# 2021 Program Schedule

## On-Line Virtual Gathering, April 10 2021

| Saturday, April 10, 2021 | | |
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| 9:30am-9:45 | **Welcome Statement**<br>D.J. Ferguson, OBASM President<br>Room: Keynote Lecture Hall | |
| 9:45-11:45 | **Concurrent Sessions** | |
| | **Session 1: Invited Speakers and Podium Presentations**<br>Moderator: Harry Kestler<br>Room: Keynote Lecture Hall | |
| | 9:45-10:15 | **Chad Rappleye**, Invited speaker<br>The Ohio State University<br>**"Stealth, survival, and proliferation of the intracellular pathogen *Histoplasma capsulatum*"** |
| | 10:15-10:30 | **David D. Sarpong**, Student Podium Presentation<br>Molecular and Cellular Biology Program, Ohio University<br>**"Characterization of a putative toxin-antitoxin locus in *Shigella flexneri*"** |
| | 10:30-10:45 | **Anika Nawar Choudhury**, Student Podium Presentation<br>Department of Biological Sciences, Bowling Green State University<br>**"Utilizing Bacteriophage to Evolve Antibiotic Susceptibility in Multidrug-Resistant *Pseudomonas aeruginosa*"** |
| | 10:45-11:00 | **Break** |
| | 11:00-11:30 | **Amanda Panfil**, Invited speaker<br>The Ohio State University<br>**"Genetic and epigenetic regulation of the oncogenic human retrovirus HTLV-1"** |
| | 11:30-11:45 | **Audra Fullen**, Student Podium Presentation<br>Department of Microbial Infection and Immunity, The Ohio State University Wexner Medical Center<br>**"*Bordetella pertussis* Exopolysaccharide Bps Provides Resistance to Antimicrobial Peptides by Functioning Both as a Protective Surface Shield and as a Decoy"** |

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| **Saturday, April 10, 2021** | | |
| | **Session 2: Invited Speakers and Podium Presentations**<br>Moderator: Laura Saltman<br>Room: Lecture Hall 2 | |
| | 9:45-<br>10:15 | **Chris Ward**, Invited speaker<br>Bowling Green State University<br>**“Revealing alga-microbiome interactions critical for advancing mass algal cultivation”** |
| | 10:15-<br>10:30 | **Fiona C. Harrigian**, Student Podium Presentation<br>Bowling Green State University;<br>**"Elucidating influences on aphelid parasitism on several strains of green alga *Scenedesmus*"** |
| | 10:30-<br>10:45 | **Rachel Crowl**, Student Podium Presentation<br>Bowling Green State University<br>**"Identifying gene regions that produce antagonistic factors against drug resistant pathogens"** |
| | 10:45-<br>11:00 | **Break** |
| | 11:00-<br>11:30 | **Jyl Matson**, Invited speaker<br>University of Toledo<br>**"*Vibrio cholerae* responses to antimicrobial peptide exposure"** |
| | 11:30-<br>11:45 | **Abigail S. Jarosz**, Student Podium Presentation<br>Department of Biological Sciences, Bowling Green State University<br>**"Expression of a young endogenous gammaretroviral lineage in canine tissues"** |
| 11:45-<br>12:45pm | **Noontime Activities:** | |
| | | **Lunch Break**<br>**OBASM Business Meeting - Breakout Room** |
| 12:45-2:15 | **Poster Session**<br>Coordinator: Chet Cooper<br>Room: Poster Hall | |
| 2:15-3:15 | **Session 3: Invited Speakers and Podium Presentations**<br>Moderator: D.J. Ferguson<br>Room: Keynote Lecture Hall | |
| | 2:15-<br>2:45 | **Justin North**, Invited speaker<br>The Ohio State University |

| Saturday, April 10, 2021 | | |
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| | | **“Bob Tabita: lessons from a pioneer in bacterial carbon, nitrogen, and sulfur metabolism”** |
| | 2:45-3:15 | **Amy Miller**, Invited speaker<br>University of Cincinnati Blue Ash College<br>**"Using concept inventories as a means of assessing learning and evaluating instruction"** |
| 3:15-3:30 | **Break** | |
| 3:30-4:30 | **ASM Distinguished Lecturer**<br>Room: Keynote Lecture Hall<br>**Jeffrey Gralnick**<br>University of Minnesota - Twin Cities<br>**"Electromicrobiology: How bacteria make electricity and how bacteria eat electricity"** | |
| 4:30-4:45 | **Poster and Podium Awards**<br>Presented by D.J. Ferguson<br>Room: Keynote Lecture Hall<br>**Words of thanks** | |

# 2021 Abstracts

## Adenike, Shittu

### PFAS toxicity studies of human microbiome bacteria

**Adenike Shittu*** and Jill Zeilstra-Ryalls
Bowling Green State University

Per- and polyfluoroalkyl substances (PFAS) are anthropogenic chemical compounds that are persistent in the environment, and resistant to biological degradation. Concerns are rising based on the emergence of epidemiological data that indicates a link between human exposure and certain diseases. This study focuses on the two most widely used PFAS, perfluorooctanoic acid (PFOA) and perfluorooctanesulfonic acid (PFOS), as well as and one of the newer PFAS, hexafluoropropylene oxide, which is also known by its Chemours (DuPont) trademark name GenX. GenX was developed as a safer replacement for PFOA due to its shorter carbon chain length. We evaluated sensitivities of two human gut bacteria using zone of inhibition assays. We found that *Escherchia coli* is insensitive to all three of these PFAS, whereas *Collinsella aerofaciens* is sensitive to all three. The discovery of this differential sensitivity to PFAS among human microbiome species is important, because it suggests that human ingestion of these bio-accumulative chemicals could lead to an imbalance in the gut microbiome. Our further investigations were directed toward *C. aerofaciens*, which is the most abundant obligate anaerobe actinobacterium in the gut of healthy humans. We have successfully isolated mutants that are reduced in in their sensitivities to PFAS. By sequencing their genomes, we expect that DNA sequence differences between the mutant and wild type strain will direct us to the candidate molecular targets of the chemicals. These candidate targets will be tested by complementation analyses. If confirmed, hypotheses regarding the mechanism of PFAS toxicity can then be formulated and investigated.

