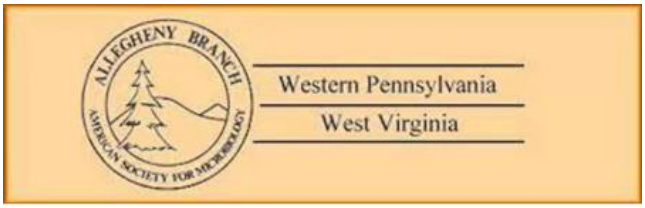
AMERICAN SOCIETY FOR MICROBIOLOGY

Annual Meeting of the Allegheny Branch American Society for Microbiology

West Virginia University
Health Sciences Center
Morgantown, WV
November 4-5, 2022

ALLEGHENY BRANCH
AMERICAN SOCIETY FOR MICROBIOLOGY
Western Pennsylvania
West Virginia

Sponsors:



Vendors:
Thank you for your contribution to this conference!

Each vendor here has made a generous donation that allowed us to host this fantastic conference. Please make sure you visit their booth and learn about their company!

Trainees: Please visit each vendor booth & obtain a STAMP, **also** you must visit at least 1 of the graduate school or medical school representatives at lunch. Please put your stamped card in the box on the table by the Fukushima auditorium (1901). (Your card is inside your name badge).

Two random cards will be drawn to win $100 each!

2022 ABASM PROGRAM (Truncated)

Friday, November 4th, 2022

2:00-5:00 PM REMNets workshop (Faculty only) – Erma Byrd G01
5:00-5:30 PM Registration/group A poster set up – Health Sciences Center – Pylon lobby
5:30-6:30 PM Dinner/opening remarks – 2940A/B
6:30-7:30 PM Post-doc keynote presentations - 2940A/B
- Dr. Maria de la Paz Gutierrez – Dr. Barbier lab – West Virginia University
- Dr. Margalida Mateu-Borras – Dr. Barbier lab – West Virginia University

7:30-7:45 PM Group A poster set-up – Pylon lobby area
*Poster set-up will also be available Saturday morning but encouraged to set up on Friday.

Saturday, November 5th, 2022

6:00-8:45AM Hot Buffet breakfast at the Hampton Inn (for those staying at the hotel)
8:00-9:00AM Registration & light breakfast – Fukushima auditorium (1901)
9:00-10:00AM Keynote presentation Dr. Timothy Cover – Fukushima auditorium (1901)
10:00-10:45AM Oral session 1 – Fukushima auditorium (1901)
10:45-11:00AM Group A poster session set up / coffee break
11:00-12:00PM Oral session 2 – (1905)
11:00-12:00PM Oral session 3 – (Fukushima 1901)
12:00-1:15PM Student lunch – 2940A/B
12:00-1:15PM ABASM business lunch (faculty/post-docs) – G119
1:15-2:15PM Group A poster session – Pylon lobby (Group A take down posters at 2:15PM)
2:15-2:30PM Group B poster set up and coffee break – Pylon lobby
2:30-3:30PM Group B poster session – Pylon lobby
3:30-3:45PM Break and take posters down
3:45-5:00PM Oral session 4 – (1905)
3:45-5:00PM Oral session 5 – (Fukushima 1901)
5:00-5:15PM Judges debriefing and decisions – (2940A/B)
5:15-5:30PM Award presentations and closing remarks – Fukushima auditorium (1901)

2022 ABASM PROGRAM (Full)

Friday, November 4th, 2022

2:00-5:00 PM REMNets workshop (Faculty only) – Erma Byrd G01

4:30-5:30 PM Registration/Group A poster set up – Health Sciences Center – Pylon lobby

5:30-6:30 PM Dinner/opening remarks – 2940A/B—Dr. Jonathan Busada / Dr. Albert Berrebi

6:30-7:30 PM Post-doc keynote presentations - 2940A/B

6:30 **Dr. Maria de la Paz Gutierrez**, West Virginia University; *"Length of protection varies between two veterinary Lyme vaccines inducing different immune responses in a murine model of vaccination and challenge"*

7:00 **Dr. Margalida Mateu-Borras**, West Virginia University; *"Generation and characterization of broadly reactive monoclonal antibodies against Pseudomonas aeruginosa and Burkholderia pseudomallei"*

Saturday, November 5th, 2022

6:00-8:45AM Hot Buffet breakfast at the Hampton Inn (for those staying at the hotel)

8:00-9:00AM Registration & light breakfast – Fukushima auditorium (1901)

9:00-10:00AM **Keynote presentation: Dr. Timothy Cover, Vanderbilt University; *"Helicobacter pylori genetic diversity and gastric cancer risk"*– Fukushima auditorium (1901)**

10:00-10:45AM Oral session 1 Medical Microbiology I– Fukushima auditorium (1901)

Session Chairs: Jonathan Busada Ph.D. and Sara Druffner; West Virginia University

10:00 Graham Bitzer: *"Investigation of a Pertussis Toxin Antigen in a Novel mRNA Pertussis Vaccine"*

10:15 Stuti Khadka: *"Loss of the Glucocorticoid Receptor promotes Helicobacter felis-induced gastric metaplasia"*

10:45 Cecilia G. Sierra-Bakhshi: *"Salmonella Infection in Diabetic Mice"*

10:45-11:00AM Group A poster session set up / coffee break

11:00-12:00PM Oral session 2 Medical Microbiology II – (1905)

Session Chairs: Jen Franko Ph.D. West Virginia University

11:00 Catharine Besch: "Modeling Microbiomes: Insight into Predicting the Role of Fungi in C. difficile infections"

11:12 Katherine S Lee: *"Evaluation of an intranasal VLP-RBD COVID-19 vaccine with the BECC470 adjuvant in K18-hACE2 mice"*

11:24 Jason Kang: *"Anti-Pseudomonas aeruginosa monoclonal antibody confers protection in a murine model of pneumonia and sepsis"*

11:36 Megan Grund: *"Identification of small compound antimicrobials against B. pseudomallei and ESAKPE pathogens using artificial intelligence and machine learning"*

11:48 Rhiannon Macom: *"Loss of intestinal alkaline phosphatase disrupts acute post-stroke intestinal homeostasis"*

11:00-12:00PM Oral session 3 Environmental Microbiology – (Fukushima 1901)
Session Chairs: Jen Gallagher Ph.D. West Virginia University

