing was used. This analysis identified genes that
had not been previously implicated in germination. To investigate their functions, a number of functional assays were performed that indicated a delay in both stage I and stage II of germination. The mutant strains showed significant reduction in germination efficiency with L-Valine and about 50% of the population failed to initiate germination suggesting a defect in the GerA-mediated response. The expression of gerA was studied using a lacZ transcriptional fusion followed by quantitative western blot analyses to determine decreased abundance of GerA in mutant strains. The mutants can be classified based upon normal or decreased gerA transcription and normal or reduced GerA protein. Further work involves understanding the functions of the identified genes and their correlation to the GerA receptor which may allow for better spore decontamination procedures.

**Exploring the role of MORN2 domain proteins in *Fusobacterium nucleatum* pathogenesis**

Ariana Umana*, Blake E. Sanders and Daniel J. Slade
Virginia Polytechnic Institute and State University, Blacksburg, VA

*Fusobacterium nucleatum*, a common inhabitant of the oral cavity, is an intracellular Gram-negative pathogen that is capable of spreading through the body causing numerous diseases including periodontitis, inflammatory bowel disease (IBD), and pre-term birth while also causing infections in heart, lungs, liver and brain. In addition, the association of *F. nucleatum* with intestinal cancer has been confirmed in numerous studies. Previous studies have reported that *F. nucleatum* host cell invasion is critical for induced cancer signaling, and invasive genotypes of *Fusobacterium* contain a genetic expansion of a class of completely uncharacterized proteins with Membrane Ontology and Recognition Nexus 2 (MORN2) domains. We recently completed the sequencing, annotation and assembly of eight genomes, including the genetically tractable and highly invasive strain *F. nucleatum* 23726. Using a custom HMMER profile, we have bioinformatically identified 26 MORN2 genes annotated in *F. nucleatum* 23726, of which 100% remain unstudied. Non-invasive *Fusobacterium* strains possess a scarce number of MORN2 domain proteins (i.e, <4), while active invader strains contain an average of 32 per genome.

Despite the proposed role of these proteins in active host cell invasion, no experiments describing its function have been reported for *F. nucleatum* MORN2 proteins. Herein, we present the first experimental study of MORN2 domain proteins encompassing biochemical, bioinformatic, and genetic approaches to determine their function and *F. nucleatum* pathogenesis.
**FusoPortal: An online database of Hybrid MinION sequenced, assembled, and functionally annotated *Fusobacterium* genomes**

Sanders, B.E.*, Umana, A., Todd, S.M., Lahmers K.K., Lemkul, J.E., and Slade, D.J. Virginia Polytechnic Institute and State University, Blacksburg, VA

Here we present FusoPortal, an online database of complete *Fusobacterium* genomes that were sequenced using hybrid MinION long-read sequencing, and assembled and annotated using a diverse portfolio of open-source software. This resource provides the first fully assembled genomes for several strains of virulent *Fusobacterium nucleatum*, many of which are associated with the development of colorectal cancer. FusoPortal has been initiated with eight complete genomes, of which 7 were previously only drafts that varied from 6-200 contigs. Significant efforts were made to provide data in easily downloadable formats, fostering a powerful and efficient experience for users. We further showcase that FusoPortal is superior for virulence factor identification, and have corrected a significant number of Type 5 secreted autotransporters that are misannotated in UniProt. In summary, FusoPortal is the first database of MinION sequenced *Fusobacterium* genomes, and this powerful resource will be expanded in the near future to include >25 genomes to aid the scientific community.

**Analysis of TpeL secretion in *C. perfringens* strain HN13 ©**

Dr. Stephen Melville, Angie Saadat*
Virginia Polytechnic Institute and State University, Blacksburg, VA

TpeL is a *Clostridium perfringens* toxin that is a Large Clostridial Toxin (LCT), a class of toxins that lack a known signal sequence. LCTs TcdA and TcdB in *C. difficile* require a holin-like protein for secretion, and the gene for this protein lies between the two toxins in the PaLoc operon. Interestingly, a gene for a holin-like protein lies immediately upstream of TpeL in *C. perfringens*. In preliminary experiments, polyhistidine-tagged TpeL was detected in supernatants only when this holin-like protein was expressed. Future studies aim to validate this holin-like protein-dependent secretion and characterize the associations between the holin and toxin with genetic, protein biochemistry and fluorescence microscopy methods.
Plasmids and antibiotic resistance genes in *Salmonella* strains isolated from stream sediments and poultry litter

*Noah Greenman, Raechel Davis, Charles Holmes, Sophie Jurgensen, Curtis Kapsak, James Herrick*
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Environmental reservoirs of Salmonella -- particularly those related to agricultural practices -- may contribute to the dissemination of these potential human pathogens. However, such reservoirs are not well characterized. Furthermore, the use of antibiotics in animal agriculture has potentially expanded the transfer and recombination of antimicrobial resistance (AMR) genes among Salmonella and other bacterial populations in environmental ecosystems. Surveillance of Salmonella in ecosystems such as streams, soils, and manure is potentially an important tool for understanding the overall distribution and epidemiology of this pathogen. Stream sediments from seven sites and chicken litter from five poultry houses in the Shenandoah Valley were sampled between October 2016 and June 2018. Modified FDA Bacteriological Analytical Manual methods for pre-enrichment, enrichment, and isolation were used to isolate 60 Salmonella strains; 55 of which were isolated from stream sediments and 5 of which were isolated from litter from a single commercial chicken house. Putative Salmonella were confirmed by PCR amplification of the Salmonella-specific invA gene. All 60 isolates were sequenced on an Illumina MiSeq and five were selected for long-read sequencing on the MinION. Fifteen different serotypes were identified (using SeqSero, SISTR and SMART PCR) among the 60 isolates. AMR profiles were determined using Sensititre MIC assays as well as surveyed in silico using KmerResistance and ABRicate utilizing the ARG-Annot database. All isolates possessed the ampicillin resistance gene ampH and at least one aminoglycoside resistance gene. Two isolates contained fluoroquinolone resistance genes. One isolate also contained a 300kb plasmid with multiple antibiotic and heavy metal resistance genes. Thirteen isolates also had resistance phenotypes and/or genes encoding resistance to other clinically or agriculturally relevant antibiotics. Populations of Salmonella in poultry litter and especially in stream sediments impacted by agricultural runoff may constitute important environmental reservoirs for antibiotic resistance genes.
(2) **Convergent DNA repair gene loss across diverse streamlined marine bacterial genomes**