## Babatunde, Oluwaseun

### Exploring the potential of near-infrared light stimulated intratumoral growth of a photosynthetic bacterial vector for photodynamic therapy

**Oluwaseun O. Babatunde*[1],** Adenike Shittu[1], and Jill H. Zeilstra-Ryalls[1]
[1]Bowling Green State University

Using bacteria in cancer therapies is an emerging area of research. Certain bacteria can target tumors, and therapy can involve either the direct (colonize, invade, and deplete metabolic nutrients) or indirect action (deliver a therapeutic payload or “uncloak”

the tumor to the immune system) of the bacteria  However, many of the best-suited bacterial species are pathogenic and require extensive genetic engineering to reduce or eliminate their pathogenicity before they can be used therapeutically.  The facultative anoxygenic photoautotroph *Rhodobacter sphaeroides* is non-pathogenic, and has been shown to target tumors.  We have been investigating its use as a vector for delivering 5-aminolevulinic acid (ALA) to tumors, where it functions as a pro-drug in photodynamic therapies.  ALA is a precursor in the formation of heme, and elevated concentrations delivered to tumor cells leads to overproduction of all products in the heme biosynthetic pathway, including precursor tetrapyrroles,.  In the presence of oxygen and therapeutic wavelengths of light, these molecules generate reactive oxygen species that destroy the tumor cells.  *R. sphaeroides* naturally produces copious amounts of ALA for heme and bacteriochlorophyll synthesis needed to support photosynthetic growth.  We have already shown it is possible to engineer these bacteria to produce and excrete ALA in amounts that are suitable for photodynamic therapy.  We now completed a survey of wild type strains to identify which one grows best phototrophically under intratumoral conditions.  By disrupting its genes that code for ALA synthase enzymes we created a mutant that relies upon exogenous ALA for growth.  We then assessed its ability to tolerate the presence of high concentrations of ALA under phototrophic conditions.  This was necessary in order to determine whether or not the mutant bacteria are suitable for the addition of a light-responsive ALA synthase gene that encodes a hyperactive enzyme, which will thereby generate the final therapeutic bacterium.

## Boss, Deeanna

### The Broken Water System in Sandusky Bay: cHABs

Jessica Anderson, **Deeanna Boss*,** Carly Conrad, Jose Modic, Ashlynn Reising, Nicole Wiltrout, Michelle Neudeck, and Kathryn Durham

Sandusky Bay is part of the Great Lakes, of which the lakes provide 20% of the world's freshwater. *Planktothrix agardhii* is the source of cyanobacterial Harmful Algal Blooms (cHAB) in the Bay. The production of the hepatotoxin, microcystin, by cHABs is the primary concern. A multitude of factors affect the proliferation of *Planktothrix* including precipitation, application of various fertilizers, and light availability. The majority of these factors are due to the Bay being an agricultural watershed. *Planktothrix* is non-diazotrophic. As nitrogen levels are replete, it stores intracellular nitrogen in the form of cyanophycin. During N depletion due to droughts, it scavenges N from cyanophycin and the phycobilisome. Tolerance to nitrogen deprivation and the rate at which *Planktothrix* recovers from nitrogen deprivation will be tested in the lab. RNA extraction and RT-PCR with primers will be performed to detect the presence of the genes: *cphB, nbl*A, and *cph*A. *cph*B encodes for cyanophycinase which causes the degradation of cyanophycin. *nbl*A encodes for the degradation of phycobilisomes for use as nutrients during nitrogen deprivation. *cph*A encodes for cyanophycin synthetase, which facilitates the production of cyanophycin. Similarly, *Planktothrix* relies on phosphorus as a macronutrient source. It is capable of utilizing both organic and inorganic phosphorus through alkaline

phosphatases and natural uptake of phosphorus from the environment. It stores P as polyphosphate in order to survive during scarce conditions. Additionally, an experiment will be conducted depriving Planktothrix of P then extracting RNA. The presence of transcripts for the genes encoding *ppk* and *ppx* will be tested. *ppk* encodes for polyphosphate kinase, an enzyme that facilitates polyphosphate synthesis. *ppx* encodes for the enzyme exopolyphosphatase, which catalyzes the degradation of polyphosphate for use by *Planktothrix* in nutrient deprived environments.

## Brechting, Peter J.

### Disruption of peroxisome function reduces iron scavenging and virulence in the human fungal pathogen *Histoplasma capsulatum*

**Peter J. Brechting***, Qian Shen, Matt J. Beucler, and Chad A. Rappleye
Department of Microbiology, Ohio State University

The fungal pathogen *Histoplasma capsulatum* causes pulmonary and systemic disease among both immunocompromised and immunocompetent individuals. During infection, *H*. *capsulatum* is found almost exclusively inside the phagosomal compartment of macrophages. A forward genetic screen for *H. capsulatum* mutants which were unable to replicate in macrophages identified four peroxisome-associated genes (*PEX5, PEX10, PEX11, PEX33*) suggesting an important role for fungal peroxisomes in *H. capsulatum* virulence. Peroxisomes are organelles of nearly all eukaryotic organisms, and house enzymes for beta-oxidation and glyoxylate metabolism. However, β-oxidation and glyoxylate pathways as well as the peroxisomal catalase are dispensable for *H. capsulatum* infection, indicating a novel function for peroxisomes in virulence. Pex5, Pex10, and Pex33 comprise the import machinery for peroxisomal matrix proteins, which are targeted to the organelle by a peroxisomal targeting sequence (PTS1). Accordingly, a PTS1-tagged GFP fails to localize to peroxisomes in *pex5*, *pex10*, and *pex33* mutants suggesting that PTS1-containing peroxisome protein(s) is/are required for pathogenesis. A bioinformatic search of the Histoplasma genome for PTS1-containing proteins identified enzymes involved in siderophore biosynthesis, which *H. capsulatum* uses to acquire iron in scarce conditions in the host. Consistent with this, low iron conditions impaired growth of the *pex5, pex10* and *pex33* mutants similar to that of a siderophore knockdown strain, and addition of exogenous siderophores restored growth of pex5, pex10, and pex33 mutants. Together, these data show that pathogenesis-enabling siderophore production relies upon peroxisomes. However, the *pex11* mutant showed near wild-type growth in low iron conditions, and peroxisome targeting of the PTS1-tagged GFP was normal, yet pex11 mutants still have attenuated virulence. These results indicate that the virulence function of the peroxisome is not limited to siderophore synthesis. To identify this function important for virulence, we are defining the peroxisome proteome through proximity labeling and capture of peroxisome proteins and interrogating the diverse functions represented.

