VIRGINIA BRANCH ASM
2026 ANNUAL MEETING PROGRAM

NOVEMBER 6-7, 2026

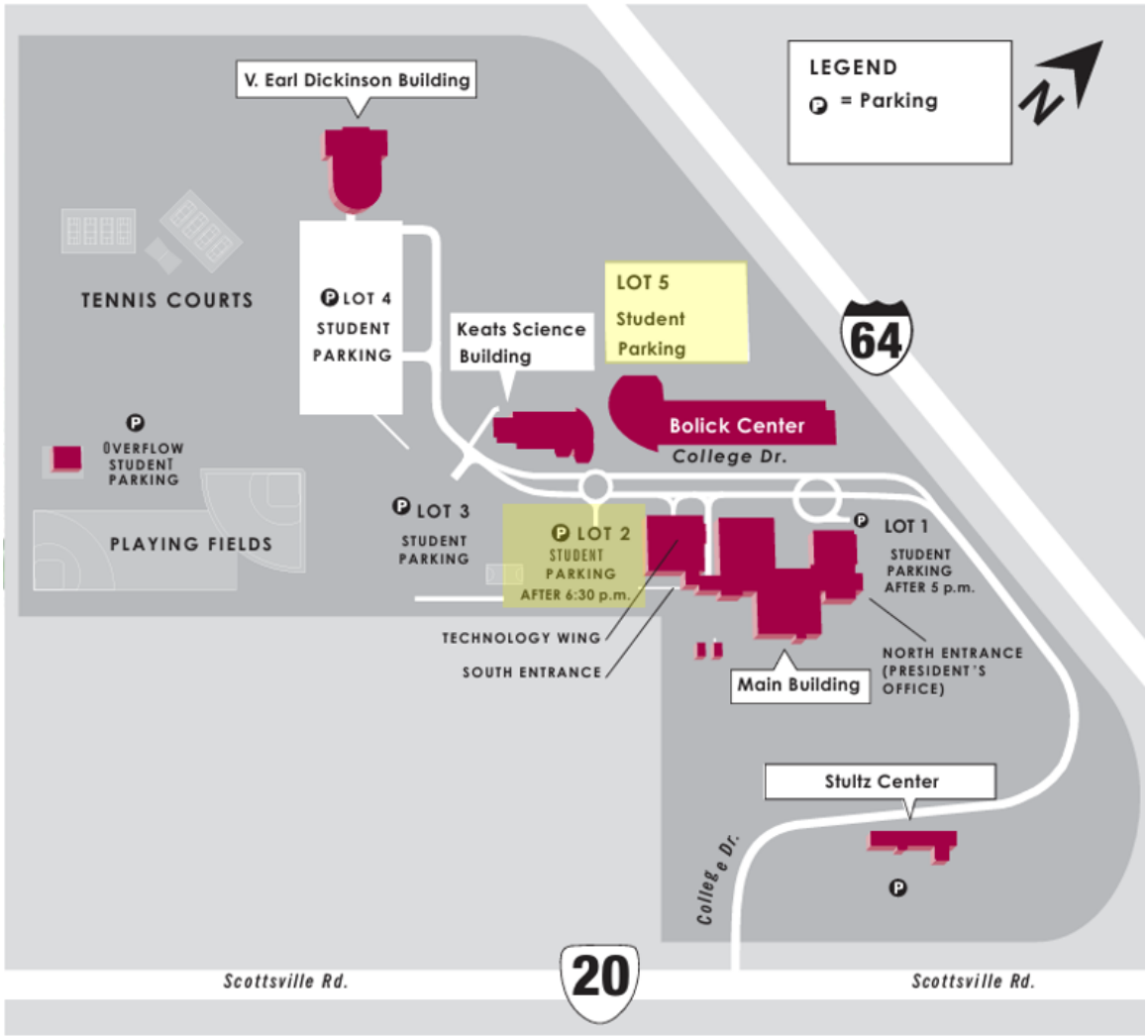
Host: Piedmont Virginia Community College

Site: Bolick Building Event Space, 500 College Drive, Charlottesville, VA, 22902
https://maps.app.goo.gl/BzQLvAKTn7Q4NZQXA



To connect to Guest WIFI:
https://www.pvcc.edu/faqs/how-do-i-connect-pvcc-wireless-network-wi-fi

***Parking & Campus Map*:** Park in LOT 5 or 2. Please register for free parking at**:**
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Access to the Bolick Event Space:
From Lot 5: Enter the Bolick Lower Lobby, turn right
From College Drive: Enter the Upper Lobby, descend stairs to lower level, turn left

***Accommodations*:**
Holiday Inn Monticello, 600 Fifth St. Landing, Charlottesville
Comfort Inn Monticello, 2097 Inn Drive, Charlottesville

VA Branch ASM Gratefully Acknowledges our Sponsors!













Edward Goyette



David Lyerly

Welcome to the Virginia Branch ASM 2026 Annual Meeting

Virginia Branch ASM Officers:

President:	Ann Stevens, Virginia Tech
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Virginia Branch 2026 Annual Meeting
Friday, November 6, 2026

12:00-5:00 PM **Registration**, **Bolick Lower Lobby**

12:30-12:45 PM **Welcome, Bolick Event Space**

HOSTS: Anne Allison and Todd Jarry, Piedmont Virginia Community College

Greeting from the Branch President: Ann Stevens, Virginia Tech

For the oral presentation sessions below, the abstracts can be found on pages 9-21 of the program. The presenters indicated by * are being judged in the oral presentation competition.

12:45-2:15 PM **Oral Presentations, Session I, Bolick Event Space**

Moderator: Joy Caughron (Radford)
Judges: Janet Adams (J. Sargeant Reynolds); Soumi Ghosh (VT); Heather Lindberg (VWCC)

1. Joshua Hueler*, Virginia Tech
2. Jasmine Cruz*, University of Virginia
3. Joud Aldaroub*, Virginia Tech
4. Colin Hawkes*, Virginia Commonwealth University
5. Jackson Wood*, Virginia Tech
6. Bryan Ericksen, University of Maryland
7. Caroline Clark*, Virginia Tech

2:15-2:30 PM **BREAK, Bolick Event Space**

2:30-4:00 PM **Oral Presentations, Session II, Bolick Event Space**

Moderator: Jared Heffron (Novonesis)
Judges: Edith Carron (Southern MD); David Freier (University of Lynchburg); Kristylea Ojeda (VWCC)

8. Foster Agyei, Virginia Tech
9. Yuang Sun*, University of Virginia
10. Abigail Heimbach*, Virginia Tech
11. Elliott Jang*, University of Virginia
12. Chrissy Walsky*, Virginia Tech
13. Jessica Miller*, Old Dominion University
14. Sarah Houser, Radford University Carilion

4:00-4:15 PM **BREAK, Bolick Event Space**

4:15-5:15 PM **Plenary Talk by ASM Distinguished Lecturer, Bolick Event Space**

Shaun Brinsmade, Georgetown University
"Lean on me (for lipids): Metabolic synergy between *Staphylococcus aureus* and *Enterococcus faecalis* during polymicrobial interaction".

5:15-5:30 PM **Branch Photograph**, **Bolick Lower Lobby**

5:30-7:30 PM **Appetizers and Posters**, **Bolick Event Space**

Poster Presentation Numbers

Odd Numbered Posters Present 5:30-6:30 PM
Even Numbered Posters Present 6:30-7:30 PM

The poster presenters who are undergraduate students or graduate students (indicated by *) will be given professional development feedback as requested by at least three other conference participants.
Abstracts on p. 21.

1. Leen Alawneh*, University of Virginia
2. Maraky Alemu*, Virginia Tech
3. Benjamin Bachmore*, Virginia Tech
4. Dale Beach, PhD, Longwood University
5. Shannon Carter, Virginia Military Institute
6. Songyu Dong, PhD, University of Virginia
7. Charlotte Halford*, University of Virginia
8. Afra Hossain, Virginia Tech
9. Joshua Huff, Virginia Tech
10. Niti B. Jadeja, PhD, University of Virginia
11. Kimora Johnson*, Longwood University
12. Jessica Kearney*, University of Virginia
13. Babatomiwa Kikiowo, Virginia Tech
14. Eli Konvalinka* and Aidan Moore*, Virginia Tech
15. Davis Kubovcik*, Old Dominion University
16. Austin Luther*, University of Lynchburg
17. Shane Mann*, Virginia Commonwealth University

18. Alex Mendez*, University of Virginia
19. Amy Nardini*, Longwood University
20. Audrey Neighmond*, University of Virginia
21. Livia Osbun, Virginia Tech
22. Bernice Owusu, Virginia Tech
23. Adriana Paci, Batten University
24. Ayla Pada, James Madison University
25. Kit Price*, University of Lynchburg
26. Steven Rolland, PhD, University of Virginia
27. Gemma Sconyers, James Madison University
28. Cooper Seidner, Batten University
29. Tori Shimozono*, Virginia Tech
30. Emma Snead* and Jackson Geeslin*, Longwood University
31. Margaret Stiker, Virginia Commonwealth University

8:00-10:00 PM **Virginia Branch ASM Evening Social**

Timberwood Tap House

245 Merchant Walk Ave Unit 100, Charlottesville, VA (10 minute drive)

1 drink ticket per person

Saturday, November 7, 2026

8:00-8:30 AM **Socializing and Coffee, Bolick Event Space**

8:30-9:00 AM **Presentation by ASM Press and ASM Journals**

9:00-9:30 AM **Invited PVCC Alumni Speaker**

Mr. Eddy Tennant, University of Virginia

"From Classroom to Cleanroom:
Navigating High School, Community College, University, and Industry"

9:30-9:45 AM **BREAK**, **Bolick Event Space**

9:45-11:15 AM **Oral Presentations, Session III, Bolick Event Space**

Moderator: Caitlin Williams (Batten)
Judges: Andrea Beyer (Richard Bland); Carmen Cantemir-Stone (Southern MD); Brady Spencer (UVA)

15. Elizabeth Burrell*, Virginia Tech
16. Mark Ciardiello *, Old Dominion University
17. Larissa Cooper*, Virginia Commonwealth University
18. Rita Makhlouf*, Virginia Tech
19. Kaitlyn Regan, Lynchburg University
20. Sharat Paka*, Virginia Tech

11:15-11:30 AM **BREAK, Bolick Event Space**

Concurrent Sessions

11:30-12:30 PM **Student Career Panel, Bolick Event Space**

Bryan Berger, Professor of Chemical Engineering and Biomedical Engineering, University of Virginia & Founder, Lytos Technologies

Shaun Brinsmade, Professor of Biology, Georgetown University

Jared Heffron, Production Microbiologist, Novonesis

Kellen Miller, Lab Technician, InBio

Sophia Stuart, Microbial Reference Group, Division of Consolidated Laboratory Services

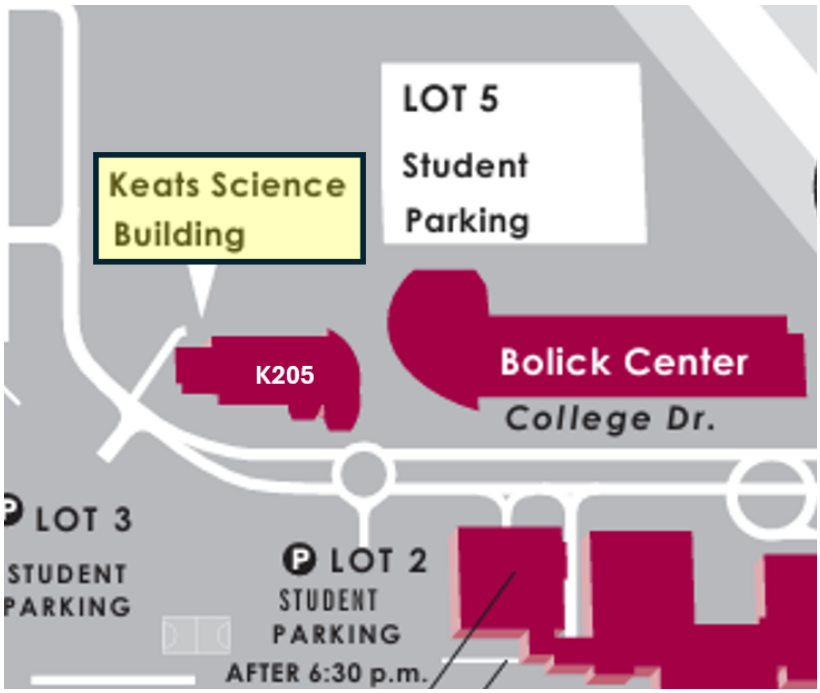
11:30-12:30 PM **Faculty Microcredentials Workshop, Bolick Second Floor West Side Open Seating Area**