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Marine microbial communities drive biogeochemical cycles throughout the global ocean, but the factors that shape their ecology and evolution are still unclear. Many abundant bacterioplankton lineages have highly reduced genomes that are thought to be the result of genome streamlining, whereby genes are adaptively lost to reduce nutrient requirements involved in RNA and protein production. However, other studies have suggested that an increased mutation rate associated with the loss of DNA repair genes is the primary cause of genome reduction in free-living bacteria. To assess the prevalence of DNA repair gene loss in marine bacterioplankton, we analyzed 368 publicly-available complete and partial genomes to explore the genomic features of several poorly-studied lineages that contain streamlined genomes. Using Hidden Markov Model annotations, calculations of broad genomic features, and a phylogenetic reconstruction based on the concatenation of 120 single-copy highly-conserved genes, we found a strong relationship between the absence of various DNA repair genes and several genomic features associated with streamlining genomes, such as low GC content, genome reduction, and short intergenic regions. These trends were particularly evident in the globally-abundant SAR11, SAR116, SAR86, and Roseobacter groups. This pattern suggests that several divergent lineages of bacterioplankton have convergently lost DNA repair genes, and that this loss may drive the subsequent evolution of streamlined genomic features.

(3) **To Grow or Not to Grow: The Question of Sodalis glossinidius in Response to Heme Stress**

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When the ectothermic, sub-Saharan tsetse fly consumes a blood meal, the endosymbiont bacteria that live within it are exposed to various environmental stressors. One type of environmental factor that affects Sodalis glossinidius, a secondary endosymbiont living in the midgut of the tsetse fly, is high levels of heme. The purpose of this study was to explore the gene-regulation mechanisms that Sodalis employs to grow in high heme and within the tsetse fly. Separate cultures of Sodalis were grown in moderate heme or high heme environments and transcription patterns were determined by RNA-sequencing data. Due to its up-regulation in high heme environments, the Sodalis gene SG2179 was selected for further study. A plasmid, pJDS1, to target and disrupt SG2179 was constructed for intron mutagenesis and electroporated into Sodalis. After induction of transcription of the intron on the plasmid, an intron fragment was inserted into SG2179, rendering the gene dysfunctional. URSOD27, which is the Sodalis mutant lacking
SG2179, showed no significant change in growth as compared to normal Sodalis in vitro, in high heme conditions. The data also showed that URSOD27 has no significant changes in growth as compared to normal Sodalis in vivo, as measured by the ability to colonize the tsetse fly.

(4) Characterization of MotF involved in Sinorhizobium meliloti flagellar motility

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The well-characterized prototypical flagellum of Escherichia coli is comprised of a basal body, which permeates the inner membrane, a rod and hook extending from the basal body through the outer membrane, and an extracellular helical filament. Within the basal body, rotor proteins rotate with the rod-hook-filament assembly with respect to stationary stator proteins, encoded by motA and motB. The stator surrounds and interacts with the rotor to induce rotational torque derived from proton motive force. In contrast to other structural genes, deletion of those encoding stator proteins results in production of flagella, but abolishes motility, thus meriting a mot- classification.

In addition to motA and motB, S. meliloti possesses two genes, motC and motE, for which deletion results in destabilization of the flagellar motor and a mot- phenotype. Here, we report a fifth gene, motF, required for efficient motility but not flagellar synthesis in S. meliloti. Deletion of motF resulted in severely reduced swim ring diameters on soft agar plates despite sustained production of flagellin proteins as indicated by Western blot analysis. Ectopic expression of motF in the ΔmotF mutant strain fully restored the motility phenotype. Bioinformatics, subcellular fractionation, and immunoblot analysis indicated that MotF is likely embedded in the inner membrane. Two suppressor mutations in MotA (G136S and Y248H) partially restored motility in the ΔmotF mutant background, but did not alter swim ring diameters in the wild-type background. Finally, we describe experiments to further investigate MotF orientation, topology, and interaction partners.

(5) Designing a Bio-Remediation Model to Control the Output of Toxic Arsenic by Environmental Bacteria

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Bacteriocins are proteins secreted by bacteria to inhibit the growth of competitive species. The use of bacteriocins to control the growth of bacteria that contribute to the mobilization of arsenic in the environment may provide a significant method for reducing the amount of arsenic that reaches surface and ground water systems.
Our research goal is to examine growth of bacteria that produce bacteriocins for the purpose of determining optimal growth conditions which we can then apply to microcosms that mimic the environment at the arsenic mine from which the cultures were originally isolated. Future work would entail quantifying the amount of toxic arsenic that each culture is capable of putting out into the environment.

(6) **Whole-Genome Sequencing of *Staphylococcus sciuri* Isolated from a Shenandoah Valley Waterway**

Raechel Davis, Max Maza, Curtis Kapsack, James Herrick
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The genus *Staphylococcus* is comprised of 41 known species of which 18 are potential human pathogens. Most of the research on *Staphylococcus* focuses on isolates from clinical settings due to its colonization of mammals and also because of potentially life-threatening infections caused by *Staphylococcus aureus*, particularly its highly oxacillin/methicillin resistant form (MRSA). Even though *Staphylococcus* species have been detected in grocery meats and salt-water environments, the environmental presence of *Staphylococcus* in freshwaters has rarely been investigated. Eleven strains were previously isolated from sediment samples collected from Muddy Creek, a freshwater stream located in Hinton, Virginia. Ten out of the eleven isolates were resistant to oxacillin/methicillin and isolate PS-5 was identified as *Staphylococcus sciuri*. Our goal was to construct and evaluate a draft assembly of the PS-5 genome using sequence data from both the MiniSeq (Illumina, Inc., San Diego CA) and MinIon (Oxford Nanopore Technologies, Oxford UK). Combining the short read data from the MiniSeq with the long read data from the MinIon resulted in a hybrid assembly consisting of a single contig representing the entire *S. sciuri* genome and a smaller contig representing a plasmid. The mecA gene, which confers resistance to oxacillin and methicillin, was detected in the genome despite our previous inability to do so using PCR. Further genomic analysis also revealed additional drug resistance genes such as tetA and ermY. The discovery of multiple antibiotic resistance genes in *Staphylococcus sciuri* could give insight into the evolution of resistance in other more pathogenic *Staphylococcus* species, such as *Staphylococcus aureus*.