11:00 Nina Olivia Tan: *"Exposure to ground level ozone induces lung inflammation in an NLRX1 dependent manner"*
11:12 Mansi Chandra: *"Effects of Walnut Consumption on the Gut Microbiota"*
11:24 The Nandar Su: *"Impacts of Hydraulic Fracturing on the Microbial Communities in Headwater Streams of Northwestern Pennsylvania in 2019-2020"*
11:36 Chansotheary Dang: *"Nitrogen deposition strongly influence the soil microorganisms and carbon biogeochemistry in arbuscular mycorrhizal ecosystem than in ectomycorrhizal ecosystem"*
11:48 Anna Vietmeier: *"Iron Bioremediation in Passive Remediation Systems Treating Acidic Abandoned Mine Drainage"*

12:00-1:15PM Student lunch – 2940A/B

12:00-1:15PM ABASM business lunch (faculty/post-docs) – G119

1:15-2:15PM Group A poster session – Pylon lobby (Group A take down posters at 2:15PM)

2:15-2:30PM Group B poster set up and coffee break – Pylon lobby

2:30-3:30PM Group B poster session – Pylon lobby

3:30-3:45PM Break and take posters down

3:45-5:00PM Oral session 4 Molecular Microbiology – (1905)
Session Chairs: Deanna Schmitt Ph.D. West Liberty University

3:45 Annalee Schmidt: *"Isolation of natural products from soil samples in Pennsylvania identifies two compounds with potent antibacterial activity against Bacillus anthracis"*
3:57 Samantha Anderson: *"Herbs and Spices Modulate Gut Bacterial Composition in Adults at Risk for CVD: Results of a Prespecified Exploratory Analysis from a Randomized, Crossover, Controlled-Feeding Study"*
4:09 Jillian Leister: *"Going Nutty: Are Walnuts Beneficial for CV Health?"*
4:21 Justin Wright: *"Comparative meta-omics for identifying pathogens associated with prosthetic joint infection"*
4:33 Bethann Wilson: *"Purification and characterization of the iron-sulfur cluster containing ArxB2 from Alkalilimnicola ehrlichii"*
4:45 Victoria Verhoeve: *"Import of a unique host-derived sugar N-acetylglucosamine 1-P by rickettsiae for biosynthesis of cell envelope glycoconjugates"*

3:45-5:00PM Oral session 5 Medical Microbiology III – (Fukushima 1901)
Session Chairs Tim Driscoll Ph.D. West Virginia University

3:45 Benjamin Rosiello: *"Mucosal Antibodies in Mild and Asymptomatic COVID-19"*
3:57 Kendyl Berry: *"Defining the Mechanism by which Mycobacterium tuberculosis Inhibits TRAP-positive Syncytial Formation in RAW 264.7 Cells"*
4:09 Megan DeJong: *"Exploration of new pertussis booster formulations with the BECC438b adjuvant"*

4:21 Paris Taylor: *“Quantification of Minimum Biofilm Eradication Concentration (MBEC) of Clinical Prosthetic Joint Infection Isolates of Staphylococcus aureus”*
4:33 Nour Bouji: *“Efficacy of intra-articular versus systemic vancomycin and Mitochondrial Response to In Vivo Prosthetic Joint Infection”*
4:45 Daniel Evans: *“Empirical genomic methods for tracking plasmid spread among healthcare-associated bacteria”*

5:00-5:15PM Judges debriefing and decisions – (2940A/B)

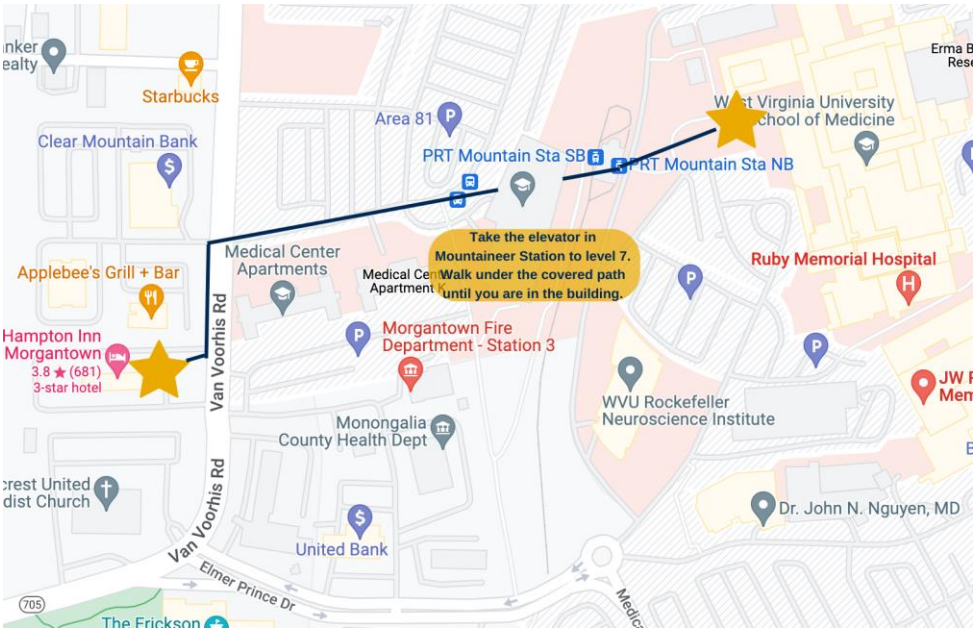
5:15-5:30PM Award presentations and closing remarks – Fukushima auditorium (1901)

Getting to West Virginia University Health Sciences Center:

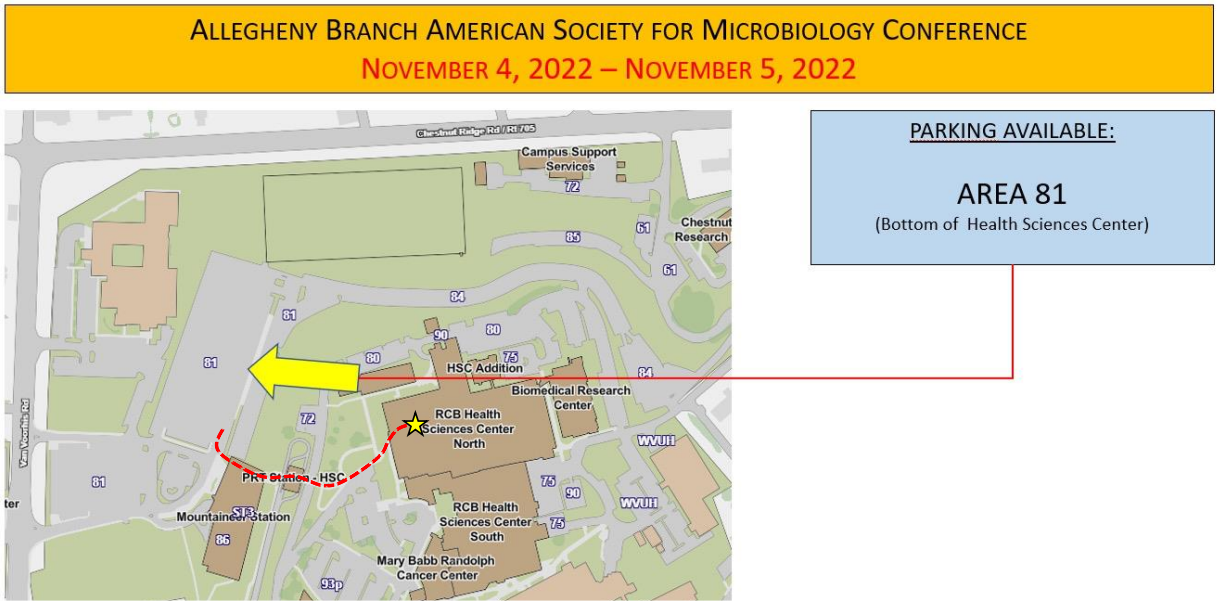
- Hampton Inn address: 1053 Van Voorhis Road, Morgantown, West Virginia, 26505, USA

- Area 81 parking lot: https://goo.gl/maps/cHgHcqkb6LXHfhYG6

- For those staying at the Hampton Inn: you can either leave your car at the Hampton Inn and walk across the street OR you can drive across the street and park in “Area 81” directly in front of the Mountaineer Station parking garage. go into the main building and take the elevator to level 7, walk under the covered path until you are in the building.



- For those not staying at the Hampton Inn: Park directly in Area 81, go into the main building and take the elevator to level 7, walk under the covered path until you are in the building.

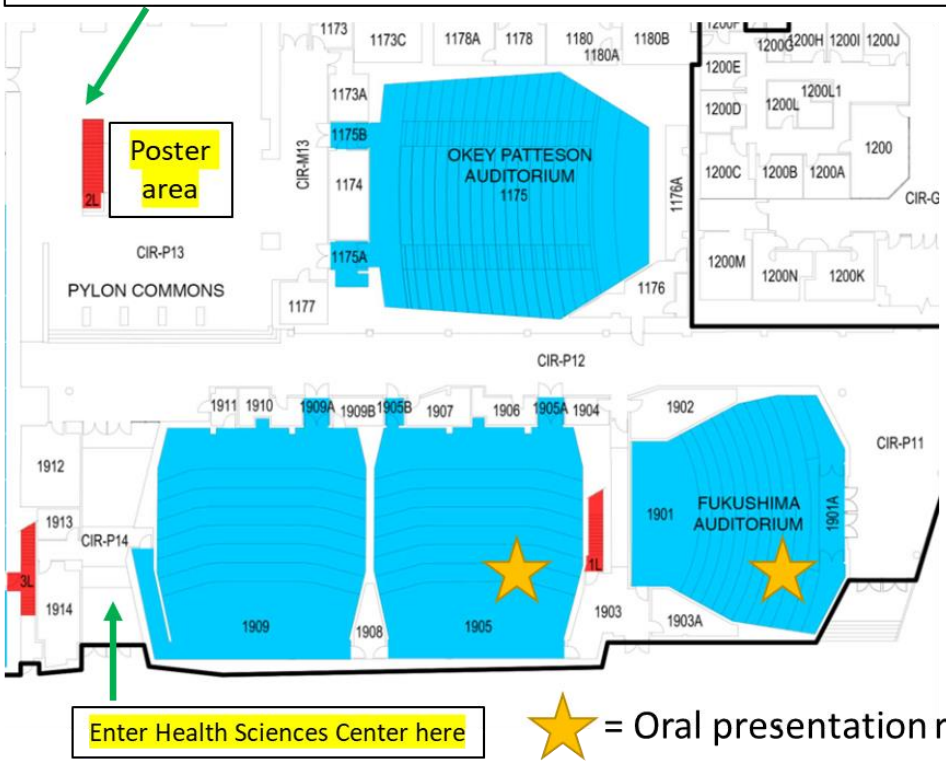


- If there is anyone with disabilities or mobility issues, please reach out to jubevere@hsc.wvu.edu for further assistance and directions.

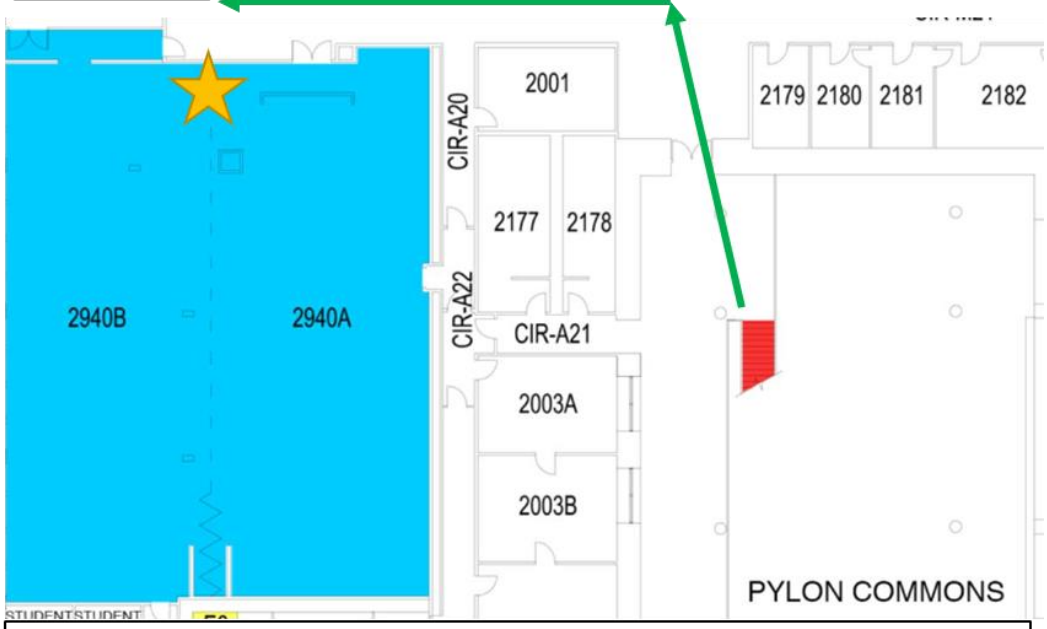
Inside the WVU Health Sciences Center:

First Floor

Stairs to get to 2nd floor for Friday dinner and Saturday Lunch



2nd floor



Come up stairs, go through double doors and turn left to get to 2940A/B

Student Lunch:

We will have the following representatives onsite during lunch on Saturday for students to be able to ask questions about graduate school and medical school.