12:30-1:30 PM **Lunch and Business Meeting, Bolick Event Space**

Secretary Report, Jamie Brooks
Treasurer Report, Alison Burke
Election of Executive Board Positions: President-Elect, Treasurer, Branch Counselor
Position Descriptions & Duties
Determination of next meeting host

1:30 PM **Closing Remarks, Student Travel Award Checks Distributed, and Adjourn**

1:45 PM Faculty Microcredentials Exams, Keats Science Building Room K205



ABSTRACTS: Oral Presentations

(In order of presentation. Presenting author is underlined and * = participating in judging)

Session I Oral Presentations

1. Influence of bacteriophages on acute inflammation in the mammalian gut
Joshua Heuler*, Hollyn Franklin, Rita Makhlouf, Zachary Baker, Bryan Hsu
Virginia Tech, Blacksburg VA

Inflammatory bowel disease (IBD) is characterized by a dysregulation of the gut immune response. Increasing IBD prevalence raises the need for treatments that restore immune tolerance. Although studies suggest a link between gut phage dysbiosis and IBD, it is still unclear how phages contribute to immune regulation. Based on prior literature demonstrating that the microbiome helps the human host utilize metabolites that can suppress inflammation, we hypothesize that phage-released intracellular metabolites may similarly restrain colitis. In antibiotic-treated mouse models with selective bacterial and phage colonization we show the presence of phage protected

against DSS-induced colitis. In bacteriophage-conditional mouse models, we similarly found that phages were important during DSS-induced colitis as their absence led to higher disease activity index (DAI) scores and elevated fecal lipocallin-2 levels. Overall, the presence of lytic phages appears to have an anti-inflammatory effect on the gut immune system. This work could improve our understanding of how phages regulate the gut immune system, which could be exploited for novel IBD therapy development.

2. Defining the role of PDZD8 in preserving *Chlamydia* replicative niche
Jasmine Cruz*, Elaheh Najafianashrafi, Isabelle Derré
University of Virginia, Charlottesville, VA

Chlamydia trachomatis (Ct) is the leading cause of sexually transmitted infection of bacterial origin, worldwide. Infection is frequently asymptomatic and if left untreated, can lead to pelvic inflammatory disease, ectopic pregnancy and infertility. These outcomes depend on Ct ability to establish and maintain its intracellular replicative niche. Ct is an obligate intracellular bacterium that replicates in a membrane-bound vacuole termed the inclusion. To support inclusion growth, Ct manipulates host organelles, such as the host endoplasmic reticulum (ER), through the formation of ER-Inclusion membrane contact sites (MCS). At ER-Inclusion MCS, protein complexes tether the ER to the inclusion membrane and facilitate lipid transfer between the ER and the inclusion. We identified PDZD8, an ER integral membrane protein implicated in lipid transfer between host membranes, as a potential ER-Inclusion MCS protein. I validated PDZD8 localization to ER-Inclusion MCS via co-localization with known ER-Inclusion MCS proteins. Functional analysis revealed an accelerated spread of infection in HeLa PDZD8 knockout (KO) cells compared to HeLa wild-type (WT) cells that was rescued by expression of WT PDZD8. Live Imaging of infected WT and PDZD8 KO HeLa cells demonstrated that the accelerated spread of infection in PDZD8 KO cells correlated with premature lysis of infected cells. Ongoing studies are investigating if this phenotype results from impaired inclusion membrane integrity caused by loss of PDZD8-dependent lipid transfer activity from the ER to the inclusion. Together this data will define the role of PDZD8 in preserving the integrity of Ct replicative niche.

3. Co-isolation and characterization of novel jumbo phages that infect phytopathogens in the bacterial genera *Pantoea* and *Erwinia*
Joud Aldaroub*, William M. Hanger, Ann M. Stevens, Frank O. Aylward
Virginia Tech, Blacksburg, VA

Large bacteriophages with genome sizes exceeding 200 kbp have recently been shown to inhabit a wide range of biomes across the planet. These phages can be isolated from different environments including water, soil, marine sediments, and other habitats using a variety of hosts. Currently, only 2.17% of all sequenced, isolated phages have genome sizes >200 kbp, which may be due to the common use of 0.2 µm filters to sterilize phage samples during isolation. Here, we isolated phages from four different agricultural soil samples collected from an onion field, apple orchard, soybean field, and corn field using different strains of phytopathogenic *Pantoea* and *Erwinia* as hosts. We developed a modified protocol for phage isolation that was more permissive to large

phage recovery. One phage named ERP was isolated against *Erwinia* and had a genome size of 369,351 bp. Surprisingly, our sequencing results of the other phage stocks isolated against *Pantoea* revealed the presence of not one, but two jumbo phages denoted as phage A (368,227 bp) and phage B (262,449 bp) that co-exist. The co-existence of those large phages was confirmed by plaque PCR where a single plaque had both phages, which does not fit the conventional definition of a plaque. Plaque assay protocols are routinely used for the isolation of individual phages, and it is therefore unclear why two large phages appear linked in most of our stocks. Further investigation is needed to understand why these phages are co-existing in different samples when infecting *Pantoea*. The isolation of novel large phages is important for expanding the potential use of phages as biocontrol agents to prevent or treat plant diseases caused by *Pantoea* and *Erwinia* species.

4. The underappreciated pathogen *Selenomonas sputigena*, right under our noses
Colin G. Hawkes*
Virginia Commonwealth, Richmond, VA

Periodontitis (PD) is a disease of the oral cavity where the subgingival plaque biofilm activates chronic immune dysregulation that destroys the tooth-supporting tissue. According to the traditional dogma of periodontal bacteriology, a small group of bacteria are responsible for causing this immune dysbiosis. This small subset of PD etiological organisms is comprised of only three species and is termed the “red complex.” Since their discovery, the red complex organisms have received the vast majority of funding and research. The red complex bacterium *Porphyromonas gingivalis*, in particular, is the best characterized periodontal pathogen and continues to receive the majority of interest from researchers in the field.

With this said, it is critical to highlight that a great variety of taxa is present within the plaque biofilm during PD. And to more completely understand the etiology of this disease, it is essential to investigate other oral pathobionts outside of the classical grouping of the red complex organisms. The topic of this work, *Selenomonas sputigena*, is one such organism.

S. sputigena is an abundant organism in periodontitis and numerous *S. sputigena*-disease associations have been reported. But, thus far, host-pathogen interactions in *Selenomonas* remain largely uninvestigated. Therefore, the objective of this research is to characterize host-pathogen interactions in *Selenomonas sputigena*. Specifically, this work includes the first published characterization of host-pathogen interactions between *S. sputigena* and gingival epithelial cells (GECs). In brief, these data illustrate the immunogenic potential of the bacterium and display intracellular invasion of *S. sputigena* within GECs, each of which phenotype is a hallmark event in PD etiology.

5. Evolutionary trials with bacteriophage Chi reveal that a single mutation in a gene of unknown function results in host range expansion
Jackson Wood*, Birgit Scharf
Virginia Tech, Blacksburg, VA

Bacteriophages (phages) are viruses that exclusively infect bacteria. Due to an increase in antibiotic resistance, there is renewed interest in using bacteriophages as therapeutics against bacterial pathogens. Phage Chi belongs to the group of phages, which require an actively rotating flagellum to infect their hosts. Unlike many other phages, which only target a single species, Chi infects three species of pathogenic bacteria: *Escherichia coli*, *Salmonella enterica*, and *Serratia marcescens*. Due to Chi's reliance on swimming motility, an important bacterial virulence factor, it is a promising candidate for phage therapy. In addition to Chi, our lab has acquired three closely related phages, namely iEPS5, Utah, and YSD1. In this study, we carried evolutionary trials, to expand the host range of Chi and its related phages. We used a cocktail of these phages to infect a coculture of *S. enterica* serotypes Arizonae, Enteritidis, Infantis, and Typhimurium, where Enteritidis was resistant to all four phages while all other serotypes were susceptible. After 10 passages, we isolated an evolved phage that infected all serotypes from the coculture. We carried out the same protocol with only Chi and received the same result. Sequencing revealed that our isolated evolved phages had point mutations to the gene Chi_35 leading to a Thr31Lys substitution in the protein gp35. Gp35 is a hypothetical protein with no known function which has homology to proteins with carbohydrate and glycan binding domains, suggesting a role in host interaction. For future work we aim to identify the molecular mechanisms behind host range expansion.

6. Evidence that the resistance mechanism of biofilm formation is relevant to the inoculum effect of the defensin HNP1 against bacteria

Bryan Ericksen, Corinne Delaney
University of Maryland School of Medicine Institute of Human Virology, Baltimore, MD

Background: Biofilms have been observed in 96 well plates used in virtual colony count (VCC) studies of the positive control antimicrobial peptide, the defensin HNP1, assayed against the standard Gram positive strain, *Staphylococcus aureus* ATCC 25922, at the standard inoculum of 5x105 CFUv/mL throughout the history of VCC assays since 2003. Biofilms were present on the bases of wells that had been scored as zero virtual survival by the VCC assay, which showed no sign of planktonic survivors in the 200 microliter batch cultures after the 12-hour plate reader incubation.

Methods: The virtual colony count (VCC) microbiological assay was adapted for varying inocula. 96-well plates were read for 12 hours in a Tecan Infinite M1000 plate reader and photographed under 10x magnification. Threshold times were determined using a Visual Basic script and these values were converted to virtual lethal doses and virtual survival values using a published Microsoft Excel spreadsheet.

Results: Biofilms were observed when HNP1 was exposed to inocula of *S. aureus* ranging from 1250 to 1x108 CFUv/mL. In addition, growth kinetic plots showed that this *S. aureus* strain produced nonparallel curves when survivors of defensin exposure were compared to 10-fold calibration dilution series of bacteria that had not been exposed to

any antimicrobial agent. VCC results demonstrated inoculum effects at the high inocula of 1x107 CFUv/mL and 1x108 CFUv/mL.
Conclusions: The inoculum effect may be caused by extracellular polymeric substances (EPS) such as DNA, RNA and capsular polysaccharides that inhibit defensins and that are structural components of biofilms.

7. Behavior of *Salmonella enterica* on fresh-cut tropical and subtropical fruits
Caroline Clark*, Laura Strawn
Virginia Tech, Blacksburg, VA

Fresh-cut tropical fruits have previously been associated with foodborne outbreaks of salmonellosis. Information regarding *Salmonella* growth on many fresh-cut tropical fruits remains limited; as a result, regulators lack the data needed to support time and temperature control for safety (TCS) guidance. This study aimed to compare the growth of *Salmonella enterica* on fresh-cut watermelon, papaya, dragon fruit, guava, and kiwi. Commodity pH was measured prior to inoculation. Cut commodities (10 g) were spot inoculated with a five-strain cocktail of *Salmonella* at 2 to 2.5 log CFU/mL to mimic low levels of contamination. Inoculated samples were air-dried for 30 min, transferred to sterile Whirl-Pak bags, and stored at 23 ± 2°C and 55 ± 2% relative humidity. *Salmonella* concentrations were enumerated hourly for the first 8 h of storage and at 24 h intervals until visible mold growth occurred. Data were fitted to a non-linear regression model in RStudio (version 4.5.2) to determine significant differences among commodities (n=6). The time required for *Salmonella* to increase by 1 log CFU/g differed among the commodities. On watermelon, papaya, dragon fruit, and guava *Salmonella* concentrations exceeded a 1-log increase by 7-8 h of storage. In contrast, populations on kiwi remained near or below initial inoculum levels over 24 h, with a greater than 1-log growth observed between 24 and 48 h. None of the evaluated commodities exceed a 1-log growth increase within 4 h under the tested conditions, suggesting they would not require addition to the current TCS framework based on *Salmonella* growth.