(7) **Use of Direct-Fed Microbes (Probiotics) to Enhance Shrimp Resistance to *Vibrio parahaemolyticus* ©**

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Early mortality syndrome (EMS) or acute hepatopancreatic necrosis syndrome (AHPNS) is an epizootic bacterial infection of shrimp. This disease, attributed to pathogenic *Vibrio parahaemolyticus* EMS strains, threatens aquaculture production and global food security. A valuable and alternative approach to using antibiotics for
pathogen control is the practice of incorporating direct-fed microbes (DFM) or probiotics into aquaculture feeds. Once established, these beneficial bacteria can improve host survival and overall animal health. Here we examine our overarching hypothesis that DFM’s (specific strains of Bacillus subtilis spores) are able to provide shrimp protection to the EMS/AHPNS disease via natural microbial exclusion of pathogenic Vibrio parahaemolyticus strains. This assessment of shrimp involves twenty-one, 20 L glass tanks, treated with one of two DFM products at different concentrations with two control treatments preformed in triplicate. Inoculation will be through feed-borne delivery or delivery directly into tank water. Shrimp will be fed daily (3% body-weight) with a DFM-coated feed for seven days, before a challenge with V. parahaemolyticus EMS. Growth curves have established that V. parahaemolyticus is capable of achieving enumerations of ~3 x 10e9 CFU/mL over 18 hours. In previous studies, using an LD50 method, probiotic treated groups showed an average of 54% survival in comparison to a control group (no probiotic treatment), which had 10% survival, P (<0.05). We expect to refine the LD50 dosage methodologies from our new studies and using various probiotic strains and delivery methods, reproduce and improve upon these results.

(8) Investigating Potential Interactions of *Legionella pneumophila* and *Pseudomonas aeruginosa in Non-Potentable Water Sources®*

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Bacteria of the genus Legionella, specifically L. pneumophila, are responsible for a number of diseases, such as Pontiac fever and a severe type of pneumonia called legionellosis. Legionella spp. live in freshwater habitats, including environmental sources and manmade structures for water storage and transport. Another bacterium responsible for serious infections is *Pseudomonas aeruginosa*, which also inhabits freshwater environments. In this study, we investigated the possibility of occurrences of and interactions between L. pneumophila and P. aeruginosa over an extended period in non-potentable water sources. Water samples were collected from non-potentable sources in Roanoke, VA from May 2016 to July 2018. DNA was extracted from the samples, and selected sequences of Legionella spp., L. pneumophila, and P. aeruginosa were amplified using polymerase chain reaction (PCR). Results from PCR were confirmed with gel electrophoresis and DNA sequencing. Seventy-four samples tested positively for the Legionella genus; seventy-six samples tested positively for L. pneumophila. This difference in positives could be the result of poor DNA quality. The sequencing data for *Pseudomonas* spp. were not as reliable. Eleven out of thirty-six samples tested for *Pseudomonas* had positive results for the genus, and from those, two were P. aeruginosa. This indicates the need for further analysis of the PCR primers to determine if the primers are specific enough for the needs of this study.
(9) **Direct Acute Effects of Caffeine on RAW 264.7 Murine Macrophage Response to Inflammatory Stimulus by Bacterial Lipopolysacharide**

Abigail Kaufmann, David O. Freier  
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There are mixed findings of the effects of caffeine in human and animal experiments. The amount of caffeine consumed may determine the behavior of cellular and molecular inflammatory responses. Direct, in vitro studies suggest caffeine may have anti-inflammatory effects. Previous unpublished work in this lab suggested pre-treatment of macrophages with caffeine may reduce nitric oxide responses to bacterial lipopolysaccharide (LPS) from E. coli. RAW 264.7 macrophages are seeded in 24-well plates at 4x10^5 cells per well in a 500 uL of DMEM complete. After acclimation overnight, appropriate wells are treated with caffeine at 0, 50, 100 and 200 ug/mL for 24 hours. Cells are co-stimulated with LPS for 24 hours. Nitrites in solution, as a measure of iNOS activity, are measured by the Greiss Reaction to determine the level of inflammatory activity of the RAW 264.7 cells. Production of the inflammatory cytokine interferon gamma will also be measured. Additionally, pre-treatment studies with caffeine are also planned. We expect caffeine will have anti-inflammatory effects based upon current literature. Our analysis may open the doors to a better understanding of what effect caffeine has in modulating the inflammatory response.

(10) **Influence of a dietary yeast supplement on intestinal epithelial-associated microbial communities in rainbow trout (Oncorhynchus mykiss)**

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In order to meet the food needs of a rising global population, aquaculture is an important sustainable alternative to wild-caught fisheries. The microbiome of a fish is an important aspect of the animal’s health, growth rate, and nutrition. The fish microbiome is influenced by exogenous factors such as diet and the rearing environment. As a sustainable alternative to fish meal, yeast (Saccharomyces cerevisiae) has been shown to yield fish with similar growth rates and fillet quality as ones fed commercial fish meal. In order to investigate the effect of supplemented yeast on the structure of the intestinal epithelial-associated microbiome, rainbow trout (Oncorhynchus mykiss) were fed either a commercial diet as a control, or one of four experimental diets containing 0%, 20%, 40%, or 60% of menhaden fishmeal substituted with yeast. Prior work has shown that the 0% supplement-fed fish had decreased survival versus the other groups for unknown reasons. Additionally, the 20% supplement-fed fish had the highest average weight gains in comparison to the other supplemented groups. To understand the effect of the yeast-supplemented diet on the intestinal epithelial-associated microbiome, intestinal samples were harvested and DNA from those
samples was subsequently extracted. Following DNA extraction, the V4 region of the 16S rRNA gene was PCR amplified. Samples were then gel purified and later sequenced using the Illumina MiSeq platform. Preliminary QIIME results have shown little community variation between treatment groups.

(11) Investigating jumbos of jumbo bacteriophages and their roles around the globe

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Ubiquitous in nature, viruses dominate as the most abundant biological entities on the planet, and recent advances in high-throughput sequencing and metagenomics have substantially enhanced our understanding of their abundance and diversity in the environment. Identifying viral sequences in metagenomic data remains challenging and requires new methods to expand our knowledge of viral diversity, however. Toward this end, we applied a marker gene approach to identify novel viral sequences in metagenomic data.