Lauren Wamsley, MPH
Director of Outreach and Recruitment
West Virginia University School of Medicine
Department of Medical Education
lwamsle2@hsc.wvu.edu

Erin Fields, MS
Program Director
Office of Graduate Admissions & Recruitment
West Virginia University
erin.fields@mail.wvu.edu

Nicole Beason
Executive Director, Office of Research and Graduate Education
Biomedical Sciences PhD and MS in Health Sciences representative
West Virginia University Health Sciences Center
nicole.beason@hsc.wvu.edu

Mountaineer Undergraduate Research Review (MURR)

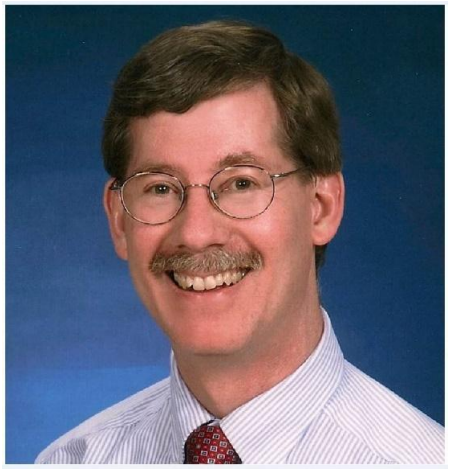
- MURR advances opportunities for WVU students by providing an outlet for publishing scholarly and creative works while deepening their engagement within their field of study. By contributing to MURR, students play an active and critical role in reinforcing WVU's reputation as a very high research-intensive, land grant university.
- **We are currently accepting research from all undergraduates participating in research within the state of West Virginia. This includes all colleges within WV.**
- Our research journal is the sole undergraduate journal at WVU and we are looking for submissions for our upcoming edition. We are accepting original research papers, literature reviews, policy briefs, and cover art submissions for MURR, Volume 8, with publication anticipated in spring 2023. Submissions can be made online via our webpage (https://undergraduateresearch.wvu.edu/research-opportunities/wvu-opportunities/murr)

Annual Business Meeting – Faculty and post-doc lunch:

The annual ABASM business meeting will be held in G119. Faculty should obtain lunch in room 2940A/B. Take stairs/elevator to the ground level and then follow signs to the room.

Keynote Speaker Biographies

Dr. Timothy Cover
Professor of Medicine,
Professor of Pathology, Microbiology, and Immunology
Vanderbilt University School of Medicine



Timothy Cover is Professor of Medicine and Professor of Pathology, Microbiology and Immunology at Vanderbilt University School of Medicine. His research interests are directed toward bacteria-host interactions relevant for development of cancer, focusing in particular on the bacterium *Helicobacter pylori* and its role in the pathogenesis of stomach cancer. His laboratory has made important contributions to our understanding of genetic variation among *H. pylori* strains and strain-specific *H. pylori* constituents that are determinants of gastric cancer risk. These include a pore-forming toxin (VacA), the effector protein CagA (regarded as a bacterial oncoprotein), and a type IV secretion system that mediates entry of CagA into host cells.

Dr. Margalida Mateu-Borras
Postdoctoral Associate
Barbier Laboratory
West Virginia University School of Medicine

Margalida Mateu-Borras earned her MS in Advanced Microbiology and a Ph.D. in Environmental and Biomedical Microbiology from the University of Balearic Islands. Her graduate research focused on *Pseudomonas aeruginosa* virulence factors and their interactions with the human innate immune system. Specifically, her thesis project addressed how the chronic presence of *P. aeruginosa* in the lung of cystic fibrosis patients impacted the complement system C5a. As a postdoctoral fellow at West Virginia University, her research focus is on developing new strategies to treat and prevent infections caused by antimicrobial-resistant (AMR) pathogens, such as the generation of broadly reactive monoclonal antibodies able to bind to *Burkholderia pseudomallei*, *P. aeruginosa.* Her long-term goal is continue to study AMR as an independent investigator.

Dr. Maria de la Paz Gutierrez
Postdoctoral Associate
Barbier Laboratory
West Virginia University School of Medicine

Maria de la Paz hails from Argentina. She graduated with a degree in Biotechnology and Molecular Biology from the National University of La Plata in 2016. As an undergraduate, she studied bacteria from the *Bordetella* genus, which cause respiratory infections. She earned her Ph.D. in Microbiology at the National University of La Plata in 2021. Her dissertation research was on factors that regulate *Bordetella bronchiseptica* pathogenesis. Shortly after graduation, she joined Dr. Mariette Barbier's laboratory at West Virginia University. Her current research is focused on the development of vaccines against *Borrelia burgdorferi*, the causative agent of Lyme disease.

Oral presentation information:

Name:	College or university you are attending from:	Oral Session	Oral Session Order
Graham Bitzer	West Virginia University	Session 1	1
Stuti Khadka	West Virginia University	Session 1	2
Cecilia G. Sierra-Bakhshi	Marshall University	Session 1	3
Cati Besch	Juniata College	Session 2	1
Katie Lee	West Virginia University	Session 2	2
Jason Kang	West Virginia University	Session 2	3
Megan Grund	West Virginia University	Session 2	4
Rhiannon Macom	West Virginia University	Session 2	5
Nina Olivia Tan	West Virginia University	Session 3	1
Mansi Chandra	Juniata College	Session 3	2
The Nandar Su	Juniata College	Session 3	3
Chansotheary Dang	West Virginia University	Session 3	4
Anna Vietmeier	Duquesne University	Session 3	5
Annalee Schmidt	The Pennsylvania State University	Session 4	1
Samantha Anderson	Juniata College	Session 4	2
Jillian Leister	Juniata College	Session 4	3
Justin Wright	Juniata College	Session 4	4
Bethann Wilson	Duquesne University	Session 4	5
Victoria I. Verhoeve	University of Maryland Baltimore	Session 4	6
Benjamin Rosiello	West Virginia University	Session 5	1
Kendyl Berry	West Virginia University	Session 5	2
Megan A DeJong	West Virginia University	Session 5	3
Paris Taylor	West Virginia University	Session 5	4
Nour Bouji	West Virginia University	Session 5	5
Daniel Richard Evans	EpiCenter Genomics LLC	Session 5	6