## Choudhury, Anika Nawar

### Utilizing Bacteriophage to Evolve Antibiotic Susceptibility in Multidrug-Resistant *Pseudomonas aeruginosa*

**Anika Nawar Choudhury***, Hans Wildschutte, Kaylee Wilburn, Ray A. Larsen
Bowling Green State University Department of Biological Sciences

Due to low-profit margins, pharmaceutical companies have stopped research on antibiotic compound discovery. Therefore, Antibiotic discovery is at an all-time low. In addition, pathogens are becoming resistant to all currently used antibiotics. Therefore, multi-drug resistant (MDR) bacterial infections are becoming more difficult to treat. Cystic fibrosis (CF) patients are immunocompromised and susceptible to MDR infections. CF is a genetically inherited disease that inhibits or decreases ion transport across mucus membranes. This results in a buildup of mucus that leads to chronic bacterial lung infections. A common pathogen that infects CF patients is *Pseudomonas aeruginosa* which dominates the lung over time. A possible alternative treatment against MDR *P. aeruginosa* is bacteriophage therapy which has several advantages compared to antibiotics. First, phage therapy exhibits minimal side effects because phage are highly host-specific and do not inhibit other bacteria that are part of the human microbiome. Second, phage replicate itself exponentially when killing its host; and third, phage can be applied directly to the site of infection. However, like antibiotics, bacteria can evolve resistance to phage. To circumvent the problem of evolved phage and drug resistance, tradeoff effects may promote opportunities for new methods to treat MDR infections. We hypothesize that the effects of antibiotics can be restored when combined with phage therapy. To test our hypothesis, bacteriophage were isolated on MDR *P. aeruginosa strains and wereused to perform the trade-offexperiment*to select for antibiotic susceptibility. For pathogens that evolve phage resistance, the minimal inhibitory concentration was shown to decrease with some antibiotics. Comparing pre and post phage pathogen genomes will give insight into the mechanism involved in the observed tradeoff genotypes.

## Crowl, Rachel

### Identifying gene regions that produce antagonistic factors against drug resistant pathogens

**Rachel Crowl*** and Hans Wildschutte
Bowling Green State University

Multidrug resistant (MDR) bacterial infections are currently estimated to result in 700,000 deaths per year worldwide. By the year 2050, it is predicted that MDR infections will account for 10 million deaths and cost 100 trillion dollars per year, surpassing all current leading causes of death; including cancer. Additionally, no major

pharmaceutical companies are pursuing novel antibiotic discovery which further compounds the MDR crisis. MDR pathogens not only cause dangerous infections in humans, but have evolved many different mechanisms of resistance to evade all currently available antibiotics. We hypothesize that environmental *Pseudomonas* isolates that inhibit many of these MDR pathogens will produce novel antibiotic compounds with different bacterial targets from currently used drugs. To test this hypothesis, 384 strains were isolated from the Maumee River in Northwest Ohio, as polluted environments provide the ideal habitat for bacteria to develop new ways to compete for nutrients. To test for competitive activity, the environmental isolates were competed against MDR *Pseudomonas aeruginosa* and *Burkholderia spp.* using the Burkholder plate assays. Of 384 environmental isolates, 232 were able to inhibit the growth of the 28 pathogens tested. To identify genes involved in antagonistic activity, we optimized transposon mutagenesis to identify loss of inhibition (LOI) mutants, of which eleven were found. Arbitrary PCR with the LOI mutants and full genome sequencing of the wild type strains was used to identify the biosynthetic gene clusters involved in antibiotic production, revealing a possible lipopeptide antimicrobial as well as potential sideromycin activity in two different environmental isolates. This research aims to facilitate the discovery of antagonistic compounds that inhibit MDR pathogens through novel targets by the continued identification of distinct gene clusters.

## Cui, Jerry J.

### The Landscape of Phosphonic Acid Natural Product Biosynthesis in Actinobacteria

**Jerry J. Cui**,[*1] Chase Kayrouz,[1] and Kou-San Ju[1-4]
[1]Department of Microbiology, The Ohio State University
[2]Division of Medicinal Chemistry and Pharmacognosy, The Ohio State University
[3]Center for Applied Plant Sciences, The Ohio State University
[4]Infectious Diseases Institute, The Ohio State University

Phosphonic acid natural products are promising compounds for drug discovery, as their C-P bonds enable mimicry of phosphate ester and carboxylic acid compounds essential to metabolism. These natural products have found commercial success as antibiotics, herbicides, and antiviral agents, and recent studies suggest bacteria encode for hundreds of unknown phosphonic acids. While genomic advancements have facilitated discovery of new phosphonic acids by targeting strains encoding unique biosynthetic gene clusters (BGCs), thorough understanding of the occurrence, abundance, and diversity of these pathways is required for directed discovery of desired compounds. Here we describe the biosynthetic landscape of phosphonic acids across Actinobacteria, a vital source of clinically used medications. Analysis of all Actinobacterial genomes (>30,000) within NCBI identified 548 strains containing >1 gene for phosphoenolpyruvate mutase (PepM), which catalyzes the essential first step in all known phosphonic acid biosynthesis: rearranging phosphoenolpyruvate to phosphonopyruvate. As PepM similarity correlates with shared BGC content, that metric

was applied to form gene cluster groups (GCGs). Within GCGs, *pepM* genomic neighborhoods were analyzed for synteny, operonic structure, and protein function, resulting in 592 discrete BGCs. Phosphonic acid biosynthesis is driven by the second step of the biosynthetic pathway and is categorized accordingly. Organismal phylogeny revealed that the second-step enzymes are well-conserved within genera (non-*Streptomycetales*) or species (*Streptomycetales*), but do not form monophyletic groups, suggesting that horizontal gene transfer or independent evolution underlies their taxonomic distribution. The majority (>85%) of the BGCs are uncharacterized and potentially encode phosphonic acid peptides, polyketides, and other small molecules. Interestingly, several GCGs clearly contain multiple distinct BGCs, whereas other separate GCGs contained near-identical BGCs, indicating that PepM similarity is an imperfect metric and adjustments based on taxonomy, biosynthetic branchpoints, or functional context are needed. These findings broaden our knowledge of and enable further construction of a greater framework for phosphonic acid biosynthesis.