Considering that several phages encode RNA polymerase (RNAP), we selected this gene for phylogenetic analyses. We examined >1500 publicly-available metagenomes for divergent, virus-like RNAP and identified 74 candidate scaffolds ranging in size from 10-408 Kbp. Many of these scaffolds encode phage hallmark genes, such as base-plate proteins, and some belong to clades containing known jumbo bacteriophages, which have genomes > 200 Kbp. Jumbo bacteriophages are thought to have evolved independently of one another from smaller phages, which may explain the observed phylogeny of the putative viruses we detected.

Our work demonstrates that RNAP can be used as an effective phylogenetic marker to identify novel viruses in nature. In the future, we aim to uncover the roles of these novel putative viruses in the environment. We will investigate their putative hosts, functional capacity, and abundances around the globe. These putative viruses have been detected in metagenomes from a variety of environments, such as hydrothermal vents and elephant rumen. This diversity in range suggests universal roles yet unexplored in our understanding of viral ecology.

(12) A Functional Comparison of Murine Bone Marrow Derived Macrophages to RAW 264.7 Murine Macrophages

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The RAW 264.7 murine macrophage cell line is commonly used to investigate inflammatory functions of innate immune responses with bacterial lipopolysaccharide (LPS) serving as the inflammatory stimulus. The purpose of this
research is to determine similarities and differences in functional performance between bone marrow derived macrophages (BMDM) and the RAW 264.7 cell line. The bone marrow from two femurs of a Female Swiss mouse is collected in ice cold PBS, panned to eliminate macrophages and other monocytic cells, and the remaining cells are stimulated with murine M-CSF (PeproTech) over the course of 7 days to produce sufficient quantities of BMDM for direct comparison experiments to the RAW 264.7 cell line. Both BMDM and RAW 264.7 cells are seeded at 4x10^5 cells per well in 500 uL of complete DMEM. After acclimation overnight, cells are stimulated with 0, 1, 10, and 100 ng/mL of LPS for 24 hours. A sample of supernatant is used in the Greiss reaction to determine nitric oxide production as a measure of inflammatory function. In an initial experiment the RAW 264.7 cells produced a 10-fold greater response to LPS at the 100 ng/mL concentration of LPS than the BMDM did. Continuing studies will examine this relationship, including the general morphology of both cells, cytokine production, and the potential to stimulate the BMDM to develop the M1 (classical macrophage) phenotype using interferon gamma.

(13) Molecular Strain Typing for the Tick-Borne Pathogen Rickettsia parkeri ©

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Rickettsia parkeri is one of a group of bacteria that cause Spotted Fever Group Rickettsioses (SFGR) in humans. Spotted Fever Group Rickettsioses can cause fever, headaches, rashes, muscle aches, and an eschar and some, such as Rocky Mountain Spotted Fever (R. rickettsii) can be fatal. R. parkeri, which is transmitted to humans by the Gulf Coast Tick (Amblyomma maculatum), causes a milder SFGR. In its historic range along the US Gulf Coast and the southeastern US, R. parkeri has prevalence of 1-15% in A. maculatum. However, in the Tidewater region of Virginia, where A. maculatum is a recent invader, prevalence reaches 50-60%. One hypothesis for this disparity is that there are different strains of R. parkeri present in these separate areas. Previous studies have not been able to demonstrate strain variation in R. parkeri; however, most of these studies have only performed typing at single genes. In this work, we aim to identify additional genetic markers for strain typing of R. parkeri. To accomplish this goal, we performed comparative genomics on four complete R. parkeri genomes, locating variable regions between strains. This analysis yielded 14 variable intergenic spacer regions and >30 SNP loci. We are currently optimizing primers and PCR conditions for these loci and assessing their variability in R. parkeri infected ticks from the Tidewater area. Preliminary data from the DKS-xerC intergenic spacer locus will be presented.
The Effects of Endocrine Disrupting Chemicals Virus Infectivity of Vero

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Endocrine disrupting chemicals (EDCs) are used in plastics, food packaging, receipts and other human care products. EDCs are becoming a possible threat to animals and humans because they cause morphology changes and affect hormone function. As pollution has increased and more garbage and plastic is dumped into our waterways, EDCs have become increasingly prevalent because they enter our food chain through ingestion by fish and other aquatic organisms. Our goal is to test if EDCs like Bisphenol A (BPA) and Bisphenol S (BPS) disrupt regular viral replication ability of green monkey cells (Vero cells). Vero cells are infected with LaCrosse Virus and the EDCs are introduced after infection. Virus is then quantified at several points by plaque assay in Vero cells. These EDCs have been observed to cause changes to infectivity of Vero cells. Time and amount of infection both may play a role in results.

Uncovering the Role of Trimeric Autotransporter Adhesins in the Pathogenesis of Fusobacterium nucleatum

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F. nucleatum is an anaerobic, Gram-negative bacterium that disseminates from its native oral cavity and contributes to the progression of numerous human diseases including: colorectal cancer, periodontitis, preterm birth, & tissue abscesses. Although F. nucleatum is associated with multiple disease states, little is known about the molecular mechanisms that enable F. nucleatum to migrate and persist in a wide spectrum of environmental conditions. Interestingly, this bacterium lacks most major protein secretions systems (Type 1, 2, 3, 4, 6, 9); however, genomic analyses revealed an overabundance of Type 5 secreted proteins (autotransporters). To aid in virulence factor identification and characterization, an efficient and selectable genetic system has been developed in the Slade Lab that allows for multiple markerless gene deletions and complementation of tagged proteins of interest. Additionally, a novel suite of in vitro tools for studying autotransporters and bacterial cell surface proteins have been implemented to uncover the role of these virulence factors. Our recent completion of the F. nucleatum 23726 genome resulted in the correct annotation of the autotransporter protein family, including five genes from the Type Vc secreted trimeric autotransporters; herein renamed Fusobacterium Type Vc proteins (FvcA, FvcB, FvcC, FvcD, and FvcE). Using gene deletion strains, we reveal trimeric autotransporters are critical for binding & invasion of host cells, and recombinant expression of these proteins in E. coli conferred an invasive phenotype from a
previously non-invasive bacterium. Through our work, we present an innovative set of genetic and molecular tools for F. nucleatum 23726 that has revealed a critical role for Type 5c secreted autotransporters in F. nucleatum virulence. Ultimately, by addressing the significance of trimeric autotransporters we will further our understanding of the mechanisms involved in F. nucleatum host-pathogen interactions and how this contributes to the onset of various human diseases.