Session II Oral Presentations

8. Characterizing transition metal sensing in *Sinorhizobium meliloti* mediated by the chemoreceptor McpW
Foster Agyei*, Afra Hossain, Nisha Dhiman, Birgit Scharf
Virginia Tech, Blacksburg, VA

Sinorhizobium meliloti is a Gram-negative, free-living soil bacterium that possesses eight chemoreceptors. These chemoreceptors, or methyl-accepting chemotaxis proteins (MCPs), play a pivotal role in its symbiotic relationship with alfalfa by sensing compounds released by host plant roots. Among these chemoreceptors, the ligand-binding profiles of McpU, McpX, McpV, and McpT have been characterized. However, the remaining four chemoreceptors, McpW, McpZ, McpY, and IcpA, remain uncharacterized. Preliminary observations suggested that McpW may recognize transition metals, motivating investigation of its ligand-binding properties and the behavioral responses of *S. meliloti* to metal ions. Differential Scanning Fluorimetry of

the purified ligand-binding domain of McpW (McpWLBD) with Zn2+, Mn2+, and Ni2+ resulted in a significant thermal shift, which suggested that metal ions may be ligands of McpW. Isothermal titration calorimetry of McpWLBD and metal ions yielded dissociation constant (Kd) values in the nanomolar range. AlphaFold3 analysis of McpWLBD with metal ions predicts that Asp 118 and Asp122 residues are required for successful interaction. To determine whether metal ions also influence swimming behavior as either attractants or repellents, swimming speeds of *S. meliloti* were measured in the presence of Ni^{2+}, Mn^{2+}, and Zn^{2+} at varying concentrations. Ni^{2+} produced a substantially greater reduction in swim speed than the other transition metals, and agarose chemical in-plug assays suggest that Mn^{2+} elicits an attractant response in *S. meliloti*.

9. *Shigella flexneri* undergoes niche-specific adaptation during infection
Yuang Sun*, David W. Lazinski, Lauren K. Yum, Andrew Camilli, Hervé Agaisse
University of Virginia, Charlottesville, VA

Shigella flexneri is a leading cause of diarrheal disease worldwide, yet bacterial factors required within distinct host niches remain poorly understood. Here, we used genome-wide transposon sequencing in an infant rabbit model of shigellosis to identify genes promoting bacterial fitness in vivo. The screen identified virulence factors on the large virulence plasmid and novel chromosomal fitness factors. Characterization of *zitB*, encoding a cation diffusion facilitator family Zn transporter, revealed a niche-specific fitness contribution. The Δ*zitB* mutant showed no growth defect in vitro in rich media or in vivo during the early infection phase (8 hpi) in epithelial cells. However, bacterial burden was reduced during the late infection phase (24 hpi) in vivo, when bacteria interact with immune cells, in both competitive and mono-infection assays. Reduced bacterial burden was associated with increased MARCO-specific macrophages, suggesting impaired colonization and killing by the Δ*zitB* mutant. Consistently, Δ*zitB* fitness was impaired in THP-1-derived macrophages. Metal chelation increased bacterial burden, indicating a role for macrophage-mediated metal toxicity. The Δ*zitB* mutant also showed increased sensitivity to zinc and copper. Finally, macrophage depletion in vivo restored bacterial burden to wild-type levels. Together, these findings identify ZitB as a niche-specific bacterial factor promoting *S. flexneri* fitness during macrophage-associated metal stress.

10. Metabolites in the maize xylem environment utilized by *Pantoea stewartii* subsp. *stewartii* for growth
Abigail M. Heimbach*, David M. Mackey, Ann M. Stevens
Virginia Tech, Blacksburg, VA

The insect vector-borne bacterium *Pantoea stewartii* subsp. *stewartii* (Pss) is the causative agent of Stewart's wilt disease in susceptible maize varieties in the USA. Wilt disease occurs when Pss invades the xylem and forms a biofilm, thereby blocking water transport. The resulting plant wilting and necrosis lead to stunted growth or death. To study the carbon and nitrogen sources available to support growth of Pss in the xylem, the composition of B73 maize xylem fluid was defined using targeted metabolomics. The most abundant (top ~90%) sugars, amino acids, and organic acids identified in

extracted xylem fluid (EXF) were used to construct synthetic xylem medium (SXM). SXM supports the growth of Pss and serves as a model medium for the in planta environment. Targeted metabolomics analysis of EXF or SXM before and after Pss growth revealed changes in metabolite concentrations. Depleted compounds, including glucose, fructose, and several amino acids, indicate preferred metabolites used by Pss. Accumulated compounds, such as pyruvate, were likely products of bacterial metabolism. A parallel analysis of inorganic nitrogen utilization is being completed to determine whether Pss preferentially utilizes organic or inorganic nitrogen. Since mycorrhizae provide inorganic nitrogen to maize roots, metabolites in EXF from seedlings grown in soil with or without mycorrhizae were compared. Overall, higher amino acid and lower sugar and organic acid concentrations were observed in plants grown with mycorrhizae compared to without. Information gained about how Pss grows in the maize xylem environment will reveal strategies for improving disease resistance in maize.

11. Biofilm formation in *Clostridioides difficile* isolates from a tertiary academic hospital
Elliot E. Jang*, Maria Luana Gaudencio dos Santos Morais, Deiziane V. S. Costa, Cirle A. Warren
University of Virginia, Charlottesville, VA

Clostridioides difficile is a Gram-positive, spore-forming pathogen responsible for *C. difficile* infection (CDI), a nosocomial and community-acquired diarrheal disease. Biofilm formation in *C. difficile* strains has been linked to high CDI recurrence rates. Despite its potential role in recurrence, biofilm formation in clinical isolates of *C. difficile* remains underexamined. This study aimed to evaluate biofilm-formation capacity among *C. difficile* isolates from CDI-diagnosed patients at the University of Virginia Hospital, with approval from the UVA IRB. Samples from adults positive for CDI were cultured for *C. difficile*, and clinical data were obtained from electronic medical records. Biofilm formation in vitro was estimated by crystal violet staining. Antimicrobial susceptibility assays were assessed using e-test strips (Liofichem). We found that all clinical isolates (n=165) were biofilm producers, and formation profiles varied over time, with trends shifting from weak producers (54.5%) at 24h to strong (60%) at 48h and settling at weak and medium (36.4% and 45.5%) at 72h. Most risk factors, including age, recurrence, PPI, and prior antibiotic usage, were similar across categories. No significant difference in antimicrobial susceptibility was observed between biofilm categories. However, mean hospital stay was significantly shorter in strong compared to weak producers in the 24hr and 72hr biofilm cohorts. The 48hr cohort exhibited the opposite trend but lacked statistical significance ($p=0.230$). We concluded that biofilm formation profiles are dynamic and both time- and strain-dependent. Trends observed in hospitalization, biofilm biomass capacity, and clinical relevance of biofilm in *C. difficile* warrant further investigation.

12. Comparative analysis of wet and dry inocula on the survival of *Salmonella* Tennessee on fresh-cut ‘gala’ apples and green bell peppers post freeze-dry processing
Chrissy M. Walsky*, Claudia Clinchard, Jennifer C. Acuff, Rachel A. Cheng, and Alexis M. Hamilton

Virginia Tech, Blacksburg, VA

Freeze-drying (FD) is a non-thermal drying method used to reduce water activity of high-moisture foods to prevent microbial growth. Freeze-dried produce may pose food safety concerns due to multiple wet- or dry-routes of contamination, lack of a microbial kill step, and the ability of foodborne pathogens like *Salmonella* to survive in these foods over time. Elucidation of the effects of FD, inoculation method, and commodity choice on *Salmonella* survival can inform microbial challenge studies to determine safety risks associated with these products. The objective of this study was to determine the effect of FD, time in storage, inoculation method, and commodity on *Salmonella* survival on freeze-dried produce. Fresh-cut, 'Gala' apple and green bell pepper slices (n=156) were inoculated with *Salmonella* Tennessee using a wet or dry (sand-based) inoculum (ca. 8 log CFU/g), freeze-dried, and stored at 23ºC for 28 days. *S.* Tennessee populations were enumerated on 0, 3, 7, 14, 21, and 28 d in storage. *Salmonella* reductions were fit to a linear regression model with a three-way interaction between time (days), inoculum type, and commodity. There were no significant ($p>0.05$) changes in *S.* Tennessee population on dry inoculated bell pepper only during FD and throughout storage. Wet inoculated populations reduced by ca. 0.52-1.46 log CFU/g higher than dry inoculated populations ($p<0.0001$). Populations on apple reduced by ca. 0.23-0.92 log CFU/g higher than on bell pepper at 14-28 d in storage ($p<0.05$). Inoculation method (i.e., contamination simulation, particularly by commodity type) should be considered a critical design factor in challenge studies for FD products.

13. Temperature-dependent survival of *Rickettsia parkeri*: Implications of tick expansion and avian hosts

Jessica Miller*, Wayne Hynes, Isaura Simões
Old Dominion University, Norfolk, VA

The Gulf Coast Tick (*Amblyomma maculatum* Koch) is a growing public health threat in the U.S. serving as both a vector and reservoir of *Rickettsia parkeri*, a causative agent of Spotted Fever Rickettsiosis (SFR). Expansion of this three-host tick in the U.S. has coincided with movements of cattle and migratory birds from states surrounding the Gulf Coast. However, mechanisms enabling *R. parkeri* persistence amid the expansion of *A. maculatum* and increasing incidence of SFR cases remain enigmatic. Unlike mammals, birds maintain higher body temperatures, potentially exposing *R. parkeri* within feeding ticks to elevated thermal conditions. However, their impact on rickettsial survival remains unknown. Herein, embryonic chicken fibroblasts were infected with *R. parkeri* expressing GFPAusFP1 (kindly provided by Dr. Erin Goley, Johns Hopkins University) and incubated at their optimum temperature, 39C. *Rickettsia* growth was assessed at one hour and daily up to six days post-infection via IFA and qPCR. IFA revealed clear changes in *R. parkeri* morphology over infection; however, no growth or decline was detected via qPCR. To further assess infectivity following exposure to 39C, titration assays were performed in Vero cells at 34C. Comparable amounts of *Rickettsia* /mL were recovered one hour post infection but declined steadily over time. However, infectious bacteria were recovered up to four days post-infection, suggesting at least partial survival at 39C. We are currently assessing the morphological changes in *R.*

parkeri observed in chicken fibroblasts. These data aim to illuminate an epidemiological factor that may influence the spread of *R. parkeri* in the U.S.

14. Surveillance for antimicrobial resistance (AMR) gene targets within sewersheds in southwest Virginia
Jessica Stevens, Sara Houser, Susan Tolliver, Danielle Folks, Mark Frazier, Jayasimha Rao
Radford University Carilion, Roanoke, VA

Antimicrobial resistance (AMR) is a critical public health issue with global implications. Wastewater-based surveillance of AMR genes is increasingly being used to provide insight into resistance patterns at the community level. This study focuses on detecting AMR gene targets present in local sewersheds across Roanoke and Salem, VA. Samples were collected over a 12-week period from eight locations between February and May 2023. For comparison, five of the original eight locations were sampled over a 12-week period from March to May 2025. Locations included acute care facilities, a retirement facility, community sewersheds, and influent from the wastewater treatment plant. Total Nucleic Acid extracted from these samples was quantified using qPCR for 13 AMR gene targets. These targets include beta-lactamase and carbapenemase-producing genes, a tetracycline repressor gene, and a gene associated with a quaternary ammonium compound efflux pump. The subsequent copy numbers were represented in Log10. In 2023, qacΔE (15.94 copies/L), blaKPC (13.74 copies/L), blaOXA-10 (12.81 copies/L), and tetR (12.46 copies/L) were among the most abundant targets detected, while blaCTXM-15 (11.58 copies/L), blaPER-1 (11.58 copies/L), blaOXA-2 (10.38 copies/L), and blaNDM-1 (9.37 copies/L) were detected at lower concentrations. In 2025, most gene targets increased in concentration across the five repeated collection sites, while tetR (11.58 copies/L) and qacΔE (13.43 copies/L) decreased. Future surveillance of local sewersheds can identify changes in AMR patterns over time. This study is extended to a 2026 sample cohort, which will continue to lay the foundation for future research and antibiotic stewardship efforts.