## D'Silva, Susanna

### High Light Response and Recovery in the Extremophile *Chlamydomonas* sp. UWO 241

**Susanna D'Silva*[1]**, Devon Popson[1], Rachael Morgan-Kiss[1]
[1]Miami University

The Antarctic algae *Chlamydomonas* sp. UWO 241 (UWO) grows in harsh conditions in its native environment: Lake Bonney, Antarctica. UWO 241 is found deep in the water column, where the light levels are permanent shade (<50 µmol $m^{-2}s^{-1}$), temperatures are permanently cold (~2-5$^{o}$C), and salinity is high (~700 mM NaCl). The mechanisms UWO employs to deal with extreme environmental stress have been of interest to researchers. In this study, we asked whether UWO has the capacity to grow and respond to high light stress. We demonstrate that UWO can grow under light levels up to 250 µmol $m^{-2}s^{-1}$, which is >5-fold native conditions. High light-acclimated cultures exhibited a longer lag phase and higher levels of nonphotochemical quenching (NPQ) but ultimately reached a cell density and photosynthetic efficiency similar to control cultures. Next, we asked whether pre-acclimation to high light conferred increased resistance to high light-induced photoinhibition (1000 µmol $m^{-2}s^{-1}$). Relative to control-grown cultures, high light-acclimated cells exhibited higher levels of Photosystem I-driven cyclic electron flow and NPQ during photoinhibition and recovery phases. Both control- and high light-acclimated cells exhibited comparable PS II activity recovery post-photoinhibition. In conclusion, UWO demonstrates growth and resistance to short- and long-term light stress at levels much higher than that found in its native environment. Future studies can also look at how UWO response to multiple stressors, and how long-term adaptation to these conditions can affect its photoprotective abilities in the short term.

## Fullen, Audra R

## *Bordetella pertussis* Exopolysaccharide Bps Provides Resistance to Antimicrobial Peptides by Functioning Both as a Protective Surface Shield and as a Decoy

**Audra R Fullen**[1], Jessica L Gutierrez-Ferman[1], Piyanka Sivarajah[2], Deepa Raju[2], P Lynne Howell[2,3] and Rajendar Deora[1]
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The Ohio State University Wexner Medical Center, Columbus, OH, USA
[2]Program in Molecular Medicine, The Hospital for Sick Children, Toronto, ON, Canada.
[3]Department of Biochemistry, University of Toronto, Toronto, ON, Canada

The incidence of whooping cough, caused by the Gram-negative bacterium *Bordetella pertussis* (*Bp*), is increasing despite high vaccination coverage. The current acellular pertussis vaccines, while protective against severe disease, remain ineffective against nasopharyngeal colonization, carriage, and transmission. *Bordetella* polysaccharide (Bps), encoded by the *bpsABCD* locus, is a poly-β-1,6-*N*-acetyl-D-glucosamine exopolysaccharide that is important for biofilm formation and colonization of the murine respiratory tract. In this work, we tested the hypothesis that Bps promotes respiratory tract colonization and persistence by resisting killing by antimicrobial peptides (AMPs), a critical component of host innate immunity. Compared to the wildtype laboratory-adapted strain Bp536, the D*bpsABCD* mutant strain was more susceptible to killing by cationic AMPs LL-37 and polymyxin B. Furthermore, treatment of Bp536 with the glycoside hydrolase Dispersin B resulted in enhanced AMP-mediated killing. Ectopic production of Bps in *E. coli*, independent of other *Bordetella* factors, was sufficient to confer resistance to AMPs. Additionally, we found that, in addition to producing Bps on the cell-surface, Bp536 also secreted Bps in the culture supernatant. Addition of both supernatants containing Bps and purified Bps restored the resistance of the D*bpsABCD* strain to wildtype levels. Both cell-associated Bps and purified Bps bound AMPs. We propose that Bps can neutralize AMP activity by either trapping them or by reducing the amount of soluble peptides that can reach the bacterial cells. To our knowledge, our results document for the first time the dual activity of a bacterial polysaccharide in promoting AMP resistance by functioning both as a protective shield on the bacterial surface and as a decoy.

## Gluck

The study of the Brain-Gut-Microbiome axis is an exciting avenue of research. The microbiome has been shown to modulate behaviors and physiology associated with activity and movement including Parkinson's Disease, locomotion, and sleep. There are competing studies which argue whether or not the microbiome modulates the sleep homeostasis response. Sleep homeostasis in response to sleep deprivation in *Drosophila melanogaster* is regulated by *nur*, a gene which produces the secreted peptide nemuri. In order to test the effect of the microbiome on the sleep homeostasis response, wild-type, dechorionated, streptomycin-treated, and *L. brevis*-treated flies were sleep deprived. Relative expression of *nur* was compared across treatment groups

to determine if one of the key regulatory mechanisms of sleep homeostasis is affected by microbial composition. Semiquantitative gel electrophoresis PCR revealed that differences in microbial composition modulated baseline sleep levels and rebound sleep, specifically in dechorionated flies. Differences in relative expression level of *nur* could not be detected between experimental groups, indicating that there is a relatively small difference in expression of *nur* induced by sleep deprivation. Microbial composition had no observable effect on expression of *nur.* For more precise differentiation between experimental groups, future studies should compare exact levels of transcription. Much more work needs to be done to probe the mechanisms through which the microbiome can regulate the sleep homeostasis response.

## Hamilton, Lyndsy

### The N-terminal portion of the CCR5 Protein as a Potential Gene Therapy for AIDS

**Lyndsy Hamilton.*[1,3]**, Brielle McCarthy[1], Raquel Dauch B.A.[1.2], Gary Dodson B.A.[1], and Harry Kestler[1]
[1] Lorain County Community College, Elyria, Ohio, [2] MetroHealth Medical Center, Cleveland, Ohio, [3]CCP Keystone High School, LaGrange, Ohio

The AIDS pandemic in humans has persisted for over 40 years. The causative agent, Human Immunodeficiency Virus (HIV) is a virus that degrades the immune system and provides opportunity for further infections. For HIV to infect a cell, it first binds the primary receptor on the human T-cell, CD4, to one of two secondary receptors, CCR5 or CXCR4. After HIV glycoprotein 120 successfully binds, it uses glycoprotein 41 to pierce the cell membrane, allowing for insertion of its genetic material; creating an infected cell.

There is a mutation known to affect HIV infectivity. Delta32 is a deletion mutation of 32 base pairs of the *ccr5* human gene sequence. The 32 base deletion results in a frameshift ending the protein sequence after 184 amino acids. The truncated *CCR*5 receptor effects surface localization. The mutation confers resistance to HIV in homozygous individuals. The truncated protein may prevent full length CCR5 and CXCR4 from surface expression.