(16) **Investigation into the Role of Manganese in the Growth of the Opportunistic Pathogen Streptococcus sanguinis**

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While Streptococcus sanguinis plays a beneficial role in the oral cavity as a competitor of Streptococcus mutans and Streptococcus sobrinus, the bacteria that produce dental caries, it can cause deadly infective endocarditis if given the opportunity to colonize the vegetations that form over damaged endocardial tissue. Pre-existing heart conditions, surgery, and intravenous drug use predispose individuals to endocarditis. S. sanguinis growth and consequential virulence is significantly impeded by restriction to manganese. This is due to the resulting overwhelming oxidative stress and formation of reactive oxygen species which damage DNA and other cellular components. Manganese is essential for S. sanguinis proteins involved in DNA synthesis and is predicted to play other important roles. This study investigates the importance of the previously identified manganese transporter SsaACB, of the Lral family of conserved metal transporters, in combination with other proteins, such as ComCDE, SSA_0872, LguL, SSA_1625, and NrdD, for strain survival in vitro. These proteins of interest were selected because notable accumulation or reduction of associated metabolites in a metabolomic study suggested causation for the loss of virulence of the ssaACB knockout strain in vivo. A serum growth study of strains with single and double knockout mutations incubated at physiological conditions was conducted and the results were further supported by observation of fermenter culture growth after chelation of free manganese ions to exacerbate the effects of manganese starvation. This study identified combinations of proteins which are not essential for manganese-dependent S. sanguinis growth.

(17) **Characterizing the mycobacteriophage Bassalto, TreeD and Jambo**

Joedy N. Boyd, Malcolm Z. Bass, and Nazir Barekzi
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The aim of the current study was to isolate and characterize novel bacteriophage using the host bacterium Mycobacterium smegmatis mc2155. M. smegmatis is a soil bacterium and an ideal host for isolating mycobacteriophage. Soil samples were collected from campus grounds at Norfolk State University, Norfolk, VA.
Bacteriophage were isolated from the soil samples through direct and enrichment procedures. The efficiency of each isolation method was determined. Direct isolation and purification methods yielded distinct phage including Bassalto and TreeD; while, enrichment isolation produced Jambo. To further investigate the characteristics of a phage, Bassalto DNA was extracted from a high titer lysate and sequenced using an Illumina MiSeq Next Generation Sequencer. The whole-genome is being annotated using an open source software called DNA Master. The overall goal of studying phage is to advance and contribute to the general knowledge of genetics, biodiversity, and microbial ecology. Beyond basic scientific research, the applications of phage therapy are being investigated to control microbial populations.

(18) **Analyzing the Role of nsrR in *Pantoea stewartii* subsp. *stewartii* during in planta Growth via Reverse Genetics ®**

Brandi J. Thomas*, Holly Packard, and Ann M. Stevens  
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Pantoea stewartii subsp. stewartii is a Gram-negative, phytopathogenic bacterium responsible for causing Stewart’s wilt disease in sweet corn and popcorn varieties. P. stewartii is primarily transmitted to the plant via the corn flea beetle vector. Upon entering the plant, the bacteria initially cause symptoms of water-soaked lesions in the leaf apoplast. After migrating to the xylem, they grow to high cell densities and produce a bacterial biofilm that blocks water flow, resulting in wilt and potentially plant death. The transcriptional regulator important for nitrogen stress response, encoded by nsrR, was discovered through Tn-Seq to be important for colonization and growth of P. stewartii in planta. Regions upstream and downstream of nsrR were amplified via overlap-extension polymerase chain reactions and cloned into Gateway system vectors to enable generation of a deletion strain via homologous recombination. The nsrR gene with its native promoter was reinserted into the chromosome of P. stewartii in a neutral region downstream of glmS to create a corresponding complementation strain. The deletion and complementation strains for nsrR will be tested in both competition and virulence in planta assays to examine the impact of nsrR on growth and survival of the bacteria and its ability to cause disease in sweet corn. This reverse genetics strategy will provide insights into the role of this bacterial gene in planta and aid in the development of future disease intervention strategies.

(19) **Coumarin Inhibition of La Crosse Virus**

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Coumarin is a plant-derived natural product known for its antibacterial, antifungal, and antiviral properties. We tested coumarin’s ability to inhibit La Crosse virus in a cell-culture assay. La Crosse virus (LACV) is a mosquito-borne virus that can cause
nausea, vomiting, and lethargy lasting a few days, and can also cause encephalitis, coma, and death. We are determining viral inhibition by infecting African green monkey kidney (Vero) cells with LACV and exposing them to Coumarin; control cells are infected but not exposed to Coumarin. This is followed by comparing titers of supernatant samples taken at daily intervals. Preliminary results suggest that Coumarin inhibits La Crosse virus replication. We are continuing to experiment with dosage and time of treatment. Coumarin could have the ability to treat viral infections.

(20) Evaluation of Pseudomonas putida bacteriophages on clinical isolates of Pseudomonas aeruginosa

Rachel D. Persinger
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Pseudomonas aeruginosa is an opportunistic pathogen and the causative agent in several acute and chronic infections. More specifically, it is the leading cause of lung infection and death in cystic fibrosis patients. This is due in part to the synergistic effects of P. aeruginosa’s strong, sticky biofilm and the thick, mucus-lined lungs of those with cystic fibrosis. P. aeruginosa also thrives in moist environments, such as tubing and catheters used in hospitals, increasing the risk of infection in patients. This resilient bacterium is becoming more antibiotic resistant, which has exhausted many options and made it increasingly difficult to treat. Thus, bacteriophage therapy has become a potential treatment of interest. In a pilot study, Pseudomonas putida bacteriophages were tested against four different clinical isolates of P. aeruginosa from the University of Virginia. Multiple positive results were obtained from a few singular phage infections. Further testing with five pools containing four phage samples in each resulted in one positive on both UVA 6 and UVA 4 isolates and two positives on UVA 5 isolate. In future experimentation, isolation and identification of the specific phages from each pool contributing most to infection of the clinical strains will be done. This will aid in developing a more effective phage cocktail. Further, the highly specific host range of phages impedes the effectiveness that it could potentially have across a broader range of hosts. Expanding the host range of the P. putida phages may be useful and increase the likelihood of infection against P. aeruginosa.