Session III Oral Presentations

15. Analysis of antibiotic resistance gene levels in hatchery-raised rainbow trout (*Oncorhynchus mykiss*) and their environment
Elizabeth Burrell*, Petra Choi, Loc Nguyen, Amy Pruden, David Kuhn, Ann Stevens
Virginia Tech, Blacksburg, VA

In aquaculture, broad-spectrum antibiotics effectively treat bacterial infections, but increase the risk of higher antibiotic resistance gene (ARG) abundance in the fish intestinal microbiome and the surrounding environment. This study was conducted to examine the effects of antibiotic treatment on ARG levels in the intestinal and external environments of hatchery-raised fish. Three genetic lines of rainbow trout, Arlee, Shasta, and Wythe, from younger (25 - 28 months old) and older (36 - 40 months old) groups were sampled. Fish in the raceway system had been treated with antibiotics (florfenicol and oxytetracycline) if they were diagnosed with a bacterial infection. Of the

six groups tested, only the younger Wythe group was directly treated with antibiotics. Intestinal mucosa swab samples were obtained from fish, and environmental samples were collected by swabbing the sides of raceways and filtering water samples. 16S rRNA V4 region gene amplicon sequencing was performed on purified DNA from intestinal samples. Beta diversity analysis was then used to select 18 representative intestinal samples for metagenomic sequencing, while 8 environmental samples were selected separately for metagenomic sequencing. It was hypothesized that (1) antibiotic-treated fish would exhibit a higher abundance of corresponding ARGs compared to untreated fish, and (2) highly abundant fish ARGs would also be present in the environment. Initial metagenomic analysis identified ARGs conferring resistance to antibiotics used at the hatchery in treated and untreated fish and in the environment, suggesting possible ARG dissemination throughout the hatchery.

16. Investigating mitochondrial dynamics during macrophage infection by pathogenic and non-pathogenic SFG *Rickettsia*
Mark Ciardiello*, Isaura Simões
Old Dominion University, Norfolk, VA

Rickettsia are obligate intracellular bacteria that rely on host cells to establish a replicative niche. Several species cause tick-borne diseases in humans, including spotted fever group (SFG) rickettsioses, which can range from mild febrile illness to severe life-threatening systemic disease, such as Rocky Mountain spotted fever. We and others have shown that pathogenic SFG *Rickettsia* can survive and replicate in human macrophages, whereas non-pathogenic species are eliminated. The mechanisms underlying these species-specific outcomes remain poorly understood. Because mitochondrial dynamics are closely linked to cellular metabolism, stress responses, and innate immunity, modulation of mitochondrial homeostasis may contribute to the ability of pathogenic *Rickettsia* to persist within macrophages.

Here, we investigate the impact of *Rickettsia* pathogenicity on mitochondrial dynamics during infection of THP-1 macrophages. We compare the mildly pathogenic species *R. parkeri* and *R. massiliae* with the non-pathogenic *R. montanensis* across a time course of infection. Changes in the mitochondrial fission machinery are assessed by monitoring total dynamin-related protein 1 (DRP1) and its phosphorylation at Ser616 and Ser637, regulatory sites associated with differential control of DRP1 activity. Mitochondrial fusion is evaluated through analysis of OPA1, while mitochondrial DNA copy number and mitochondrial footprint by immunofluorescence analysis are monitored throughout infection as additional measures of mitochondrial homeostasis.

Together, these analyses provide an initial characterization of mitochondrial responses to *Rickettsia* infection and explore whether distinct rickettsial species differentially impact mitochondrial dynamics and homeostasis in human macrophages.

17. Defining a novel c-di-AMP system in pathogenic *Leptospira*
Larissa N. Cooper*, Edward J. A. Schuler, Aidan D. Moylan, Daniel P. Miller, Richard T. Marconi

Virginia Commonwealth University, Richmond, VA

Leptospirosis is a global zoonotic disease caused by the *Leptospira* genus, leading to 1 million human cases annually. Leptospires infect vertebrate hosts through mucosal membranes or external abrasions, allowing the bacteria to rapidly disseminate into the bloodstream, establish infection in the renal tubules, and shed back into the environment through urine. Because *Leptospira* cycles between free-living and host environments, the bacteria must quickly adapt to vastly different conditions. Bioinformatic analyses have identified a putative CdaA-type diadenylate cyclase (DAC) and a PgpH-like phosphodiesterase (PDE) in *Leptospira interrogans* Fiocruz L1-130 (lic10844 and lic12106, respectively). DACs synthesize cyclic di-adenosine monophosphate (c-di-AMP) from two ATP molecules, and PgpH PDEs catabolize c-di-AMP into 5'pApA and AMP, depending on the PDE. C-di-AMP is a secondary dinucleotide messenger studied in diverse bacteria, but until recently, it was unstudied in *Leptospira*. We show that CdaA and PgpH are a functional DAC and PDE, respectively. Using size exclusion chromatography and DAC assays, we found that CdaA functions as a homodimer with cobalt or manganese as divalent cation cofactors. PDE assays revealed that PgpH requires manganese for enzymatic activity. Amino acid substitutions in both CdaA and PgpH were used to elucidate functional enzymatic residues. RT-qPCR analysis showed that lic10844 transcription is upregulated under elevated potassium levels, supporting the idea that c-di-AMP is critical for potassium homeostasis. This study represents important steps toward elucidating the biological role of c-di-AMP in Leptospira.

18. Engineered temperate phage to deliver a defective ATPase gene establishes anti-virulence via dominant negative effect

Rita Makhlouf*, Rogerio Bataglioli, Michael M. McClelland, Hiba Baaziz, Ella Drennan, Bryan B. Hsu
Virginia Tech, Blacksburg, VA

Salmonella enterica serovar Typhimurium (STm) causes an estimated 1.35 million nontyphoidal infections annually in the United States, yet no FDA-approved vaccine exists, and rising multidrug resistance has eroded the effectiveness of antibiotic therapy. These gaps highlight the need for therapeutic strategies that move beyond conventional antimicrobials.

Phage therapy offers a promising alternative, but clinical translation has been limited. Lytic phages typically require high multiplicities of infection to be effective, and bacteria rapidly evolve resistance, constraining long-term efficacy. Temperate phages present a different opportunity: rather than killing bacteria outright, they integrate into the host genome and can deliver genetic payloads that reprogram bacterial behavior, offering a way to suppress virulence while reducing the selective pressure that drives resistance.

Here, we engineer the temperate phage P22 as a vehicle for anti-virulence therapy against STm. We introduced a dominant-negative mutant of *invC*, which encodes an ATPase essential to the Type III secretion system (T3SS), into the P22 genome.

Because the T3SS enables STm to invade host cells, disrupting it directly impairs pathogenicity. Bacteria lysogenized by the engineered phage showed hemolytic activity reduced to levels indistinguishable from an *invC*-deficient mutant, indicating complete suppression of T3SS-dependent function. Unlike the lytic phage P22-H5, which selected for spontaneous resistant mutants, our engineered temperate phage generated no detectable resistance.

This work establishes temperate phages as programmable platforms for precision anti-virulence therapy, offering a way to attenuate pathogens without eradicating them, reducing disease burden while minimizing the evolutionary pressure that drives antimicrobial resistance.

19. Development of a promoter-reporter plasmid system in *Staphylococcus aureus*
Kaitlyn Regan*, Jamie Brooks
Lynchburg University, Lynchburg, VA

Staphylococcus aureus is a clinically relevant bacterium and an increasing source of antibiotic-resistant infections. As antimicrobial resistance limits the effectiveness of traditional treatments, understanding interactions between *S. aureus* and its mobile genetic elements may provide new approaches for studying and controlling infection. In addition to its chromosome, *S. aureus* commonly contains bacteriophages and pathogenicity islands, which can interact to alter bacterial gene expression. This project aims to develop a plasmid-based reporter system to investigate promoter activity and regulatory interactions between phage 80α and *S. aureus* pathogenicity island 1 (SaPI1). The *S. aureus* shuttle vector pBASE1 was previously developed, and pBASE2 was generated by incorporating a NanoLuciferase reporter. Initial experiments evaluating transformation efficiency were successfully completed. The current objective is to construct pBASE2 variants containing *S. aureus* wild-type and constitutively over-expressing promoter sequences to evaluate promoter activity. Establishing a reliable reporter system will enable future characterization of promoter activity and interactions between *S. aureus* mobile genetic elements.

20. The role of transglutaminase in the motility of *Sinorhizobium meliloti*
Sharat P. Paka*, Birgit E. Scharf
Virginia Tech, Blacksburg, VA

Sinorhizobium meliloti is a nitrogen-fixing bacterial symbiont of the alfalfa plant. An early step in this symbiotic relationship is the biased movement towards host-plant-derived chemoattractants, known as chemotaxis. Motility is driven by the flagellar system, which enables movement through the rhizosphere. LdtR, a transcriptional regulator known to regulate stress tolerance and peptidoglycan remodeling, has recently been discovered to also be required for motility. RNA-seq analysis revealed that the most strongly downregulated gene in the ldtR deletion strain encodes a putative transglutaminase. The primary reaction of this class of enzymes is the crosslinking of glutamine and lysine residues. When this transglutaminase gene is deleted, *S. meliloti* motility is severely reduced, hence, the gene was named *tgrM* or transglutaminase required for motility.

Transglutaminases have not been reported as required for motility in bacteria previously. Interestingly, suppressor mutants were frequently isolated. One had a point mutation in MotA, a subunit of the stator complex that converts proton motive force into rotational torque for the flagellum. A second set of suppressor mutations consisted of loss-of-function mutations of MotS, an uncharacterized protein hypothesized to aid in optimal stator alignment in the cell envelope. Through targeted mutagenesis, we found that the enzymatic activity of TgrM is necessary for flagellar movement We therefore propose that TgrM contributes to proper association of the stator with the cell wall, thus permitting torque generation, by crosslinking yet unknown components of the flagellar motor.

ABSTRACTS: Poster Presentations

(Number indicates your poster number; Presenting author is underlined and * = student presenter who requested feedback.)

1. Effector translocation across the Group B Streptococcal cell envelope by the Type VII Secretion System

Leen Alawneh*, Glenda de Figueiredo Sanches, and Brady Spencer
University of Virginia, Charlottesville, VA

Group B *Streptococcus* (GBS) is a Gram-positive pathogen that colonizes the female genital tract and is a leading cause of neonatal bacteremia and meningitis following maternal transmission. GBS utilizes a membrane-localized Type VII Secretion System (T7SSb) to export effector proteins that contribute to virulence; however, how these effectors traverse the cell wall remains poorly understood. The T7SSb component EsaA is hypothesized to facilitate effector translocation across the cell envelope. Structural studies of EsaA in a related species identified an elongated, arrow-shaped extracellular-loop homodimer, suggesting that this region may span the peptidoglycan layer and function as a conduit for secretion. Whether the EsaA extracellular loop performs a similar function in GBS remains unknown. This study investigates the role of the EsaA extracellular loop in secretion of the T7SSb effector EsxA. To establish the requirement for EsaA in effector export, EsxA secretion was assessed in parental and Δ*esaA* GBS strains by western blot analysis of culture supernatant and bacterial pellet fractions. Preliminary results demonstrated an absence of detectable EsxA in the culture supernatant of the Δ*esaA* strain, supporting a requirement for EsaA in EsxA secretion. We are currently mutating conserved extracellular-loop residues individually to alanine using site-directed mutagenesis. Constructs will be confirmed by DNA sequencing and western blotting to assess EsaA expression and stability. Subsequent experiments will assess EsaA surface localization and EsxA secretion to determine whether individual substitutions disrupt EsaA localization or specifically impair effector translocation. These studies will provide mechanistic insight into how GBS transports proteins across its cell wall.