Poster session information:

Name:	College or university you are attending from:	Poster Session	Poster #
Jason Kang	West Virginia University	Session A	1
The Nandar Su	Juniata College	Session A	2
Anna Vietmeier	Duquesne University	Session A	3
Bethann Wilson	Duquesne University	Session A	4
Daniel Richard Evans	EpiCenter Genomics LLC	Session A	5
Maya Scarpaci	Pennsylvania Western University	Session A	6
Natalie Giacobe	The Pennsylvania State University	Session A	7
Jenna Conty AND Karina Aragon	University of Pittsburgh at Greensburg	Session A	8
Natalie Lamagna	Duquesne University	Session A	9
Cameron Tod Trowbridge	Juniata College	Session A	10
Sarah Cook	Juniata College	Session A	11
Emma Swiger	West Virginia University	Session A	12
Evita Yu Wei Yang	West Virginia University	Session A	13
Shrinidhi Venkateshwaraprabu	West Virginia University	Session A	14
Abigail Tillema	West Virginia University	Session A	15
Claire Magill	Juniata College	Session A	16
Shveta Kalathur	Juniata College	Session A	17
Breanna Haught	West Virginia University	Session A	18
Lily Saar	Lycoming College	Session A	19
Bryce Lincoski	Pennsylvania Western University	Session A	20
Maeve Morris	West Virginia University	Session A	21
Isabella Adair Trotter	Lycoming College	Session A	22
James Johnson	Lycoming College	Session A	23
Michelle Gresser and Jazmin Farabaugh	University of Pittsburgh at Greensburg	Session A	24
Jordan Gibson	West Liberty University	Session A	25
Barrett-Anne C Briggs	West Virginia University	Session A	26
Lillie Powell	West Virginia University	Session A	27
Sara Druffner	West Virginia University	Session A	28
Cecilia G. Sierra-Bakhshi	Marshall University	Session B	1
Megan Grund	West Virginia University	Session B	2
Rhiannon Macom	West Virginia University	Session B	3
Nina Olivia Tan	West Virginia University	Session B	4
Mansi Chandra	Juniata College	Session B	5
Benjamin Rosiello	West Virginia University	Session B	6
Kendyl Berry	West Virginia University	Session B	7
Christopher Graham	Lycoming College	Session B	8
Autumn Buck AND Matthew Clippinger	Mount Aloysius College	Session B	9
Alivia R. Yauger	Pennsylvania Western University	Session B	10
Dionysios Patriarcheas	West Virginia University	Session B	11
Kennedi Lewellyn	West Virginia University	Session B	12
Sabrina Siegan	West Virginia University	Session B	13
Emily Young	West Liberty University	Session B	14
Kayla Brennan	Duquesne University	Session B	15
Tyler Chandross-Cohen	The Pennsylvania State University	Session B	16
Kassandra Hoff	West Virginia University	Session B	17
Carter Joseph Branigan	Lycoming College	Session B	18
Elizabeth Giacobe AND Aidan Donnelly	The Pennsylvania State University	Session B	19
Claire Kelly	West Liberty University	Session B	20
Gage Pyles	West Virginia university	Session B	21
Katherine Lee AND Nate Rader	West Virginia University	Session B	22
Evan Thomas	Juniata college	Session B	23
Travis Russell	Juniata College	Session B	24

Abstracts (Oral Sessions):

Investigation of a Pertussis Toxin Antigen in a Novel mRNA Pertussis Vaccine

G.J. Bitzer [1,2], M.A. Wolf [1,2], M.A. DeJong [1,2], C. Cunningham [1,2], J.A. Chapman[1,2], N.A. Fitzgerald[1,2], M.D. Warden[1,2], J.R. Bevere [1,2], M. Barbier[1,2], F.H. Damron [1,2]
[1]Department of Microbiology, Immunology, and Cell Biology, West Virginia University, Morgantown, WV, USA; [2]West Virginia University Vaccine Development center, West Virginia University, Morgantown, WV, USA,

Pertussis toxin (PT) is an AB_5 holotoxin that is the hallmark toxin of *Bordetella pertussis* (*Bp*). During pertussis (Whooping Cough) infections, PT intoxication leads to leukocytosis which can be fatal in neonates. The current acellular pertussis vaccine (aP) includes a chemically detoxified Pertussis toxoid which potentially corrupts epitope regions needed for complete protection. We sought to evaluate a mRNA-pertussis vaccine to abrogate this immunity issue by investigating two components: 1) If antibodies to the B subunits of PT were immunogenic and 2) evaluate two different PT subunit A (PTxA) mRNA antigens (soluble and membrane-tethered antigen) for inclusion in a mRNA-pertussis vaccine. Starting at 5-weeks of age, BALB/c mice were IM primed and then boosted 4 weeks later with either control vaccines delivered at 1/20th and 1/200th human dose or an mRNA vaccine delivered at 1 µg per antigen and 0.1 µg per antigen. Mice were challenged 2 weeks post-boost with 2 µg of PT through IP injection and euthanized 3-days post-challenge. Antibody titers were analyzed post-prime, post-boost, and post-challenge for different subunits of PT, as well as, whole PT. No vaccines induced antibody levels to any PT B subunits. Interestingly, while both mRNA PT antigens had IgG titers similar to our positive controls the soluble mRNA had a higher response to whole PT but the membrane-tethered PT antigen had higher titers to PTxA. Also, the low dose soluble mRNA did not have an IgG titer to PTxA. We also investigated leukocyte numbers 3-days post-challenge and found both the experimental mRNA vaccines performed equivalent to our positive controls in limiting leukocytosis. Our studies suggest that both PTxA mRNA antigens are effective at preventing leukocytosis but soluble mRNA induces higher titers to whole PT. Currently, studies are under way evaluating the soluble antigen in an aerosol *Bp* challenge in rats.

Loss of the Glucocorticoid Receptor promotes *Helicobacter felis*-induced gastric metaplasia.