(21) Determining the role of iscR during Pantoea stewartii infection of corn through reverse genetics and plant assays

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Pantoea stewartii subsp. stewartii is a bacterial phytopathogen that causes Stewart’s wilt in corn. This bacterium is vectored by the corn flea beetle. Chlorotic, water-soaked lesions initially develop on leaves infected by P. stewartii following
beetle feeding. As the infection progresses, plants have occluded xylem pathways due to a thick exopolysaccharide biofilm that disrupts water transport, leading to wilt. A previous Tn-Seq study identified genes that are important to the colonization and growth of P. stewartii in planta. A putative transcriptional regulator encoded by iscR was identified through the Tn-Seq study as being essential for survival. In other enteric bacteria, IscR senses and synthesizes iron-sulfur cluster proteins and it is hypothesized that it plays a similar role in P. stewartii. Reverse genetics techniques have been used to generate an iscR gene deletion strain (∆iscR) using the Gateway system protocol. The corresponding complementation strain is under construction. Wild type, deletion, and complementation strains will be used during in planta competition and virulence assays to study the role of IscR. Specifically, the virulence assay will evaluate the role of iscR in causing wilt disease, while the competition assay will evaluate the ability of the ∆iscR to colonize the host in comparison to the antibiotic resistant complementation strain (with wild-type phenotype). These efforts will provide insights into the function of iscR with regard to bacterial colonization and pathogenicity within a corn plant.

(22) Chemical Treatments to Inhibit Hypoxia Inducible Factor-1α in a Breast Cancer Cell Line ®

William Harrison
University of Mary Washington, Fredericksburg, VA

High expression levels of hypoxia inducible factor-1α in breast cancer cells have been correlated to poor prognosis. As a hypoxic tumor environment develops, hypoxia inducible factor-1α activates growth factors involved in tumor vascularization which influencing tumor growth. Due to this, hypoxia inducible factor-1α has the potential to be a chemotherapy target. Two chemical treatments digoxin, a cardiac glycoside, and everolimus, an inhibitor of mammalian target of rapamycin, have been identified to inhibit the translation of hypoxia inducible factor-1α. For this research a tumorigenic mouse breast epithelium cell line will be treated with digoxin and everolimus separately in a hypoxic environment. A western blot assay will be used to determine the hypoxia inducible factor-1α expression levels in each cell line, after which ImageJ can analyze the assay and produce a numerical representation for the levels of hypoxia inducible factor-1α expression. It is understood that hypoxia inducible factor-1α is downstream to the mammalian target of rapamycin kinase in the PI3K pathway. The inhibition of mammalian target of rapamycin kinase could result in a significant decrease of hypoxia inducible factor-1α expression. Based on this, I believe the treatment of everolimus will result in a significant decrease of hypoxia inducible factor-1α expression compared to digoxin. By determining a treatment to inhibit hypoxia inducible factor-1α for breast cancer, this could increase the potency chemotherapy treatments to reduce tumor growth.
(23) **CRISPRi Knockdown of dnaK Expression in *Sodalis Glossinidius***

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The tsetse fly bacterial endosymbiont *Sodalis glossinidius* is exposed to thermal stress when its host feeds on vertebrate blood. A chaperone protein called DnaK is known to be part of the heat shock response in many bacteria. Our lab has begun to show that it confers the same function in *Sodalis*, but deletion of this gene seems to be lethal to *Sodalis*. CRISPR interference (CRISPRi) has been shown to be an effective method of knocking down specific genes in bacteria, without completely deleting the gene. This technique presents a useful way to repress dnaK in order to better understand its function in *Sodalis*. Here, a CRISPRi system was developed to knockdown the dnaK gene in *Sodalis*. In initial experiments, decreasing dnaK expression allowed *Sodalis* to better survive thermal stress than a control strain.

(24) **Comparative Pan-Genomics in the Genus *Pseudomonas***

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Advancements in high-throughput sequencing technology have permitted the rapid sequencing of genomes from bacteria that are of interest for medicine, biotechnology, and basic science. This fast production of whole bacterial genome sequences has put a major emphasis on comparative genomics, in particular pan-genomic analysis of closely-related genomes. A pan-genome is a collection of every different non-redundant gene found within the sequenced genomes among individuals of the same species. Pan-genomes can provide important information regarding intrinsic characteristics of genomes, bacterial adaptation and evolution, and medicinal applications, but it is still often unclear how to calculate various pan-genomic parameters or their biological implications. For this project, we produced pan-genomes of species belonging to the genus *Pseudomonas*, which is particularly well-represented in the sequenced genome collection and therefore is an excellent group for studying pan-genomic characteristics; these bacteria are also found worldwide in soil, plants, and animals, and include well-known pathogens and strains important for biotechnology. We found that genes present in only one *Pseudomonas* genome (ORFan genes) played a critical role in determining pan-genome characteristics in all species groups analyzed, and removal of these genes alone substantially reduced overall genomic diversity. It has been speculated that many ORFan genes are not transcribed or translated, which suggests that the majority of diversity in closely-related genomes may be due to genes with little overall impact on overall bacterial physiology. Overall, these results indicate that bacterial genomes may be more cohesive than may be typically thought using traditional pan-genomic analyses.
Characterizing the Sodalis glossinidius uspA Gene Through Expression in *Escherichia coli*

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The endosymbiont Sodalis glossinidius is found in the midgut of the tsetse fly, and in this environment the bacteria is exposed to high levels of heme from the bloodmeals the tsetse fly consumes. Sodalis has evolved to survive in this high-heme environment and this study focused on the effect that one gene has on stress resistance in Sodalis. The gene of interest was Sodalis SG0074, which is homologous to the uspA gene in other bacteria where it facilitates survival from multiple environmental stressors. Preliminary work, however, shows that uspA does not affect Sodalis’ growth in a high-heme environment. This study here worked towards characterizing the uspA gene by cloning it into an *Escherichia coli* uspA mutant and determining if it confers increased stress resistance. This E. coli strain was successfully made and a series of oxidative stress tests with H2O2 were done to establish the effect that the Sodalis uspA gene has on oxidative stress resistance.