PCR primers that detect the deleted portion of the *ccr5* identified, subject GD1, as being heterozygous for the *ccr5* delta32 allele. After confirmation, PCR amplification from the subject's DNA was completed using primers that circumscribe the entire reading frame. The primers have restriction endonuclease recognition sites BamHI and XhoI allowing for the purification of both wild type and delta32 alleles. The products were ligated into the mammalian/prokaryotic shuttle vector, pLenti puro HA-ubiquitin. The plasmid DNA was analyzed by PCR amplification to confirm the presence of the *ccr*5 alleles.

The next phase in this research consists of transfecting the pLenti*/ccr5delta32* vector into HEK293FT, a lentiviral packaging cell line. The resulting lentiviral products will then be used to create stable and transient transductants into H9 and other Lymphoid cells. The transductants will tested for HIV infectability and *ccr*5 expression.

## Harper, Elizabeth D.

### Mutations in MDR *Pseudomonas aeruginosa* after exposure to environmental bacteriophage

**Elizabeth D. Harper**[*1], R.A. Larsen[1], Hans K. Wildschutte[1]
[1]Bowling Green State University

Fatalities from multidrug-resistant bacterial infections are increasing due to the spread of antibiotic resistance mechanisms via horizontal gene transfer through multiple pathogenic bacterial populations. To prevent a return to pre-antibiotic era fatality rates, new treatments are needed. *Pseudomonas aeruginosa* is responsible for a significant proportion of drug resistant bacterial infections, particularly in patients with Cystic Fibrosis. Bacteriophage therapy has been shown to successfully eliminate chronic *P. aeruginosa* infections in several Cystic Fibrosis patients. Evidence suggests that in *P. aeruginosa* trade-offs occur between resistance to infection by bacteriophage and sensitivity to antibiotics. This research investigates the genetic changes that occur in multidrug-resistant *P. aeruginosa* exhibiting phenotypic trade-offs after exposure to a bacteriophage. A population of *P. aeruginosa* is infected multiple times with a single environmental bacteriophage in liquid media. A double agar overlay assay is used to screen for qualitative changes in the ability of the bacteriophage to infect the host bacterium after each round of infection. Changes in concentration of antibiotic needed to kill the *P. aeruginosa* before and after exposure to bacteriophage is evaluated by a minimum inhibitory concentration assay in liquid media. Whole genome comparison of *P. aeruginosa* before and after exposure to bacteriophage is used to identify genetic loci that have mutated. The expected outcome of this project is the identification of bacterial genes or regulatory elements that are under selection when exposed to bacteriophage and that confer a change in antibiotic sensitivity.

## Harrigian, Fiona C.

### Elucidating influences on aphelid parasitism on several strains of green alga *Scenedesmus*

**Fiona C. Harrigian**[*1], Kensuke Seto[2], Timothy Y. James[2], Christopher S. Ward[1], Kristina Rolison[3], Ty Samo[3]
[1]Bowling Green State University; [2]University of Michigan; [3]Lawrence Livermore National Laboratory

Chytrid fungi, despite their prevalence and virulence in algal cultivation raceways, remain understudied and their interactions with algae unexplored. In order to determine how algal strain and culture conditions may alter chytrid parasitism on algae, an obligate aphelid parasite and several *Scenedesmus* strains (*S. obliquus*, *S. dimorphus* and *S. armatus* species) were co-cultured under various conditions. Despite being isolated and maintained on *Scenedesmus obliquus* UTEX 393, the aphelid can infect a number of algal strains within the *Scenedesmus* genus. It achieves greater levels of infection in *Scenedesmus obliquus* UTEX 393 cultures than in *Scenedesmus dimorphus* UTEX 1237 cultures. In lower light conditions, fewer *S. dimorphus* cells were infected but light did not alter prevalence of infection in *S. obliquus* cells. Higher temperature (30°C compared to 25°C) increased prevalence of infection in both *S. dimorphus* and *S. obliquus* cultures. In continuing work, more conditions will be tested and combined to gain a greater knowledge of factors governing chytrid-algal interactions in biofuel ponds and other aquatic ecosystems. Not only does this research provide important data for biofuel strain selection and efficacy of ecology-based pest mitigation strategies, but it will also shed light on aspects of aquatic carbon cycling, trophic interactions, and aquatic parasitology and disease ecology.

## Hunt, Landon

### Determining the Potential of the N-Terminus of CCR5 to Downmodulate CXCR4 Cell Surface Expression

**Landon Hunt*[1,2]**, Leéna Boone[1,3], and H. W. Kestler[1]
[1] Lorain County Community College Department of Biology, [2] College Credit Plus Columbia High School, [3]The Ohio State University

The Human Immunodeficiency Virus (HIV) targets and erodes the body's immune system causing an Acquired Immunodeficiency Syndrome (AIDS). HIV infection begins when GP120 interacts with the primary receptor, CD4, and one of two co-receptors, CXCR4 or CCR5. There are two variants of HIV that recognize different receptor combinations, R5 virus targets cells that express CCR5 and X4 variant targets cells expressing CXCR4.When CCR5-Delta32 is present, functional CCR5 is absent from the cell's surface. Since both versions of HIV are present in the population and Delta32 homozygotes are very unlikely to be infected, Delta32 may affect CXCR4 surface expression. This research will determine the effects of CCR5-Delta32 on CXCR4 cell-surface expression and HIV infectivity.

In order to conclusively determine the effects of CCR5-Delta32, CXCR4 must be overexpressed on T-cells. *cxcr4* was isolated from the H9 human T-cell line by PCR. After isolation, the *cxcr4* fragment was blunt-end ligated into the retroviral shuttle vector pLXSN using the restriction enzyme HpaI. The ligation product, pLXSN-*cxcr4,* was transformed into competent *E. coli* cells. The plasmid was extracted and restriction endonuclease mapped to verify pLXSN-*cxcr4*.

Once a successful clone of pLXSN-*cxcr4* is accomplished, it will then be transfected into the packaging cell line PT67 to produce retroviral particles. This process will also be carried out with various *ccr5* alleles including the Delta32 mutation; wild type *ccr5*; and TG5, a novel point mutation isolated from a possibly HIV-resistant subject. Once these retroviral particles are produced, they will then be transfected into tumor cells like CEMX174. HIV-resistance will be tested by exposing cells that are expressing *cxcr4* and various *ccr5* alleles to HIV.