2. Investigating the mode of action of micafungin as an antiphage compound

Maraky Alemu*, Aariq Huq, Tori Shimozono, Zhaomin Yang

Virginia Tech, Blacksburg, VA

The evolutionary arms race between bacteria and bacteriophages has resulted in great pressure for adaptation and survival. Bacteriophages infect bacterial cells by adhering to specific surface receptors and injecting their genetic material into the cell, where they can enter the lytic cycle. By hijacking the host's machinery, they are able to replicate and kill the cell, or the lysogenic cycle, where they integrate their genome into the host's DNA and remain dormant. As a result, bacteria have evolved to develop a range of defense mechanisms, one being antiphage compounds.

Antiphage compounds are chemical agents that are utilized by bacteria to inhibit or neutralize bacteriophages. Understanding the diversity and mechanisms of these compounds provides valuable insight into bacterial survival strategies and may inform future applications in biotechnology and phage therapy.

From HTS, we identified micafungin as a potential antiphage compound. The underlying mechanism of micafungin as an antiphage compound remains unclear. Micafungin belongs to a class of antifungals that target the fungal cell wall known as echinocandins. Its structure is unique to known antiphage compounds. Thus far we have found that micafungin has no direct effect on phage stability. Preliminary results suggest that micafungin can impact the adsorption of Luz 7. Future studies will evaluate the effects of micafungin to either confirm its effect on adsorption or downstream steps in the lytic infection cycle. These steps include, but are not limited to, entry, replication and host lysis.

3. Effects of experimental, yeast-based diet replacement on the intestinal microbiome of rainbow trout (*Oncorhynchus mykiss*)
Benjamin Bachmore*, Elizabeth Burrell, Jason Pough II, David Kuhn, Ann Stevens
Virginia Tech, Blacksburg, VA

Aquaculture is a rapidly growing industry that provides an important source of fish for recreational purposes and human dietary protein. Finding more sustainable materials to replace fish meal in fish feed is an important challenge for aquaculture. The goal of this study was to examine the physiological impacts associated with feeding fish a diet where a proprietary nutritionally-enhanced yeast strain, expressing a hypothesized beneficial nutrient, was used to replace fishmeal in the animal feed. The intestinal microbiome of fish plays an important role in the overall health of the fish, and in turn, the intestinal microbiome is regulated by both intrinsic and extrinsic factors, including diet. The animal model for this study was rainbow trout (*Oncorhynchus mykiss*) housed in a recirculating aquaculture system (RAS). Fourteen fish were housed per tank, with four replicates for each of the six diets tested. The diets included: a commercial diet, a control diet with no yeast replacement, the control diet containing the parental yeast strain, and the experimental diets containing low, medium, or high levels of the proprietary yeast strain expressing the proposed beneficial nutrient. After a five-week feeding period, the fish were euthanized, and the intestinal epithelial layer of the fish was swabbed before extraction of the bacterial

microbiome DNA. DNA extraction was completed using a Qiagen kit, and following extraction, the 16S rRNA gene V4 region was amplified through PCR using bar-coded universal primers. Following sequencing of the PCR products, a bioinformatics analysis of the sequencing data will be completed. This study will allow us to compare the intestinal bacterial microbiome across different diets alongside animal production metrics.

4. What CURE'd my cabbage: A course-based undergraduate research experience (CURE) in NGS techniques
Dale Beach
Longwood University, Farmville, VA

With the decrease in NGS costs and readily available technology for sequencing and analysis, we are developing an innovative, lab-based research experience for undergraduate courses. Commercially available, fermented food products including kimchi, sauerkraut, kombucha, yogurt, and kefir are typically sold as a source of "probiotic" nutrition can provide a well-controlled and generally consistent community of living microbes. We seek to utilize these products as a persistent and reliable biological sample to study microbial communities through a metagenomic approach, and whole genome sequencing of individual colony isolates. We will use the Nanopore DNA sequencing platform to complete in-house, hands-on student-lead sequencing of DNA isolates. Subsequent analysis will utilize both Nanopore provided predefined analysis pipelines as well as the free, online Galaxy genome analysis portal. Because fermented food products will generally provide a small and well-defined community of microbes, the expected metagenomic outcomes for the project are limited in scope and scale, and easily verified in the literature. By introducing standard microbial isolation techniques, the derivation of single colonies from the product will provide well single species template for whole genome sequencing. Isolates of the same species from different manufactures, production centers, or fermented food products may produce sufficient sequence variation to provide a genetic means to identify or track food products in the event of a foodborne outbreak.

5. Do diet and water temperature modulate skin microbiome diversity in laboratory axolotls?
Shannon Carter, Alison Kernell Burke, Moshe Khurgel
Virginia Military Institute, Lexington, VA

Mexican axolotls (*Ambystoma mexicanum*) are fully aquatic salamanders that are widely studied for their regenerative abilities. However, the relationship between axolotl microbiome and their metabolism as well as the potential interactions between these factors and regeneration have not been addressed extensively. This multifactorial study examines whether and how key husbandry conditions, diet and water temperature, modulate skin microbiome of captive-bred axolotls. Skin swabs were collected from axolotls that were maintained under different dietary and temperature conditions. Bacterial communities in the samples are being characterized and compared between the experimental groups. Compositional changes in microbiome might influence host

metabolism, and possibly have an effect on the rate and extent of post-amputation wound healing and tissue regeneration. In addition, understanding how diet and water temperature shape the axolotl skin microbiome could inform husbandry practices for this endangered species' conservation programs.

6. Dissecting the molecular mechanisms of IcsA's dual functions
Songyu Dong, Volkan Köseoğlu, Zachary Lifschin, Hervé Agaisse
University of Virginia, Charlottesville, VA

Shigella flexneri infection causes more than 270 million cases of bacillary dysentery annually, resulting in 200,000 deaths, especially in children under age five. *Shigella* is highly contagious, with a 100-1000 infectious dose. *Shigella* is transmitted through the fecal-oral route. Before reaching its site of infection in the colon, *Shigella* encounters and survives the harsh physicochemical conditions of the digestive system. Our recent work uncovered a novel function of the *Shigella* virulence effector IcsA in supporting bile salt-dependent biofilm formation, which we propose is essential for bacterial survival in the small intestine. In the colon, *Shigella* invades colonic epithelial cells and spreads intercellularly, disrupting the intestinal epithelial layer. IcsA also plays a critical role in promoting cell-to-cell spread through actin-based motility (ABM). However, the key residues supporting IcsA's individual function haven't been determined.
We identified an autotransporter family protein from *E. coli* strain M605 (M605) that displays high sequence and structural similarity to IcsA but doesn't confer ABM or biofilm formation functions when expressed in *S. flexneri*. We designed IcsA-M605 swapping chimeras to dissect IcsA's dual functions. We used a high-throughput fluorescence-based screening assay to assess *Shigella* cell-to-cell spread and biofilm formation. We identified aa271-282 as key residues for ABM. While individual substitutions preserve *Shigella* biofilm formation, we speculate that IcsA contains multiple, redundant motifs that cumulatively mediate biofilm formation, which needs further investigation. Eventually, we will use ABM- or biofilm-formation-deficient *Shigella* to uncouple IcsA's niche-specific functions during shigellosis using *Shigella* infant rabbit infection models.

7. Randomized clinical study: Contribution of alanyl-glutamine to intestinal and systemic inflammation in patients with *Clostridioides difficile* infection
Charlotte G. Halford*, Deiziane V. S. Costa, Darren Hackley, Jasmine Jacobs-Ackers, Ekta Bansal, Cirle A. Warren
University of Virginia, Charlottesville, VA

Clostridioides difficile (*C. difficile*) continues to be an important urgent threat in the United States, causing antibiotic-associated diarrhea in hospital and community settings. Mortality post-*C. difficile* infection (CDI) and recurrence are important concerns and may be associated with exacerbated intestinal/systemic inflammation and incomplete gut repair, respectively. We have shown that alanyl-glutamine (AQ) effectively prevents recurrence and reduces intestinal epithelial cell damage induced by *C. difficile* toxins in in vitro and animal models of CDI. However, it is unknown whether AQ is effective as an adjuvant treatment in patients with active CDI. The aim of our

study is to evaluate whether AQ treatment in combination with standard care is effective in decreasing intestinal and systemic inflammation in patients with CDI. A randomized, placebo-controlled clinical trial was conducted at two hospitals in Virginia (UVA and Carilion) from January 2021 to March 2026. Fecal specimens and blood were collected at three different time points (baseline, 10 days, and 70 days after AQ treatment). Fecal biomarkers of intestinal and systemic inflammation were evaluated by ELISA (Lcn2, lipocalin; S100A8/S100A9, calprotectin; IL-6; MPO, myeloperoxidase; S100B, S100 calcium-binding protein B). Levels of *C. difficile* toxin B (TcdB), one of the major virulence factors causing cell damage in humans, in fecal specimens and systemically (serum) were also evaluated. We just recently unblinded our data and are currently performing analyses. We expect to identify the potential contribution of AQ treatment in improving intestinal and systemic inflammation in patients with CDI. We shall present our preliminary findings in the meeting.

8. Expressing and purifying the *Bradyrhizobium diazoefficiens* chemoreceptor Blr2932 for ligand-binding characterization
Afra Hossain, Foster K. Agyei, Idella Collett, and Birgit E. Scharf
Virginia Tech, Blacksburg, VA

Bradyrhizobium diazoefficiens (*B. diazoefficiens*) is an agriculturally important bacterium that fixes nitrogen for its legume host, soybean. The symbiotic relationship between soybean and *B. diazoefficiens* provides a sustainable alternative for nitrogen acquisition, thereby reducing our dependence on synthetic nitrogen fertilizers that pollute our environment. Successful establishment of this symbiosis depends on bacterial chemotaxis, in which methyl-accepting chemotaxis proteins (MCPs) detect host-derived chemical signals and direct bacterial movement toward plant roots. Blr2932 is among 36 genes that encode putative chemoreceptors in *B. diazoefficiens* whose ligand-binding profiles are yet to be characterized. Blr2932 is a transmembrane chemoreceptor, and AlphaFold3 structure predictions showed that its periplasmic binding domain is a Dual Calcium Channel and Chemotaxis receptor (Dual Cache_1). Sequence alignment of the Dual Cache_1 receptor Blr2932 with experimentally characterized receptors from *Sinorhizobium meliloti* revealed conservation of key residues associated with amino acid recognition, indicating that Blr2932 is a strong candidate for amino acid sensing despite partial divergence from the established consensus motif. This project seeks to determine viable conditions for optimal expression and purification of Blr2932 for amino acid binding analysis. The periplasmic binding domain will be expressed in *E. coli* using the pQE30 expression vector under the control of a T5 promoter and purified by Ni-NTA affinity chromatography. Differential Scanning Fluorimetry and Isothermal Titration Calorimetry will be used to analyze amino acid binding.