Stuti Khadka, Sebastian Dziadowicz, Halima Akhter, Gangqing Hu, and Jonathan T. Busada
Department of Microbiology, Immunology, and Cell Biology, West Virginia University, Morgantown, WV, USA

Chronic gastric inflammation associated with *Helicobacter pylori* infection drives gastric cancer development. We have previously shown that the steroid hormone glucocorticoids are master regulators of gastric inflammation and that systemic disruption of glucocorticoid signaling by adrenalectomy causes spontaneous pathological gastric inflammation and metaplasia. These pathologies were primarily driven by infiltrating macrophages. However, the cell-intrinsic functions of glucocorticoids within macrophages are unclear. In this study, we investigated how glucocorticoid receptor (GR) deletion within macrophages affects their activation and impacts inflammation in the stomach by using the LysM-Cre system to delete GR protein from macrophages. We found that ex vivo challenge of GRKO peritoneal macrophages with LPS resulted in higher expression of *Il1b, Tnf,* and *Il6*. RNA-seq of the macrophages from mice challenged with *H. pylori* showed that GRKO macrophages exhibited a defective response and activation of cancer-associated pathways. Further, macrophage GRKO mice infected for six months with *Helicobacter felis* had increased metaplasia compared to WT-infected mice. Overall, this study suggests that GR signaling in macrophages is critical for regulating gastric inflammation and metaplasia during Helicobacter infection.

Salmonella Infection in Diabetic Mice.

Cecilia G. Sierra-Bakhshi[1], Michael E. Smith[1], Torren A. Kalaskey[1], Katelyn Perkins[1], Maria G. Winter[2], Saroj Sigdel[3], Sebastian Winter[2], Lydia M. Bogomolnaya[1]

[1]Department of Biomedical Sciences, Joan C. Edwards School of Medicine, Marshall University, Huntington, WV. [2]Department of Microbiology and Immunology, UT Southwestern Medical Center, Dallas, TX [3]Department of Pathology, Joan C. Edwards School of Medicine, Marshall University, Huntington, WV.

Type 2 diabetes (T2D) is a risk factor for bacterial infections including those caused by nontyphoidal *Salmonella*. Individuals with uncontrolled T2D often experience the unusual extraintestinal spread of *Salmonella* which can lead to life-threatening disorders. The precise underlying mechanism of this predisposition is not clearly understood. In this study 8-week-old male TALLYHO (TH) mice were maintained on a standard chow, or on a high fat (HF) diet (45% fat) for 8 weeks to promote diabetes development. As expected, mice on the HF diet gained more weight compared to the animals on a standard chow and had diabetic levels of glucose in blood (>300 mg/dL). At that time, TH mice from each diet group were orally infected with 10^6 CFU (colony forming units) of a fully virulent bioluminescent *Salmonella* Typhimurium strain to follow the pathogen spread in individual animals using *in vivo* imaging. Mice in both groups developed clinical signs of salmonellosis. However, *Salmonella* spread in mice with diabetes had an unusual pattern compared to the healthy animals. Nevertheless, infection with *Salmonella* did not induce gross pathological changes in intestinal epithelium in the animals from both experimental groups compared to uninfected mice. Because T2D patients have altered gut microbiota with decreased population of butyrate-producing bacteria, we analyzed the intestinal profile of short chain fatty acids (SCFAs), e.g., butyrate in TH mice by GC-MS (Gas Chromatography Mass Spectrometry). As expected, concentrations of SCFA, including butyrate were reduced in the gut of animals on HF diet compared to TH mice maintained on the standard chow. We hypothesized that supplementation with tributyrin (butyrate prodrug) would decrease extraintestinal spread of *Salmonella*. We found that butyrate supplementation reduced bacterial burden in animals maintained on a standard diet. Unexpectedly, oral supplementation of butyrate did not decrease the spread of *Salmonella* in diabetic mice.

(Supported by NIH Grant P20GM103434 to the West Virginia IDeA Network for Biomedical Research Excellence)

Modeling Microbiomes: Insight into Predicting the Role of Fungi in C. difficile infections

Catharine Besch, Mohini Khedekar, David Stewart, Jeremy Chen See, Peter Kruse, Regina Lamendella

As the highest-occurring hospital-acquired disease in the USA, C. difficile infection (CDI) had focused on the bacterial role in the past. This study looks at the potential role of previously understudied fungi that have newly been discovered to have an impact on CDI. 16S rRNA gene profiling and shotgun metatranscriptomics were performed on 40 CDI+/- patients to measure bacterial taxonomy and expressed microbial functions. The resulting data provide a list of active microbial taxa and genes that serve as input to downstream models. Neural networks were implemented to explore if bacterial and/or fungal taxa and expressed genes are predictive of CDI status. Our preliminary results indicate that bacterial and fungal features are predictive of disease status and point towards specific metabolic pathways of interest. Metabolic modeling was then implemented to predict important metabolites using the Melonnpan model. Our study can provide alternative approaches to disease prevention and treatment by focusing on the specific fungal and bacterial features that exacerbate pathogenesis.

Evaluation of an intranasal VLP-RBD COVID-19 vaccine with the BECC470 adjuvant in K18-hACE2 mice

Katherine S Lee[1,2], Nathaniel A Rader[1,2], Ting Y Wong[1,2], Olivia A Miller[2], Melissa Cooper[2], Md. Shahrier Amin[3], Michael Winters[1], Ivan Martinez[1,4], Mariette Barbier[1,2], Justin R Bevere[1,2], and F. Heath Damron[1,2*]
[1] Department of Microbiology, Immunology, and Cell Biology, School of Medicine, West Virginia University, Morgantown, WV, USA; [2] Vaccine Development Center at West Virginia University Health Sciences Center, Morgantown, WV, USA; [3]Department of Pathology, Anatomy, and Laboratory Medicine, West Virginia University, Morgantown, WV, USA; [4]West Virginia University Cancer Institute, School of Medicine, Morgantown, WV, USA;