## Jarosz, Abigail S.

### Expression of a young endogenous gammaretroviral lineage in canine tissues

**Abigail S. Jarosz[1]***, Erica Cech[1], Lindsey E. Davis[1], Malika Day[1], Amanda L. Pendleton[2], Jamie Modiano[3], Julia V. Halo[1]
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Despite their being constantly challenged from numerous exogenous, retroviral infection in the domestic dog has traditionally been assumed as rare or nonexistent. The domestic dog genome displays a substantially lower endogenous retrovirus (ERV) presence when compared to other mammals, at just 0.15% of their nuclear genome. Although current canids appear to evade infection, when looking at canids' genomes it is apparent their ancestors were infected. ERV-Fc1(a) appears to be the youngest of the ERVs found in canids is a canid-specific group, belonging to a recombinant sub-lineage that was spread to canine ancestors by interspecies transmission. Analysis of 19 proviral sequences identified shared disruptive mutations indicating defective proviruses were spread via complementation. The patterns of ERV polymorphism and sequence variation indicate multiple circulating viruses infected canid ancestors within the last 20 million to within 1.6 million years with a recent bust of germline invasion in the lineage leading to wolves and dogs. Further phylogenetic analysis confirmed that this lineage contains a gag and pol gene most closely related to human ERV-Fc and an env gene to ERV-W. Both lineages have elements that are associated with several human diseases. Although circulating XRVs remain elusive to canids, there have been numerous reports of gammaretrovirus-like C-type particles and enzyme activities in canine leukemias and lymphomas. We have identified a transcriptionally active gamma-like ERV group in canine tumors and cancer derived cell lines. Tissues with env expression, all clustered with the CfERV-Fc1(a) members. Remarkably, the majority of Fc1(a) *env* genes, responsible for host receptor recognition and binding, have complete open reading frames (ORF) and contain all necessary motifs to maintain viral function. Our preliminary research demonstrates some of these polymorphic insertions have retained their fusogenic ability, raising the possibility that its translated products may play roles in the development or progression of certain canine cancer.

## Kashyap, Jyoti

## Characterization of a human fecal-derived *Citrobacter amalonaticus* strain capable of degrading choline by an unknown mechanism that does not produce trimethylamine

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Gut microbiota metabolism can have profound effects on human health. Choline, a quaternary amine (QA) highly abundant in our diet, is canonically cleaved by a glycyl radical enzyme, choline trimethylamine lyase (CutC), and its SAM-dependent radical activator, CutD .CutC cleaves choline to form trimethylamine (TMA) and acetaldehyde. TMA is oxidized to TMAO by FMO3 in the liver, which plays a role in causing atherosclerosis. Our hypothesis is that alternative pathways for choline degradation occur within gut microbes. Our hypothesis has been formulated on the basis of evidence that *Desulfitobacterium hafniense* Y51, a strict anaerobe encodes pyrrolysine-lacking trimethylamine:corrinoid methyltransferase (MtgB) that carries out demethylation of a QA, glycine betaine. MtgB is a member of the widespread COG5598 superfamily predicted to be involved in QA demethylation, which is heavily represented within the human gut microbiota. Therefore, we predict that certain gut microbiota can anaerobically respire QAs such as choline. We have isolated, from enrichments of a human fecal sample, a strain of *Citrobacter amalonaticus*, strain CJ25, capable of anaerobically utilizing choline as its sole carbon and energy source. Its genome does not encode CutC/D, nor does it encode any COG5598 enzymes. We have confirmed the degradation of choline over time by using nuclear magnetic resonance 500 MHz along with production of acetate. However, gas chromatography analysis of the culture did not show any production of TMA. The culture additionally grows on TMA as well. Hence, the future goals of this project are to perform transcriptomics to identify highly expressed genes during growth of *C. amalonaticus* CJ25 on choline and further characterize the metabolic end products of its metabolism during growth on choline.

## Kirby, Hope A.

## The influence of blue light and the BlsA photoreceptor on the oxidative stress response of *Acinetobacter baumannii*

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*Acinetobacter baumannii* is a ubiquitous human bacterial pathogen with pronounced morbidity and mortality rates in immunocompromised patients. With a prominent rise of multidrug-resistant *A. baumannii* strains, understanding the mechanisms by which this bacterium regulates and maintains its virulence is imperative for the development of alternative treatments and prevention. One such form of regulation is through the blue light using flavin (BLUF) protein BlsA. At 24°C, BlsA has been linked to regulation of multiple virulence factors including motility, iron uptake, and biofilm formation. We

aimed to elucidate how *A. baumannii* regulates its oxidative stress response both generally and from the sphere of influence of BlsA. First, we aimed to demonstrate which of the four catalase homologs in *A. baumannii* ATCC 17978 were regulated by BlsA. From these data, our findings also provide insight into the mechanisms through which BlsA regulates the catalase KatE. A C-terminal lysine residue of BlsA, critical for motility regulation, was shown to also be important for light mediated catalase activity regulation. Notably, the five most C-terminal BlsA residues, despite being unnecessary for motility regulation, are necessary for catalase activity regulation independent of light. These residues were also found to be involved in a BlsA-mediated control of superoxide dismutase (SOD) activity. Beyond light-mediated regulation, our findings elucidate the general pathophysiological roles of the four catalase homologs in *A. baumannii*. Ultimately, these data provide insight into how *A. baumannii* depends on light to regulate responses to oxidative stress.

## Kraus, Eric A.

### An Examination of Antibiotic Resistance Genes in *Pseudomonas chlororaphis* in Response to Bacteriophage Therapy

**Eric A. Kraus***
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Extensive antibiotic use over recent decades has led to increased levels of antibiotic resistance among bacteria. A current alternative treatment, phage therapy uses bacteriophages to infect and lyse bacteria while leaving the host organism unaffected. Evolution of phage resistance in bacteria can lead to the deletion, mutation, or expression level changes of certain genes. Increased antibiotic susceptibility is one of these benefits, providing an exciting new treatment option when phages are administered in conjunction with antibiotics. This study examined the effects of phage-resistance on the antibiotic susceptibility of *Pseudomonas chlororaphis* 14B11. The susceptibility profile of the 14B11 parent strain was determined against 10 different antibiotics. Then, the parent strain was grown with the PC1 bacteriophage to promote emergence of phage-resistant mutants. Mutant antibiotic susceptibility was tested and compared to the parent strain. A total of 27 mutants were isolated, where 26 mutants displayed increased susceptibility to at least one antibiotic. The following genes can confer antibiotic resistance and were selected for study in the mutants: *MexE*, *MexF*, *OprN*, *MexG*, *MexH*, *MexI*, *OpmD*, and *CyoE*. Ongoing efforts to amplify candidate genes via PCR and examine parent and mutant sequences for mutations have continued.