9. Exploring phage-compound synergy as a strategy to reduce enteric bacterial growth
Joshua Huff, Hollyn Franklin, Rogerio Bataglioli, and Bryan Hsu
Virginia Tech, Blacksburg, VA

Antibiotic resistance increasingly limits conventional treatments, creating an urgent need for strategies that enhance bacterial killing and preserve antibiotic effectiveness. We evaluated synergistic interactions between bacteriophage T4 and FDA approved compounds against *Escherichia coli* C600. Using a high throughput screen, we tested 2,816 compounds from the ApexBio FDA approved drug library at 50 μM with and without T4, measuring bacterial growth by OD600. Thirty eight compounds showed synergy, producing greater growth suppression in combination with T4 than either treatment alone. Ampicillin was selected for follow up because of its strong synergistic profile. Growth curve and four hour concentration response assays demonstrated that T4–ampicillin combinations produced greater and more sustained reductions in *E. coli* growth than ampicillin or T4 alone, especially at lower ampicillin concentrations where single treatments allowed continued growth or recovery. These results support our hypothesis that phage–compound synergy can improve antimicrobial activity and highlight FDA approved compounds as promising candidates for combination phage therapy. Future work will validate additional synergistic compounds, investigate mechanisms underlying enhanced phage mediated killing, test combinations across more bacterial strains, and evaluate their effectiveness and microbiome impacts in vivo.

10. Distinct effects of neutrophil maturation on bacterial burden and tissue injury during *Shigella* infection
Niti B Jadeja and Herve Agaisse
University of Virginia, Charlottesville, VA

To investigate the contribution of terminal granulocyte maturation to *Shigella flexneri* pathogenesis, we generated CEBPE-deficient infant rabbits. CEBPE is required for terminal granulocyte maturation and specific-granule formation, providing an in vivo model of impaired specific-granule formation. Colonic infection outcomes were assessed 24 h after inoculation. RNAscope demonstrated a genotype-dependent reduction in LCN2 signal, with homozygous animals showing near-complete loss, heterozygotes showing intermediate signal, and wild-type animals showing the highest signal. Homozygous animals had lower bacterial burden and reduced hemorrhagic areas compared with wild type. Heterozygotes showed an intermediate bacterial burden, but hemorrhagic area remained comparable to wild type. In contrast, epithelial fenestration was similarly elevated in both heterozygous and homozygous animals. These findings suggest that CEBPE-dependent neutrophil maturation or granule function is associated with bacterial burden and hemorrhagic injury but does not uniformly control epithelial remodeling. The selective reduction in bacterial burden and hemorrhage in homozygous animals raises the possibility that mature, granule-competent neutrophils contribute to tissue injury and influence bacterial persistence or dissemination. Larger cohorts, cellular validation of LCN2 signal, and time-course studies are needed to distinguish causal relationships between bacterial burden, hemorrhage, and epithelial damage.

11. Fungal pathogen influence on pickerel frog microbiome
Kimora E. Johnson*, Rachel M. Goodman and Amanda M. Starr
Longwood University, Farmville, VA

Amphibian populations have declined at an alarming rate due to infectious diseases. Many damaging pathogens prey on amphibian skin, a vital organ for breathing, hydration, immunity, and homeostasis. This project aimed to collect and analyze the amphibian skin microbiome, the community of protective microorganisms that prevent and fight infection. To study the microbiome of these amphibians, 135 amphibians were swabbed from Hampden-Sydney campus from June 2024 to April 2026. DNA was extracted from the swabs using the Qiagen DNeasy Blood and Tissue Kit. In conjunction with the student network for amphibian pathogen surveillance and in-house qPCR analysis, 6% of samples tested *Batrachochytrium dendrobatidis* (Bd) positive and 0% tested positive for *Batrachochytrium salamandrivorans*. The pickerel frog's microbiome has not been explored previously with and without infection. The swab DNA from Bd+ and Bd- samples from the pickerel frog were amplified using the Nanopore microbial amplicon barcoding kit and sequenced using the MinION device. Identification and analysis of microbiomes was completed with EPI2ME and R software. The pickerel skin microbiome differed by location and infection status. Bd infection consistently reduced microbial abundance and altered community composition. Anti-Bd taxa varied across collection sites and key protective species (*Chitinophaga*, *Janthinobacterium, Agrobacterium*) were absent in Bd+ samples. Understanding how amphibian microbiomes change with and without infection, can be used to identify anti-Bd microbes with anti-fungal properties which can be used for probiotic treatments of amphibians. Future work includes expanding amphibian species and analyzing tissue samples for the presence of *Perkinsea* and Ranavirus.

12. Toxin-induced neuroinflammation during Group B *Streptococcus* late onset meningitis

Jessica N. Kearney*, Deanna C. Sekulich, Brady L. Spencer
University of Virginia, Charlottesville, VA

Bacterial meningitis remains a leading cause of neonatal mortality and morbidity worldwide, with up to 40% of surviving infants developing permanent neurological sequelae including cognitive impairment, seizures, and cerebral palsy. Despite advances in antibiotic therapy, the molecular mechanisms driving Central Nervous System injury and post-infectious neurological damage remain poorly understood, and no therapeutics exist that target these downstream consequences. *Streptococcus agalactiae* (Group B *Streptococcus*; GBS) is a primary etiological agent of neonatal meningitis, and while much research has focused on bacterial invasion of the blood-brain barrier, the host and bacterial factors that perpetuate cerebrovascular dysfunction following infection have received far less attention. We have identified the GBS Type VII secretion system (T7SS) and its secreted pore-forming effector EsxA as critical virulence determinants that promote CNS cytotoxicity, systemic dissemination, and mortality in murine models of neonatal late-onset disease. We hypothesize that T7SS effectors induce acute meningeal inflammation and cerebrovascular injury that drive long-term neurological sequelae persisting beyond bacterial clearance. To test this, we are performing transcriptomic profiling of GBS-infected neonatal brain parenchyma and leptomeninges to identify infection-induced CNS damage signatures. In the future, we will also develop a clinically relevant antibiotic treatment model of neonatal meningitis

survival to evaluate post-infectious immune landscape remodeling, glial and neuronal responses, meningeal lymphatic dysfunction, and cognitive impacts. These studies will illuminate novel bacterial and host targets for therapeutic intervention and further our understanding of the long-term neurological consequences of neonatal bacterial meningitis.

13. Identification of external signals that activate the GacS/GacA signaling pathway in *Pseudomonas aeruginosa*

Babatomiwa Kikiowo, Erin Gloag, Florian D Schubot
Virginia Tech, Blacksburg, VA

Pseudomonas aeruginosa is an opportunistic Gram-negative pathogen that uses signaling networks to regulate virulence and adaptation to changing environments. The GacS/GacA phosphorelay signaling pathway controls the transition between acute virulence-associated behaviors and chronic biofilm-associated lifestyles. Upon activation, the membrane-bound sensor kinase GacS initiates a phosphorelay that activates the response regulator GacA. Activated GacA promotes transcription of the small regulatory RNAs RsmY and RsmZ, which regulate downstream processes including biofilm formation. However, the extracellular signal sensed by the periplasmic domain of GacS remains unknown.
We hypothesize that specific environmental metabolites interact with the GacS periplasmic domain to activate GacS-dependent signaling. To identify candidate external signals that bind to and activate GacS, we engineered PrsmY-lacZ and PrsmZ-lacZ transcriptional reporters into wild-type, ΔgacS, ΔretS, and ΔgacSΔretS *P. aeruginosa* strains. Reporter incorporation was validated in all genetic backgrounds, and the strains exhibited measurable β-galactosidase activity. The PrsmY-lacZ reporter showed β-galactosidase activity patterns consistent with the observed biofilm phenotypes. The PrsmZ-lacZ reporter exhibited measurable β-galactosidase activity; however, its activity pattern did not show the same relationship with the biofilm phenotypes, even when different β-galactosidase substrates were used.
These validated reporter strains provide a quantitative platform for screening candidate environmental signals and will be used to evaluate hit compounds identified through in silico screening of the GacS periplasmic domain and determine whether binders modulate GacS signaling in cells. Identification of GacS-activating ligands will provide mechanistic insight into environmental sensing and regulation of biofilm-associated behaviors in *P. aeruginosa*.

14. Host range expansion of bacteriophage chi against *Salmonella enterica* through phage training

Eli Konvalinka*, Aidan S. Moore*, Jackson A. Wood, Birgit E. Scharf
Virginia Tech, Blacksburg, VA

In response to the antimicrobial resistance crisis, bacteriophage (phage) therapy has developed renewed interest as an alternative therapeutic option. Phages are viruses that exclusively infect bacteria, and often have a narrow host range, infecting only a few strains of bacteria. In contrast, phage Chi has a relatively broad host range, infecting

three bacterial species: *Escherichia coli*, *Salmonella enterica*, and *Serratia marcescens*. Chi is a flagellotropic phage, requiring a rotating flagellum to infect its host. This infection mechanism presents an evolutionary trade off as bacteria evade infection by losing flagellar motility, which is an important virulence factor. As such, Chi is an attractive candidate for phage therapy. This research aims to broaden Chi's host range to resistant serotypes of *S. enterica* through phage training. Previously, phage training of Chi resulted in an output phage called evolved Chi (Evo Chi), which could infect the previously resistant serotype Enteritidis. We performed a new round of phage training with Evo Chi by infecting a coculture of four susceptible clinically relevant serotypes of *S. enterica*: Arizonae, Enteritidis, Infantis, and Typhimurium, and one resistant serotype, Montevideo. After ten rounds of coculturing, we were unable to establish host range expansion. We next performed another phage training experiment with Evo Chi by only changing the resistant serotype to Oranienburg. After six rounds of coculturing, we established host range expansion of a trained phage isolate to Oranienburg. Sequencing of genomic DNA from this isolate identified it as prophage PhiESI instead of Chi, and its origin is being examined.

15. Exploring the mechanisms of *Rickettsia parkeri* entry into human macrophages
Davis Kubovcik*, Isaura Simoẽs
Old Dominion University, Norfolk, VA

Rickettsia parkeri, a member of the spotted fever group (SFG) *Rickettsia*, is the etiological agent of *Rickettsia parkeri* rickettsiosis, an emerging tick-borne disease in the United States transmitted by the Gulf Coast tick, *Amblyomma maculatum*. Rickettsia are obligate intracellular bacteria that require adhesion to and invasion of host cells to establish a successful infection. Although SFG *Rickettsia* preferentially target endothelial cells, evidence from our group and others has shown that pathogenic species are also capable of invading and proliferating within human macrophages. However, the mechanisms by which SFG *Rickettsia* associate with and invade human macrophages remain largely unknown. To begin elucidating these mechanisms, we are employing a pharmacological approach using PMA-differentiated THP-1 macrophages and *R. parkeri* expressing GFPAusFP1 (kindly provided by Dr. Erin Goley, Johns Hopkins University) as experimental models. Our current results show that inhibition of Na+/H+ exchangers with two different amiloride derivatives, 5-(N,N-Dimethyl)amiloride hydrocholoride (DMA) and 5-(N-Ehtly-N isopropyl)amiloride (EIPA), markedly impairs *R. parkeri* association with macrophage-like cells, suggesting that macropinocytosis-like pathways may contribute to rickettsial entry into macrophages. We are currently expanding our panel of pharmacological inhibitors to further characterize this previously unrecognized route of invasion. By defining the mechanisms underlying SFG *Rickettsia* entry into macrophages we expect to reveal new targets for therapeutic intervention.

16. Effect of glucose levels in DMEM on RAW 264.7 murine macrophage response to bacterial lipopolysaccharide
Austin Luther*, David Freier
University of Lynchburg, Lynchburg, VA

Macrophages are white blood cells responsible for fighting pathogens, promoting tissue repair, initiating inflammatory responses, and performing other functions depending on their location. Murine macrophages are often grown in Dulbecco's Modified Eagle Medium (DMEM) or Roswell Park Memorial Institute 1640 Medium (RPMI). DMEM is typically supplemented with 4.5 g/L of glucose, while RPMI 1640 contains a more moderate glucose composition of 2 g/L. The higher glucose content commonly found in DMEM is presumed to support the aerobic metabolism of the proinflammatory phenotype of macrophages. Previous evidence in the lab demonstrated that the RAW 264.7 murine macrophages did not perform as well in RPMI 1640 when grown in low glucose. The addition of glucose in RPMI 1640 at 4.5 g/L returned macrophage response levels to normal. Our current experiment was designed to test the effect of glucose levels on RAW 264.7 responses to bacterial lipopolysaccharide (LPS) in DMEM only. RAW 264.7 cells were grown in high glucose, low glucose, and low then high glucose to test nitric oxide response recovery. After an incubation period of 12-14 hours, the cells were stimulated with LPS in a dose-responsive fashion (0–100 ng/mL) for 24 hours. Nitrite levels in the supernatants were then determined using the Griess reaction. The results of this experiment suggested that RAW 264.7 cells do not perform as well in low-glucose DMEM following stimulation with LPS. This is consistent with previous experiments using low-glucose media.