The intranasal route of administration for vaccines against SARS-CoV-2 and other respiratory pathogens is an attractive alternative to intramuscular delivery due to its potential to stimulate highly protective IgA-dominated immune responses in the upper airway's mucosal surfaces. As seasonal COVID-19 boosters become a plausible reality, we hypothesize that intranasal vaccines will afford the best protection and prevent transmission. We developed a virus like particle (VLP) based vaccine which utilizes hepatitis B surface antigen (HbSAg) conjugated using SpyCatcher technology to SARS-CoV-2 receptor binding domain (RBD) proteins from the Wuhan ancestral strain. The VLP-RBD vaccine is adjuvanted with BECC470 which has been utilized previously by our lab and determined to be a potent adjuvant for intranasal COVID-19 vaccines. In this study, K18-hACE2 mice were vaccinated with two doses of intranasal VLP-RBD+BECC470 and compared to mice vaccinated with two doses of intramuscular mRNA-1273. Pre-challenge, IN VLP-RBD mice had slightly lower IgG levels than IM mRNA-1273 mice. Serum antibodies from the IN group also displayed less RBD-ACE2 binding inhibition and neutralizing activity *in vitro*. After SARS-CoV-2 Delta variant challenge, IN VLP-RBD+BECC470 prevented the development of severe disease phenotypes and morbidity. These phenotypes were accompanied by high IgA production in the lung supernatant and nasal wash, as well as limited viral RNA burden in the nasal wash, lung, and brain. Interestingly, lung inflammation was limited at early timepoints as well as late in IN VLP-RBD mice. Together, these data prove that VLP-RBD+BECC470 vaccine is a highly effective intranasal COVID-19 vaccine in K18-hACE2 mice. Future studies will be performed to 1) evaluate the protection of VLP-RBD+BECC470 against SARS-CoV-2 Omicron challenge; 2) assess the VLP+BECC470 platform with novel antigens; 3) characterize the immune response to IN VLP-RBD+BECC470 in a preclinical comorbidity model.

Anti-*Pseudomonas aeruginosa* monoclonal antibody confers protection in a murine model of pneumonia and sepsis

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The emergence of antimicrobial resistance among *Pseudomonas aeruginosa* species has diminished the efficacy of antibiotics and driven a search for non-conventional treatment options. One alternative to the treatment of antibiotic-resistant infections is monoclonal antibodies (mAbs), which can target virulence factors, rendering bacteria non-virulent, and subject to immune system clearance. In this study, we developed anti-*P. aeruginosa* mAbs by immunizing mice with *P. aeruginosa* grown on *Pseudomonas* isolation agar supplemented with ammonium metavanadate, which induces a mucoid colony morphology. Monoclonal antibodies were formed using hybridoma technology, and we identified two IgG2b antibodies (S1F9 and S3D4) directed against the O-antigen of *P. aeruginosa* from serogroup O5. Bacterial survival assays revealed that these mAbs cause bacterial aggregation, which can enhance clearance by immune cells. To assess the efficacy of S1F9 and S3D4 *in vivo*, we used murine sepsis and pneumonia models of infection. Prophylactic treatment of mice with S1F9 and S3D4 markedly improved survival in a lethal sepsis model ($p<0.0001$). Furthermore, the number of viable bacteria was significantly reduced in the blood ($p<0.01$), spleen ($p<0.01$), and kidney ($p<0.01$). Mice were similarly protected in a pneumonia model of infection as prophylactic administration of S1F9 and S3D4 significantly reduced the bacterial burden in the lungs ($p<0.05$) and nasal passages ($p<0.01$). Overall, this study shows that our anti-*P. aeruginosa* mAbs directed against the O-antigen can be used to treat bloodstream and lung infections in mice caused by *P. aeruginosa*.

Identification of small compound antimicrobials against *B. pseudomallei* and ESAKPE pathogens using artificial intelligence and machine learning.

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Background: A pressing global issue today is the rise of antimicrobial resistant pathogens and halt in identifying novel antimicrobials that surpass gold standards. While there is a plethora of compounds to test for antimicrobial activity, screening compound libraries takes an insurmountable amount of time and resources. Objective/Hypothesis: Our objective is to utilize a high-throughput workflow for screening and identifying novel antimicrobials against resistant pathogens by employing the expansive power of artificial intelligence and machine learning. Methods: A library of 2200+ small compounds was screened for growth inhibition against *Burkholderia pseudomallei* Bp82, a multidrug resistant bacteria that is classified as a Tier-1 select agent. Growth conditions were optimized for cultures in 96-well plates using Mueller Hinton Broth. Compounds were added to a diluted overnight culture of Bp82 and OD_{600nm} was measured continuously for 24 hours. 200 antimicrobial compounds were selected based on 75% growth inhibition compared to normal growth and used as a training set for deep machine learning on compound structure and inhibitory activity. Results: Of the compounds screened, 109 non-antibiotics and 98 antibiotics were identified as having inhibitory activity. Compound substructures that were identified by deep machine learning on compound structural formulas were used to screen a larger library of ~10^6 compounds *in silico* for predicted inhibitory properties. Compounds were narrowed down based on predictive score and feasibility and assayed for minimum inhibitory concentrations. The selected compounds were next evaluated for growth inhibition against ESKAPE pathogens with promising levels of effectiveness. Conclusions: Here, we have employed a combination of experimental testing with machine learning to identify novel small compounds with antibacterial activity against antimicrobial resistant pathogens. Six candidates were selected based on low minimum inhibitory concentrations for future investigations into synergy with current treatment options or combination of selected candidates, effects on bacterial cell phenotype, and *in vivo* studies.

Loss of intestinal alkaline phosphatase disrupts acute post-stroke intestinal homeostasis

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Strokes are a leading cause of death and disability worldwide. While strokes are typically associated with the brain, it is widely known that stroke affects many peripheral organ systems. The gastrointestinal (GI) tract is a critical organ system that is affected during and after stroke, and the interaction between the GI tract and the brain in stroke is attributed to the modulation of the gut-brain axis. Intestinal alkaline phosphatase (IAP) is a brush border enzyme localized in the intestinal epithelium that regulates intestinal homeostasis by modulating inflammation and intestinal integrity. We hypothesized that IAP regulates the early post-stroke changes within the gut-brain axis, and that loss of IAP disrupts the gut-brain axis to worsen post-stroke outcomes. We utilized 4-6 month old male and female mice with a genomic deletion of *Akp3*, the gene that encodes for IAP in mice, and their wild type (WT) littermate controls. Following the induction of ischemic stroke by photothrombotic stroke (PTS), stroke-injured mice and their sham controls were for evaluated for bacterial load, intestinal permeability, intestinal motility, neurological deficits, and cerebral blood flow over the next 24 hours. Clark's neurological score, laser speckle flowmetry, and 2,3,5-triphenyltetrazolium chloride (TTC) staining showed a main effect of stroke but no genotype differences. While, intestinal motility was not altered at this early timepoint, *Akp3-/-* stroke mice showed increased intestinal permeability to 3kD-FITC-dextan, but not 10kD-Cascade blue-dextran compared to controls. Bacterial burden was also altered in the ileum with a loss of anaerobic bacteria in *Akp3-/-* mice post stroke compared to WT. Finally, *Akp3-/-* mice also showed a genotype specific increase in IgA levels in the plasma showing increased inflammation. The current results support the hypothesis that IAP shapes early post-stroke outcomes and modulates the gut-brain axis in ischemic stroke.