*RetS*-mediated regulation of infection states in *Pseudomonas aeruginosa*

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*Pseudomonas aeruginosa*—a ubiquitous, Gram-negative bacterium—is an opportunistic human pathogen. *P. aeruginosa* is able to quickly adapt to environmental changes using an impressive array of regulatory networks. One such network, the Gac/Rsm signal transduction pathway, allows *P. aeruginosa* to rapidly switch between an acute infection state, characterized by the expression of a type III secretion system (T3SS), and a chronic infection state, characterized by biofilm formation. At the heart of the pathway sits the GacS/GacA phosphorelay. The hybrid sensor kinase, GacS, has been found to phosphorylate GacA, its cognate response regulator. Once phosphorylated, GacA acts as a transcriptional activator, indirectly upregulating the production of the biofilm-associated polysaccharides Psl and Pel, and indirectly downregulating expression of the T3SS, ultimately leading to a chronic infection state. RetS, a hybrid sensor kinase, has been shown to inhibit GacS through direct binding and via dephosphorylation, thereby promoting an acute infection state. To date, RetS has not been observed to possess any canonical kinase activity. Current work focuses on elucidating the molecular mechanism by which RetS interacts with GacS and the effects of this interaction on the Gac/Rsm pathway in *P. aeruginosa*. 
(27) Dose Response Effect of Lipoarabinomannan from Mycobacterium smegmatis Stimulates RAW 264.7 Murine Macrophage Response

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Understanding the immune response to tuberculosis infection is critical to developing better methods of prevention and treatment. Macrophages are a key leukocyte in defense against Mycobacterium tuberculosis infection. The precise mechanism by which M. tuberculosis is able to evade acidification by the phagosomes of macrophages is not yet known, so understanding the interactions between macrophages and cell wall components of Mycobacteria is of great relevance. Lipoarabinomannan (LAM) is a glycolipid Toll-Like Receptor-2 ligand found on the cell wall of mycobacteria. The purpose of this research is to evaluate the optimal dose of LAM to elicit an inducible nitric oxide synthase (iNOS) and Tumor Necrosis Factor-alpha (TNF-α) response in RAW 264.7 murine macrophages. Concentrations of LAM at 10 ng/mL, 100 ng/mL, and 1000 ng/mL, are compared to a positive control of 100 ng/mL Lipopolysaccharide (LPS) from E. coli(O55:B5). Nitrite response is measured using the Greiss reaction. LAM from M. smegmatis exhibited a dose response effect in RAW 264.7 macrophages that is less potent than bacterial LPS. Understanding LAM as a stimulus to macrophage activation will advance our understanding of Mycobacterial cell wall as macrophage stimuli, and may be a model for further investigation of evasion. This could lead to research that can explore new avenues for the treatment of M. tuberculosis infection.

(28) Investigation of factors influencing flagellotrophic bacteriophage lysis patterns

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Bacteriophages, viruses that attack bacteria, are found in many different niches including human intestinal flora. Bacteriophages have very specific host ranges and binding mechanisms. Flagellotropic bacteriophages are phages that infect only motile bacteria and do so by binding to rotating flagellar filaments. Filaments must be actively rotating for infection to occur. In this study, we used phage drop assays to investigate factors influencing the pattern of phage-mediated bacterial lysis. For each experiment, Salmonella enterica subsp. enterica serovar Typhimurium strain 14028s was inoculated with flagellotrophic bacteriophage χ on swim plates. The low agar concentration in swim plates allows for the formation of a swim ring, corresponding to motile bacteria swimming through the medium. Various factors hypothesized to impact the lysis pattern were altered in separate trials. We determined that virus titer, nutrient concentration, multiplicity of infection, configuration of phage inoculation, and deletions of bacterial chemoreceptor genes all play a role in determining the pattern of lysis. These results help elucidate the poorly understood dispersal mechanism of bacteriophage viruses in various microbiological environments.
Effects of ibuprofen on replication of La Crosse virus in mammalian cells

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La Crosse Virus (LACV) is a mosquito-borne virus found throughout much of the eastern United States. It commonly causes La Crosse encephalitis in children, with symptoms that include fever, headache, nausea, vomiting, fatigue, and lethargy. Ibuprofen is a common over-the-counter, non-steroidal anti-inflammatory drug that works by inhibiting pain- and inflammation-inducing hormones called prostaglandins. Headaches and fevers, two common symptoms of LACV infection, are frequent reasons taking ibuprofen, so it is likely that the virus may be exposed to ibuprofen during an infection. To determine whether ibuprofen affects virus replication in mammalian cells, ibuprofen will be added to African green monkey kidney (Vero) cells at various concentrations and/or time points. Vero cells will be infected with LACV at a given concentration (depending on whether flasks or plates are used). During the course of infection in flasks, samples will be taken of the culture supernatant fluid for later quantitation of virus in cell-culture plates. A comparison will be conducted looking at plaque numbers in ibuprofen-treated vs. untreated cells to determine whether ibuprofen affects virus replication, with the concentration and time-of-addition studies providing different outcomes. If ibuprofen inhibits or enhances virus replication, it could potentially be valuable information for health care providers treating viral infections.

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Investigating the Microflora in Feline Oral Biofilms

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Feline oral biofilms are diverse microbial environments which remain poorly examined. This is problematic because poor knowledge of oral diversity can lead to dental caries, gingivitis, and periodontal disease, without understanding their contributors. Understanding these contributors would provide better means of understanding pet oral health care for veterinarians. The oral microbiota of felines is rarely investigated for individual species or phylum-level taxonomic composition. In order to understand the ecological and biochemical nature of feline oral biofilms, isolated organisms and phyla will be identified. Microbial isolates are collected by direct swab of gingival tissue. Swabs are grown overnight in Thioglycolate broth. Individual isolates are then grown on Columbia agar in aerobic and anaerobic conditions. Biochemical and genomic techniques will be used to isolate and identify individual bacterial species present. Previous work in our lab identified bacterial
species such as Staphylococcus epidermidis, Bacillus subtilis, Kytococcus schroeteri, and others that profile in the genus Streptococcus or Neisseria. Results will be used to support further research for use by others to develop a better understanding of feline oral biofilms and diversity.