## Kunch, Austin

### The Requirement of the N terminus of the CCR5-delta 32 for *in vivo* HIV infectivity

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CCR5 and CXCR4 are integral membrane proteins found on T-cells. Two HIV variants known as HIV-R5 and HIV-X4 infect different subsets of T-cells. HIV-R5 infects T-cells by binding to receptor CD4 and CCR5. HIV-X4 enters T-cells through receptor CD4 and CXCR4. The C-terminus of CCR5 isn't expressed due to a 32 base pair deletion in the *ccr5* gene. This truncation is known to confer resistance to HIV infection *in vitro*. The first person cured of HIV, Timothy Ray Brown (TRB) harbored both HIV R5, and X4 variants. He received a bone marrow transplant from a donor, homozygous for the *ccr5-delta 32* alleles. Brown was cured of both variants of HIV, suggesting resistance is conferred *in vivo.* Both TRB and the donor were wild-type for CXCR4 yet TRB was cured of both variants. It's hypothesized that the CCR5 delta 32 gene products can down-modulate the CXCR4 protein, rendering cells uninfectable. The hypothesis is to remove the N-terminus of the *ccr5* gene and to determine CXCR4 surface expression. There should be a correlation of CXCR4 to the surface and X4 variant infectivity.

Using CRISPR/cas9, the *ccr5* gene will be modified in a variety of human T-Cells. A retroviral particle will be generated using pLlentiCRISPR v2. A guide RNA was designed to edit *ccr5* at the N-terminus of the gene. A deoxyoligonucleotide representing the guide RNA will be cloned into pLlentiCRISPR. The clone pLlentiCRISPR/*ccr5-KO* will be cotransfected, with needed plasmids to build the Lenti-Retroviral particle (psPAX2; pMD2.G), into HEK-293 Cells. The assembled viral particle can infect the T-cell lines such as H9 to deliver the knockout mechanism. The gene expression of the knockout cell line can be determined using flow cytometry and SDS-PAGE.

## Pacini, Cole H.

### Evaluating how Adenovirus proteins regulate DNA-PK

**Cole H. Pacini*,** Christopher Chen, Eileen Bridge
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Chemotherapy induces double-stranded breaks (DSB) in DNA to kill cancer cells, however certain types of cancer cells may upregulate the cellular DNA damage response (DDR) to repair DSB, which can lead to chemoresistance. While several DSB repair pathways exist, non-homologous end joining (NHEJ) is primarily utilized. NHEJ regulatory factors, such as DNA-dependent protein kinase (DNA-PK), have been implicated in facilitating chemoresistance. However, oncolytic viruses are genetically modified viruses that have been used to circumvent chemoresistance by selectively infecting and killing cancer cells. Adenovirus (Ad), a DNA virus, has been studied extensively and tested in clinical trials for its therapeutic potential to sensitize cancer cells to radiation and chemotherapy. Ad infection may activate DDR, which, if left

unchecked, will lead to concatenation of viral genomes via DNA-PK mediated NHEJ. Genome concatenation can hinder viral replication, but Ad encodes the 11kDa and 34kDa proteins in early region 4 (E4) to overcome NHEJ interference. However, the effects that the Ad E4 proteins have on DNA-PK remains to be studied. We hypothesized that both the E4 11kDa and 34kDa proteins are functionally redundant in regulating DNA-PK during Ad infection in cervical cancer cells. Our results suggest that the expression of either the E4 11kDa or 34kDa can reduce DNA-PK activation. We further investigated if the Ad E4 proteins affected the subcellular localization of activated DNA-PK during viral infection. Our data indicate that the presence of either E4 protein alters the localization of activated DNA-PK. These findings fill the previous gap in knowledge regarding how Ad E4 proteins regulate DNA-PK. Understanding these basic molecular mechanisms provides the platform for future research towards oncolytic virotherapy.

## Raja, A.

### Torin-1-Mediated Inhibition of the Viability and Locomotion in *Naegleria gruberi*

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*Naegleriafowleri* is a pathogenic unicellular protist of free-living ameba commonly found in warm freshwater and undergoes three stages of the life cycle, amebic trophozoite, flagellate, and metabolically dormant cyst, depending on the environmental conditions. However, the trophozoite is the only form that invades the central nervous tissues through the nasal cavity and causes highly fatal primary amebic meningoencephalitis (PAM). In this study, the non-pathogenic species, *Naegleriagruberi*(NEG-M strain, ATCC 30224), was assessed its sensitivities to *Streptomyces*-derived macrolides rapamycin and FK506, and the ATP-competitive TOR (target of rapamycin) inhibitor, Torin-1. Although expressing the common macrolide receptor, FKBP12, *N.gruberi* was resistant to the allosteric inhibitors for TOR kinase (rapamycin) and calcineurin phosphatase (FK506) in terms of its viability and locomotion, while showing the vulnerability to Torin-1 in a dose-dependent manner ($IC_{50}$ = 7.79 nM). Moreover, Torin-1 treatment induced the formation of cyst-like structure and greatly affected ameba's locomotion, suggesting TOR's potential regulatory role in the morphological differentiation and actin filament polymerization in the *Naegleria* genus.

## Ray, Stephanie C.

### Host restriction of copper serves as an intracellular environmental signal driving *Histoplasma* virulence

**Stephanie C. Ray*** and Chad A. Rappleye
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The dimorphic fungal pathogen *Histoplasma capsulatum* replicates within the phagosomes of host macrophages, where it must scavenge nutrients such as copper from the host cell to survive. After the onset of adaptive immunity, copper becomes restricted in the macrophage phagosome necessitating upregulation of the high-affinity copper transporter Ctr3 for full *Histoplasma* virulence. To ascertain which regulatory elements are responsible for upregulation of *CTR3* in response to low copper, we fused *CTR3* promoter truncations to a GFP reporter and found 335bp was sufficient for copper-regulated induction of the *CTR3* promoter. Interrogation of this 335bp region identified putative binding sites for Mac1, a transcription factor with copper-dependent DNA binding. To assess the functional role of these putative binding sites for *CTR3* expression in low copper, we scrambled the binding sites individually or in combination. Simultaneous disruption of at least two putative Mac1-binding sites abolished copper regulation of *CTR3* expression. In addition, we tested if Mac1 was required for copper regulation of the CTR3 promoter. A *MAC1*-RNAi strain showed loss of induction of CTR3 expression in response to low copper and had increased sensitivity to the copper chelator BCS, confirming the role of Mac1 in the regulation of CTR3 expression. In a mouse model of respiratory histoplasmosis, loss of Mac1 function caused more severe attenuation than the loss of Ctr3 function suggesting that Mac1 controls additional genes important for *Histoplasma* virulence. RNAseq analysis of wild-type and *MAC1*-deficient *Histoplasma* yeast grown in high and low copper conditions revealed Mac1-dependent regulation of genes related to acquisition of other metals in addition to copper and genes involved in reactive oxygen detoxification. Thus, Mac1-dependent sensing of limitation of phagosomal copper induces the expression of multiple virulence determinants for intracellular survival and proliferation of Histoplasma yeasts.