17. OD600 and dPCR method for calculating the approximate number of Dense Core Ehrlichia Chaffeensis in the supernatant of co-cultures of RF/6As and DH82s

Shane N. Mann*, Nicholas Cramer, Daniel A. Counts, Richard T. Marconi
Virginia Commonwealth University, Richmond, VA

Ehrlichia chaffeensis is the primary agent of Ehrlichiosis in humans. Accurately quantifying live *E. chaffeensis* is vital for reproducible in vitro and in vivo studies, but current methods largely rely on infected host counts. T75 flasks were seeded with either 1x105 /cm2 DH82s or 3x105 /cm2 RF/6As, then inoculated with supernatant from an *E. chaffeensis* infected culture. Each day post-inoculation, supernatant was collected, and the OD600 and genome copies/uL were recorded. The OD600s were plotted against genome copies/uL and a nonlinear regression was performed. Serial dilutions, inoculation of DH82s and RF6As, and immunofluorescence assays were performed to determine the ID50. To confirm consistency, we prepared control cultures weekly 4 times and recorded the OD600 and genome copies/uL each day 7. A strong correlation was observed between the OD600 of controlled *E. chaffeensis* infected DH82 (R2=.97) and RF/6A (R2=.05) cultures and genome copies/uL of *E. chaffeensis*. This allowed the generation of formulas to estimate the number of *E. chaffeensis* in the supernatant of a culture with a fixed number of host cells, between days 6 and 8 post-inoculation. The ratio of *E. chaffeensis* per host cell to obtain ID50 for DH82s and RF6As is 0.5-1 and 2.4, respectively. After checking repeatability and comparing our estimated counts based on the acquired formulas with actual counts from dPCR, we found a 20% error rate. The correlation between OD600 and *E. chaffeensis* can be used to estimate the amount of *E. chaffeensis* in the supernatant. This fills a much-needed niche for quantifying *E. chaffeensis*.

18. Characterizing dose-dependent pathology and infection dynamics in an infant rabbit model of shigellosis

Alex Mendez*, Herve Agaisse
University of Virginia, Charlottesville, VA

Shigellosis is a highly contagious bacterial infection, responsible for more than 270 million cases annually, resulting in ~ 200,000 deaths, including more than 60,000 children under age 5. With the rise of extensively drug-resistant strains, identifying novel therapeutics is an urgent priority. A major challenge of studying bacillary dysentery is the lack of a small-animal model that mimics the symptoms observed in infected individuals. Our lab has previously established a novel infant rabbit model of bacillary dysentery that shows symptoms similar to humans (e.g., severe inflammation, massive ulceration of the colonic mucosa, and bloody diarrhea). With a unique small-animal model, we aim to contribute to the field by deciphering the mechanisms supporting the recovery process from acute infection to complete bacterial clearance, with an emphasis on the role of macrophages. Here, we establish the parameters regulating infection dynamics and recovery of the colon by studying the effects and symptoms from various bacterial infection doses on disease progressions. Using diarrhea-blood scoring alongside H&E- and immunostained paraffin- colonic sections, we quantified disease severity based on bacterial burden, mucosa edema, and epithelial fenestration. Establishing the dosage threshold defines the experimental window to investigate the resolution process spanning from acute infection to complete bacterial clearance, setting the stage to study the role of macrophages during tissue repair.

19. Unbe-leaf-able: Can a frog fungus survive on a leaf?

Amy L. Nardini*, Amanda M. Starr
Longwood, University, Farmville, VA

Batrachochytrium dendrobatidis (Bd) infects amphibians and is prevalent within the United States. The fungus is transmitted through water systems through the direct contact of amphibians with uncleaned equipment, crayfish carapaces, and the keratin of snake skin and bird feet. During extended cold periods, such as Winter, frogs enter hibernation and are not available hosts for the fungus. It is unclear how long Bd remains viable on plant matter, a possible reservoir for Bd in the absence of its host. Within this study, the underside of detached tomato (*Allium cepa*) leaves on water agar were inoculated with the Bd fungus. The leaves were monitored and imaged every twenty-four hours for 7 days to check fungal ability to damage the plant. The leaves were also washed and DNA was extracted from the wash. The wash DNA was tested for the presence and concentration of Bd DNA using qPCR. The wash was dyed with propidium iodide and DAPI to assay Bd viability with fluorescence microscopy. The aim of this research study was to test the length of time for which the Bd fungus could survive on a leaf, outside the host amphibian. The data from this study may be used to further our understanding of Bd, and contribute to the conservation of biodiversity within the global frog population, which has been deeply and irreversibly impacted by the spread of Bd.

20. The role of the Type VII Secretion System and hemolytic pigment in Group B *Streptococcus* vaginal colonization

Audrey A. Neighmond*, Abigail E. Glenn, Brady L. Spencer
University of Virginia, Charlottesville, VA

Group B *Streptococcus* (GBS) colonizes the female genital tract (FGT) asymptomatically but is a leading infectious cause of infant mortality due to vertical transmission during childbirth. The GBS Type VII secretion system (T7SS) has four subtypes, which export different effectors, that result in different vaginal colonization outcomes (T7SS-I promotes GBS vaginal persistence, and T7SS-III impairs it). Recent work utilizing a T7SS-III representative strain, CNCTC 10/84, suggests that T7SS-III effectors promote inflammation, resulting in GBS clearance. CNCTC 10/84 also overproduces a toxic pigment known to promote GBS virulence. However, the contribution of this pigment to T7SS activity and inflammation in the FGT is not known. Here, we use Δ*essC* (lacking the ATPase that powers T7SS), Δ*cylE* (lacking the hemolytic pigment), and Δ*essC*Δ*cylE* mutants to examine the role of the T7SS-III and hemolytic pigment in vaginal colonization. We hypothesized that both the T7SS-III and pigment promote vaginal epithelial interactions and subsequent inflammation resulting in GBS clearance. Upon infection of vaginal epithelial cells, we observed CylE-dependent cytotoxicity as well as a CylE-dependent increase in GM-CSF but decrease in Il-1α production. In our murine model of in vivo vaginal colonization, we observed a trend for all mutants of increased GBS persistence and tissue burden compared to our parental strain. This indicates that for CNCTC 10/84, both the T7SS-III and pigment are related to faster clearance, potentially due to changes in inflammation and cytokine secretion. These findings highlight mechanisms by which the T7SS and pigment impact GBS vaginal colonization.

21. Determining how tryptone simulates Type IV pilus–mediated interstitial biofilm expansion

Livia Osbun, Zhaomin Yang
Virginia Tech, Blacksburg VA

The bacterial type IV pilus (T4P) is a critical virulence factor for surface sensing, motility, adhesion, biofilm formation, and host colonization in many clinically relevant pathogens. Because these processes contribute to the establishment and persistence of infection, the T4P represents an attractive target for antivirulence therapeutics that can disarm pathogens without applying the selective pressures associated with traditional antibiotics. Although T4P-targeting antivirulants are an active area of research, significant gaps remain in our understanding of the regulatory mechanisms governing T4P biogenesis and function, particularly the chemical signals that modulate T4P-dependent behaviors. Here, we identify tryptone as a positive regulator of T4P-mediated interstitial biofilm expansion in *Acinetobacter* and *Pseudomonas*. Tryptone is a common media supplement used primarily as a rich source of amino acids and peptides. Unexpectedly, free amino acids did not stimulate expansion, suggesting that the active component of tryptone may instead be bioactive peptides. We aim to isolate the active component of tryptone and determine the mechanism by which they stimulate T4P-

dependent expansion. Understanding the regulatory mechanisms governing the T4P and its associated functions may aid the development of more efficacious anti-T4P therapeutics.

22. Molecular and functional characterization of C-reactive protein under pathogenic conditions
Bernice Owusu, Soumi Ghosh
Virginia Tech, Blacksburg, VA

Pathogen infections remain a major global health challenge, and many pathogens are rapidly evolving new strategies to attack host cells. The innate immune system is the host's first line of defense. It uses powerful tools known as lectins, carbohydrate–binding proteins that recognize and bind microbial glycans and initiate pathogen destruction. Among these lectins is C-reactive protein (CRP), an evolutionarily conserved lectin from the pentraxin protein family that is widely used as a clinical biomarker of inflammation and infection. However, its function as a pathogen-recognition molecule remains poorly understood. CRP binds C1q on pathogens to activate the complement system. CRP is pentameric, composed of a PTX domain that mediates ligand recognition, and each subunit contains two calcium ions. It has long been believed that CRP functions in a calcium-dependent manner; however, this is largely based on its inflammatory functions. Using thermal shift and biolayer interferometry, our studies show that CRP retains structural stability and pathogen-binding activity under pathogenic conditions such as acidic pH and low or no calcium and can bind Chikungunya virus glycoprotein E1E2 under these conditions. Therefore, our findings challenge the claim that CRP is strictly calcium-dependent for maintaining stability and pathogen binding. We hypothesize that CRP may not be strictly calcium-dependent and can recognize and bind pathogen-associated ligands through calcium-independent interactions. Given that CRP's role as a pathogen- recognition molecule remains underexplored, these findings provide new insight into CRP's role in innate immunity and serve as a potential target for future therapeutic applications, while elucidating host-pathogen interactions.

23. Deletion and overexpression of CDR20291_1748 in *C. difficile*
Adriana Paci and Caitlin L. Williams
Batten University, Virginia Beach, VA

C. difficile is a pathogenic bacteria that typically induces illness after taking antibiotics, causing diarrhea, nausea, fever, and loss of appetite. It is considered an 'urgent threat' by the CDC as of 2019, killing at least 12,800 people in the US in 2017 alone. Current treatment options are limited to additional antibiotics, which leads to frequent recurrence of infection. Because this is considered a threat, we want a better understanding of how *C. difficile* operates. We are interested in the gene CDR20291_1748, a yet uncharacterized transcription factor. In a previous experiment, transposon mutants without the CDR20291_1748 gene were unable to survive long-term in a mouse infection model, suggesting an important role in persistence. The main goal of this project is to investigate the role of CDR20291_1748 in *C. difficile* biology by deleting

and overexpressing the gene and analyzing how this affects *C. difficile*. Deletion will be accomplished via homologous recombination of upstream and downstream arms constructed into plasmid pMSR0. Overexpression will be achieved using a pRPF vector with an aTc-inducible promoter. Once constructed, mutant strains will be assessed with growth assays, surface motility assays, swimming motility assays, and biofilm assays.

24. Effects of chytridiomycosis on the Arizona tiger salamander microbiome
Ayla Pada, Matthew Rowe, Megan Zerger, Howard H. Whiteman, and Kristin M. Yoshimura
James Madison University, Harrisonburg, VA

Chytridiomycosis has been a major driver of population decline worldwide, caused by fungal pathogens *Batrachochytrium dendrobatidis* (Bd) and *Batrachochytrium salamandrivorans* (Bsal). The skin microbiome plays a crucial role in amphibian health, including potential protection against chytridiomycosis. In this study, we investigate differences in the skin microbiome of *Ambystoma mavortium nebulosum* (Arizona Tiger Salamanders) with and without visible signs of chytridiomycosis. Salamanders were sampled from pond sites in Gunnison Valley within Gunnison County, Colorado, and swabbed for microbiome analysis. DNA was extracted using the QIAGEN DNeasy PowerSoil Kit, and 16S rRNA gene sequencing was performed on the Illumina MiSeq platform. Sequences were analyzed with QIIME 2 to be able to assess microbial diversity and taxonomic composition between Bd-positive and Bd-negative individuals. The research shows that the skin microbiomes of both groups were dominated by Proteobacteria, Bacteroidota, Cyanobacteria, Firmicutes, and Actinobacteria. However, distinct differences in microbial composition were detected: only two bacterial ASVs, both from Actinobacteria, were significantly enriched in Bd-positive samples. These findings suggest that infection status influences the skin microbial community and highlight candidate bacterial taxa that may provide antifungal protection. Continued re-sequencing and expanded sampling will provide further insight into microbial taxa associated with disease resistance and may inform conservation strategies for amphibians threatened by chytridiomycosis.