(31) **The Isolation and Annotation of DrFeelGood: A Unique Mycobacteriophage**

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At Virginia Western Community College, over the course of the 2017-2018 academic year, we isolated, characterized and annotated the genome of a unique bacteriophage, DrFeelGood. Dr FeelGood was isolated from soil collected from a compost pile comprised of potting soil mixed with clay in the Roanoke Valley using Mycobacterium smegmatis mc155 as the host bacterium. Isolation and purification of Dr. FeelGood occurred during the fall semester in Biology 101, while genome annotation took place in Cellular Biology during the spring semester. Sequencing done by the University of Pittsburgh revealed DrFeelGood to be an A1 cluster phage. For genome annotation, several databases were used to investigate the sequence of DrFeelGood’s 83 genes and their correlative functions. The overall goal is the submission to GenBank with expectations of contributing to the comprehensive knowledge of phage genetics, biodiversity, and microbial ecology.

(32) **Examining the Effects of Bacteriocins to Inhibit the Growth of Environmental Bacteria from an Arsenic Mine**

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The Brinton Arsenic Mine in Floyd County, VA provides a unique opportunity to study environmental bacteria and their mobilization of arsenic compounds in the environment. These microbial processes involve conversion between different forms of arsenic, the two most prevalent being arsenate (AsV) and arsenite (AsIII). Both types of compounds interfere with protein structure and metabolic processes in humans and bacteria alike. Our work is aimed at isolating and identifying bacteria that have important impacts on concentrations of environmental arsenic, and determining their arsenic-resistance gene repertoires. Furthermore, we are designing a laboratory model system in which we use bacteriocins to reduce bacterial production of these toxic forms of arsenic. Bacteriocins are proteins secreted by bacteria to inhibit the growth of competitive species. The use of bacteriocins to control the growth of bacteria that contribute to the mobilization of arsenic in the environment may provide a significant method for reducing the amount of arsenic that reaches surface and ground water systems.
Characterization of VrsA, a novel small RNA in Agrobacterium tumefaciens

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VtlR is a LysR-type transcriptional regulator that contributes to the virulence of the bacterial plant pathogen Agrobacterium tumefaciens. While VtlR regulates the expression myriad genes, over 250 genes total, this regulator has been shown to directly activate the expression of only three genes. These three genes are small regulatory RNAs (sRNAs). Therefore, we hypothesize that VtlR indirectly regulates a majority of the genes in the regulon via direct regulation of these three sRNAs. Small RNAs (sRNAs) are non-coding short transcripts found in bacteria that play a critical regulatory role in many biological processes via activation or repression of target genes expression. VtlR directly activates the small RNAs (sRNAs) AbcR1, Atu1667, and a novel sRNA, which has been named VrsA for VtlR regulated sRNA A. It has previously been shown that AbcR1 regulates approximately half of the genes in the VtlR regulon and a deletion of abcR1 has no effect on the virulence of A. tumefaciens. Hence, our hypothesis is that the sRNAs Atu1667 and/or VrsA regulate the rest of the VtlR regulon and could be responsible for the attribution of VtlR to the virulence of A. tumefaciens.

The objective of this project is to characterize the sRNA VrsA in A. tumefaciens by determining target genes and understanding the role of VrsA in A. tumefaciens biology and pathogenesis. To date, we have confirmed the existence of VrsA by northern blot analysis. Currently studies are focused on construction of a vrsA deletion strain of A. tumefaciens to utilize for phenotypic and RNAseq analysis.

Season and climate impact soil microbial community substrate utilization

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Microbial communities in soil are influenced by temperature, water content and vegetation. These factors are dependent on season and climate. During the summer and spring seasons when temperature and vegetation is relatively high, microbial biomass and activity are high due to available substrate. The changes in seasonal rainfall, due to climate or drought, also impact availability of substrate through alteration of diffusion rates. Drought reduces diffusion resulting in stress on the microbial community. We assessed the impact of season and long-term drought on microbial activity and biomass using microrespirometry. We hypothesized that short-term drought would decrease the microbial populations and thus decrease microbial activity, however long term drought would result in a drought-adapted community. Additionally with season, overall microbial activity would be greatest during spring and summer due to warm temperatures, high rainfall and vegetation.
Soil samples were collected monthly from a prairie site at Natural Bridge State Park in Rockbridge County, Virginia over the past two years to measure substrate utilization, using 11 different substrates, and microbial biomass under natural and drought treatments. Overall there was no effect of long-term drought on microbial respiration. However, season did significantly impact the microbial community with greater respiration for most substrates and increased biomass in spring and summer compared to fall and winter (ANOVA, p<0.05). Despite the overall higher respiration, there was no difference between seasons when looking at Rmass, the respiration per unit microbial biomass, indicating similar physiological responses throughout the year.

(35) **Co-Infection of Bordetella avium and B. hinzii from Sick Turkeys Alters Biofilm Features**

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Bordetellosis is a disease in turkeys commonly caused by the Gram negative bacterium, Bordetella avium. The closely related species, B. hinzii, was known to colonize turkeys but not thought to cause disease. However, over the past four years, B. hinzii was also isolated from turkeys diagnosed with Bordetellosis. This study intends to explore the possibility that B. hinzii and B. avium participate in co-infection and whether or not this behavior increases attachment rate. The main focus of this project is on biofilm formation, which functions as a virulence factor in regards to both attachment to the host and to protection from antimicrobial chemicals. Biofilms were grown and qualitatively evaluated using glass tubes and King’s medium. Strains used were 197N (B. avium) and 14-3425 (B. hinzii), both inoculated from sick turkeys. Strength of biofilms were measured using drop bead tests, which measures the amount of 1 mm glass beads that a biofilm can hold before breaking. Results show that individually, B. avium cultures form a very thin and weak biofilm, while B. hinzii cultures form a much stronger and more extensive biofilm. However, when cultured together, the biofilm formed is larger than both individual biofilms, but not necessarily stronger than B. hinzii alone. These findings support the hypothesis that bordetellosis may colonize more efficiently when both species are present together. Bordetellosis continues to cause financial losses in the poultry industry, so our work with detailed analysis of virulence factors could be useful information in dealing with the problem.