## Sarpong, David D.

### Characterization of a putative toxin-antitoxin locus in *Shigella flexneri*

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*Shigella* are gram negative bacilli that cause shigellosis, a bloody diarrheal disease in humans. Currently, no vaccine or universally available and effective antibiotics exist to treat shigellosis. To develop novel anti-*Shigella* therapeutics, the molecular strategies that underlie the pathogen's physiology and virulence must be known. Small RNAs (sRNAs) are regulatory molecules which play a critical role in bacterial gene expression. RyfA is a sRNA which was originally discovered in *Escherichia coli* and found to be conserved in *Shigella*. Two copies of *ryfA* exist in *S. dysenteriae*, *ryfA1* and *ryA2*. Upstream of each *ryfA* is a divergently encoded sRNA named RyfB1and RyfB2,

respectively. *In silico* analyses have revealed that *S. flexneri* contains a single *ryfA* and the duplicate *ryfB1* and *ryfB2* genes. Sequence complementarity has been shown to exist between RyfA and both RyfB transcripts in *S. flexneri*. Additionally, three putative open reading frames are predicted within the sequence of RyfA. Strikingly, increased production (800-fold) of RyfA from an inducible plasmid inhibits growthof *S. flexneri,* while a ten-fold increase does not inhibit growth but does inhibit plaque formation. It is therefore proposed that the *ryf* locus represents a Type 1 Toxin/Antitoxin system in which *ryfA* encodes a small toxic peptide whose deleterious expression is tightly regulated by two complementary sRNA antitoxins, RyfB1 and RyfB2. These studies are designed to understand the regulation and function of each gene within the *S. flexneri ryf* locus, and their contribution to the physiology of this important human pathogen.

## Seder, Juliana

### Analysis of the Ability of a Novel *ccr5* Mutation to Downmodulate Alleles of CCR5 and CXCR4

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A novel mutation in the *ccr5* gene, which codes for the CCR5 protein, was found in a child of an African-American family. The mother contracted HIV before the birth of her first child in the early 1980s. After she was infected she unknowingly exposed all five of her children to HIV through vaginal delivery. One of the five children, the second born, did not acquire the infection even though they were exposed to the virus at birth. The mother and the infected children all carried genetically related HIV and the degree of similarity was inversely correlated with birth order. A missense point mutation in the cytoplasmic domain of the uninfected child's *ccr5* gene was discovered. The mutation (TG5), changes a lysine codon at position 314 into an arginine codon. The allele containing the TG5 mutation was cloned into pLenti puro HA-Ubiquitin. Clones made from pLenti-TG5 will be used to transfect the packaging cell line HEK293. The HEK293 cell line will assemble viral particles containing the TG5 mutation. The retroviral particles will be recovered and introduced into an H9 cell line as well as other cells. The expression of the TG5 gene in H9 and its effect on HIV infectivity will be tested. It has been reported that CCR5 delta 32 can down-modulate wild-type CCR5 and CXCR4. The ability of TG5 to down-modulate wild-type CCR5 and CXCR4 will be determined.

## Shittu, Adenike

### PFAS toxicity studies of human microbiome bacteria

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Per- and polyfluoroalkyl substances (PFAS) are anthropogenic chemical compounds that are persistent in the environment, and resistant to biological degradation. Concerns are rising based on the emergence of epidemiological data that indicates a link between human exposure and certain diseases. This study focuses on the two most widely used PFAS, perfluorooctanoic acid (PFOA) and perfluorooctanesulfonic acid (PFOS), as well as and one of the newer PFAS, hexafluoropropylene oxide, which is also known by its Chemours (DuPont) trademark name GenX. GenX was developed as a safer replacement for PFOA due to its shorter carbon chain length. We evaluated sensitivities of two human gut bacteria using zone of inhibition assays. We found that *Escherchia coli* is insensitive to all three of these PFAS, whereas *Collinsella aerofaciens* is sensitive to all three. The discovery of this differential sensitivity to PFAS among human microbiome species is important, because it suggests that human ingestion of these bio-accumulative chemicals could lead to an imbalance in the gut microbiome. Our further investigations were directed toward *C. aerofaciens*, which is the most abundant obligate anaerobe actinobacterium in the gut of healthy humans. We have successfully isolated mutants that are reduced in in their sensitivities to PFAS. By sequencing their genomes, we expect that DNA sequence differences between the mutant and wild type strain will direct us to the candidate molecular targets of the chemicals. These candidate targets will be tested by complementation analyses. If confirmed, hypotheses regarding the mechanism of PFAS toxicity can then be formulated and investigated.

## Zhang, Yeying

### Biosynthesis of Valinophos, an Unusual Phosphonopeptide Produced by *Streptomyces durhamensis*

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Phosphonic acids, characterized by a direct carbon-phosphorus bond, are a class of natural products with wide-ranging bioactivities. Valinophos, a recently discovered phosphonopeptide from *Streptomyces durhamensis*, is composed of (R)-2,3-dihydroxypropylphosphonic acid (DHPPA) esterified with N-acetyl valine at the terminal alcohol. The structural novelty of valinophos and inhibitory potential of DHPPA prompted us to deduce the genetic and biochemical basis of their biosynthesis. Genomic analyses identified a neighborhood encoding seven genes suggestive of a

pathway for phosphonic acids. Heterologous expression of these genes within *S. lividans* 66 resulted in the production of DHPPA and valinophos, affirming their necessity in biosynthesis. We determined the function of each gene and their encoded proteins through deletion analysis, identification of accumulated phosphonic acid intermediates, and biochemical reconstitution using purified substrates and recombinant protein. Overall, we demonstrate synthesis of DHPPA from phosphoenolpyruvate requires five distinct enzymatic transformations, with the ligation of L-valine occurring as the sixth and last step. Similarities between DHPPA biosynthesis and conical pathways for serine and glycolysis provides support that many phosphonic acid pathways evolved from primary metabolism.