25. Environmental regulation of J774A.1 macrophage plasticity: Linking culture conditions, inflammatory function, and STAT3 signaling.
Kit Price*, David Freier
University of Lynchburg, Lynchburg, VA

Macrophages are immune cells that respond to changes in their environment and can take on different roles during inflammation and tissue repair. This project examines how environmental conditions and cell signaling affect the activation and function of J774A.1 mouse macrophages. The first part of this research focused on how culture conditions, including growth medium, glucose concentration, and whether cells were attached to a surface, affected J774A.1 macrophages. Cells were treated with different concentrations of lipopolysaccharide (LPS), and nitric oxide production was measured as an indicator of inflammatory activity. The results showed that changes in culture

conditions affected both macrophage morphology and their inflammatory response to LPS. These findings led to further questions about what signaling pathways may be responsible for these changes. The next phase of the project focuses on STAT3, a protein involved in regulating inflammation and macrophage function. J774A.1 macrophages will be examined under untreated conditions and after treatment with LPS or 8-bromo-cAMP to produce proinflammatory (M1) or tissue repair (M2) macrophages. Western blotting will be used to measure STAT3 and phosphorylated STAT3 (p-STAT3), which represents the activated form of the protein. STAT3 and p-STAT3 levels will then be compared between the treatment groups and evaluated alongside the parallel nitric oxide results. This research aims to better understand how the culture environment and signaling pathways work together to influence macrophage activation and may help identify a role for STAT3 in regulating inflammatory responses.

26. Screening the NCI diversity set VII for anti-intracellular *Shigella* activity
Steven Rolland, Tsuyoshi Miyake, Eric Devairakkam, Daniel Engel, Hervé Agaisse
University of Virginia, Charlottesville, VA

Multidrug-resistant *Shigella* poses a major global health threat, as resistance to first-line antibiotics and emerging resistance to newer drugs compromise treatment efficacy. Combined with *Shigella*'s high transmissibility and lack of a licensed vaccine, this highlights an urgent need for novel therapeutic strategies. We previously showed that inhibiting PIK3C3, a host kinase, in vivo reduced *Shigella* dissemination in the tissue. Compounds targeting the host to control shigella dissemination are a potential new therapeutic strategy, as the probability of generating resistant strains remains low.
To identify new host-targeted compounds that reduce *Shigella* dissemination without directly targeting *Shigella* or killing the host, we screened the National Cancer Institute diversity set VII (1,581 compounds) against *Shigella* infection foci formation in a monolayer of HT-29 colonic epithelial cells. We identified 19 compounds that decreased *Shigella* foci number or size, of which 13 showed direct antimicrobial activity. Importantly, six compounds affected infection without detectable activity against *Shigella* or HT-29 cells, making them promising candidates.
A characterization of these compounds was performed to better understand the molecular mechanisms of *Shigella* dissemination inhibition. While two compounds remain uncharacterized, two compounds were linked to metalloprotease inhibition and CDK2 inhibition. The two remaining compounds act in the purine and pyrimidine metabolism, impairing *Shigella* intracellular growth. Ongoing work will aim to better characterize the host pathways targeted by these compounds and define their mechanisms of action. Lead candidates will be tested in vivo using our infant shigellosis model, with the goal of developing novel therapeutics for antibiotic-resistant *Shigella* infection.

27. Microbial community responses to flood disturbances in a freshwater rock pool ecosystem
Gemma C. Sconyers, Benjamin H. Falls, Karen B. Barnard-Kubow, James Vonesh, Kristin M. Yoshimura
James Madison University, Harrisonburg, VA

Rock pools are small, ephemeral aquatic ecosystems that provide an excellent natural laboratory to study microbial dispersal and community succession. These habitats are intermittently flooded, which can connect individual rock pools to each other and the source river. Replacement of the water in the pools during the flood can flush out resident microbial communities while also introducing new microbes to the habitat, significantly changing the pool community composition, which could also lead to changes in community function. As storms and flooding events are predicted to get stronger and more frequent with the changing climate, it is important to understand how microbial communities respond to disturbance events to predict shifts in these aquatic ecosystems. 15 rock pools in Belle Isle (Richmond, VA) were sampled regularly for 16S rRNA gene amplicon sequencing following a flooding event in the summer of 2024. Results show boom and bust cycles of dominant taxa in all pools following the flood, particularly within the Firmicutes. However, some rarer taxa appeared in a single pool over the timeseries, indicating that seeding of the pools is a stochastic process that could lead to periodic shifts in microbial community in a single pool.

28. Finding the binding sites of transcription factor CDR20291_1748 from *C. difficile*
Cooper Seidner, Lieselotte J. Hazelwood, Caitlin L. Williams
Batten University, Virginia Beach, VA

Clostridioides difficile is a pathogenic bacteria that typically induces illness after taking antibiotics, causing diarrhea, nausea, fever, and loss of appetite. It is considered an ‘urgent threat' by the AR Threat Report as of 2019, killing ~13,000 people every year in the US alone. Many factors involved in *C. difficile* biology have been characterized, Although the functions of many genes remain poorly understood. CDR20291_1748 is a transcription factor that has not yet been characterized. In a transposon mutant screening experiment, mutants lacking functional CDR20291_1748 were less able to colonize mice for multiple days, suggesting a potential role in regulating gene expression that is important for long-term infections. CDR20291_1748 is an OmpR-family transcription factor with a conserved receiver domain expected to enable dimerization. The goal of this study is to find out if CDR20291_1748 can form a homodimer and bind to its own promoter, and if so, where precisely the protein binds. We will use DNA pull-down assays to assess how these proteins bind to various DNA fragments. SDS-PAGE and western blotting will be used to determine if CDR20291_1748 is retained by the various DNA fragments. Our initial studies involve the likely promoter region for CDR20291_1748 divided into two overlapping fragments. If binding is observed, smaller fragments will be used to narrow down the binding region. Determining whether CDR20291_1748 associates with these DNA fragments will provide insight into its function as a transcriptional regulator, and aid in future research identifying its regulatory targets.

29. Investigating the mode of action of tuspetinib as a Type IV pilus inhibitor
Tori M. Shimozono*, Zhaomin Yang
Virginia Tech, Blacksburg, VA

The bacterial type IV pilus (T4P) is a dynamic, hair-like, surface appendage that is critical for the initiation and dissemination of eye infections caused by the ESKAPE pathogen *Pseudomonas aeruginosa*. Its critical role in *P. aeruginosa* pathogenesis makes the T4P an attractive target for the development of small molecule therapeutics as potential preventatives and/or treatments for these eye infections.
We previously identified and validated tuspetinib as an inhibitor of T4P functions in *P. aeruginosa*. However, the mode of action (MOA) of tuspetinib as a T4P inhibitor, has yet to be established. To elucidate its MOA, we first assessed the effects of tuspetinib on T4P dynamics. We found that tuspetinib inhibited T4P-mediated phage adsorption and reduced surface piliation levels without altering total PilA protein levels, indicating inhibition of T4P assembly. Because tuspetinib targets the ATP-binding pocket of select human kinases to treat myeloid leukemia, we hypothesized it might similarly target the T4P assembly ATPase, PilB. Computational molecular docking demonstrated favorable predicted binding of tuspetinib within the ATP-binding pocket of modeled *P. aeruginosa* PilB (PaPilB). In vitro enzymatic assays using purified protein showed that tuspetinib directly inhibits PaPilB ATPase activity at low micromolar concentrations.
Collectively, these results support a working hypothesis that tuspetinib disrupts T4P dynamics by inhibiting the assembly ATPase PilB. Future work will assess candidate PaPilB point mutations in vitro and in vivo to validate target specificity, alongside evaluating therapeutic efficacy in human cell culture models to advance a discovery-to-preclinical pipeline for identifying and assessing T4P inhibitors against *P. aeruginosa*.

30. Up close with chytrid and plastic: SEM and physiological evidence of microplastic efects on *Batrachochytrium dendrobatidis*
Jackson J. Geeslin*, Emma M. Snead*, Amanda M. Starr
Longwood University, Farmville, VA

Amphibian populations are declining due to multiple environmental stressors including pollution and infectious pathogens. The most devastating pathogen is *Batrachochytrium dendrobatidis* (Bd), a fungus that infects amphibian skin disrupting electrolyte balance and leading to cardiac arrest. Meanwhile, microplastics (<5 millimeters) are environmentally widespread pollutants. Although microplastics are known to alter amphibian development and physiology, their effects on pathogens (including Bd) remain poorly understood. This study investigates how microplastics influence Bd growth and virulence. Two common polymers, polyethylene and polystyrene, were introduced into two strains of Bd culture. Growth was quantified using hemocytometer counts and optical density measurements. Polyethylene increased zoospore production and fungal growth, whereas polystyrene had minimal impact. The fungus appeared to integrate the microplastics into its biofilm-like structures as observed through Scanning Electron Microscopy. Microplastics may also alter Bd virulence, as assessed through enzyme activity and adhesion proteins important for host infection. After culturing the zoospore and zoosporangium stages with API ZYM test strips, zoospores produced more enzymes than zoosporangia cells in the presence of plastics, but plastics decreased overall enzyme production. After allowing zoospores time to adhere to skin-based molecules, neither plastic interfered with Bd attachment as measured with the XTT assay, but may provide a competitive location to attach. Future work will test plastic

size, antifungal response, and conduct gene expression analysis. By integrating environmental biology and genetics, this research aims to clarify how pollution influences pathogen behavior, offering insight into the complex interplay between contaminants and disease that threatens amphibian survival.

31. The first characterization of *Selenomonas sputigena* ATCC 35185 Δ*fliF* mutant and comparison to other *Selenomonas* species

Margaret E. J. Stiker, Colin G. Hawkes, Daniel P. Miller
Virginia Commonwealth University, Richmond, VA

The genus *Selenomonas* contains sixteen cultivable species, nine of which are primarily associated with the human oral cavity and upper respiratory tract, including *S.sputigena*, *S.noxia*, *S.flueggei*, *S.infelix*, *S.artemidis*, *S.dianae*, *S.felix*, *S.timonae*, and *S.massiliensis*. *Selenomonas sputigena* is an obligate anaerobic, Gram-negative oral pathobiont that transitions from a low-abundance commensal organism to a structural component of oral biofilms associated with early childhood caries and periodontitis. Although the flagellar tuft characteristic of the genus is well documented, its contribution to *S. sputigena* growth, motility, and adherence remains poorly characterized. Prolonged observation of *S. sputigena* ATCC 35185 cultures with reduced motility prompted genomic analysis of these isolates. An incidental knockdown of selsp_1702, encoding the flagellar M-ring protein FliF, was identified. The resulting mutant, *S.sputigena* ATCC 35185ΔFliF, was compared with wild-type *S. sputigena* and other *Selenomonas* species to assess growth, flagellar morphology, motility, and adherence. Both strains grew most robustly in TYGVS medium, with no growth defect detected. Transmission electron microscopy demonstrated a complete absence of flagella in *S.sputigena* ATCC 35185ΔFliF, whereas wild-type *S.sputigena* displayed the multiple flagella characteristic of the genus. Consistent with the absence of flagella, the mutant exhibited decreased motility and slightly increased adherence relative to the wild type. These findings provide the first phenotypic characterization of *S.sputigena* ATCC 35185ΔFliF and associate disruption of selsp_1702 with loss of flagellar structures, decreased motility, and increased adherence without a detectable growth defect. Further characterization may clarify how FliF contributes to motile and surface-associated states in *S.sputigena* and its biofilm behavior within dysbiotic oral communities.