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Exposure to ground level ozone induces lung inflammation in an NLRX1 dependent manner

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A significant increase in cardiorespiratory morbidity and mortality is associated with acute episodes of air pollution increase. Ground level ozone is one of the most toxic gaseous components of the air pollution. NLRX1, a recently discovered NOD-like receptor, is implicated in multitude of cellular responses including inflammatory responses. However, its effect in mediating environmental toxicant induced lung insult is yet to be studied. We hypothesized that exposure to ozone can significantly modulate the lung inflammation via NLRX1. We exposed $NLRX1^{+}/^{+}$ and $NLRX1^{-}/^{-}$ mice to filtered air (controls) or O_3 (1 ppm) for 3 hours. The mice were euthanized 24 hours post exposure to quantify lung lavage cellularity, cell death, and lung inflammation. Our studies demonstrated significant increase in BALF neutrophil as well as inflammatory gene and protein (TNF-α, IL-1β, KC, TRAF-6, IFN-γ, IL-4, IL-6). The lack of NLRX1 gene was also associated with increase in myeloperoxidase activity and oxidant generation in myeloid cells, lung tissue and BALF. Mitochondrial function studies demonstrate attenuated mitochondrial function in O_3 exposed $NLRX1^{-}/^{-}$ mice compared to $NLRX1^{+}/^{+}$. In conclusion, these results indicate a significant role of NLRX1 in mediating O_3 induced lung inflammation. In our ongoing studies we are elaborating molecular mechanisms implicated in NLRX1 mediated altered immune cell trafficking and lung injury.

Effects of Walnut Consumption on the Gut Microbiota

Mansi Chandra and Regina Lamendella

It is still unknown as to which bacterial genes are active and what metabolic pathways areemployed by our gut microbiome which leads to potentially beneficial effects of walnut consumption on human health. This project is designed to study the effects of walnut consumption on the gut microbiome at both taxonomic and metabolic level.

In our experimental design, we put individuals on a random controlled feeding trial consisting of four groups: RunIn (standard western diet), WD (walnut derived), WFMD (fatty-acid matched diet devoid of walnuts) and ORAD (diet replacing ALA with oleic acid). Fecal samples were collected from these individuals, and samples were subjected to metatranscriptomics analysis to evaluate differential microbial gene expression across the diets.

According to our analysis, taxonomic richness did not differ significantly among the diet groups, but the functional gene richness did differ. Differential gene expression analysis revealed the

enrichment of an interesting microbe, Gordonibacter in the WD group. This bacterium is responsible for converting ellagitannins and ellagic acid to urolithins which is the primary form in which ellagitannins are absorbed by our body. The analysis of microbial gene expression data

showed differential abundance of various metabolic and biosynthetic pathways in the WD diet group, informing us more about the modulatory effects of walnut consumption on gut microbiota at the functional level.

This study has helped us in studying the effects of walnuts on human health and gain an insight as to what pathways are being active and which genes are being expressed that is leading to changes in the gut microbiota.

Impacts of Hydraulic Fracturing on the Microbial Communities in Headwater Streams of Northwestern Pennsylvania in 2019-2020

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The U.S recorded the highest natural gas export in 2021 and is projected a continuous growth through 2025. This is being made possible from the advancements in unconventional oil and gas extraction (UOG) processes such as horizontal drilling and hydraulic fracturing where highly pressurized complex fluids are injected deep into the ground to extract the trapped resources between rock formations. To date, there are over 13,000 UOG wells drilled with over 1,293 documented fracking spills in Pennsylvania. It has been well-studied that UOG activities negatively impact the environment and public health. Some environmental studies have investigated the effects of fracking on the surrounding stream ecosystems using microbial analysis and found significant differences in microbial communities between healthy streams vs UOG impacted streams. For this study, we are interested in investigating on active functional genes and species beyond the microbial community analysis, antimicrobial resistance genes (ARGs), and identify biomarkers for UOG activity. Thirteen Northwestern Pennsylvania streams were selected for water, sediment, and water chemistry samplings in the summers of 2019 and 2020. DNA and RNA were extracted from the samples and were processed for 16S rRNA and metatranscriptomic (MT) sequencing. Species richness analysis showed that there was a significant difference in microbial community between UOG sites in both data sets. Distinct clustering of samples was observed in the microbial community structure analysis plot, showing that there was a significant difference between streams. The order *Burkholderiales*, as previously discovered to be enriched in the UOG impacted sites, were also differentially abundant in this study and the ARGs enriched in this order include those associated with aminoglycosides, macrolides, and tetracyclines. These analyses provide a deeper understanding of how UOG activities impact the environment such as possible early detection in environmental changes due to UOG activities and better inform on environmental health and management.

Nitrogen deposition strongly influence the soil microorganisms and carbon biogeochemistry in arbuscular mycorrhizal ecosystem than in ectomycorrhizal ecosystem

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Globally, tree species and mycorrhizal fungi symbiosis have shaped soil environment and mediate belowground carbon processes. In temperate forest ecosystem, most tree species associate with arbuscular (AM) or ectomycorrhizal (ECM) fungi. The differences in physiology and nutrient acquisition of AM or ECM-ecosystem have resulted in distinct microbial communities, carbon biogeochemistry, and soil organic carbon stock. N-deposition is predicted to increased and can impact plant-microbe interaction. Since terrestrial ecosystem represent a large proportion of the C-pool, it is imperative to understand how N-deposition will influence microbial processes and soil organic matter formation within the AM and ECM-framework. This experiment examined the response of microbial community (activity, biomass, carbon use efficiency and composition) by utilizing soil from a long-term experimental watershed (fertilized and ambient) with established AM and ECM-stands. Additionally, quantitative stable isotope probing (qSIP) with ^{18}O-H_2O was used to explore taxon-specific growth efficiency. We found that fertilization (simulated N-deposition) had a stronger effect on AM than ECM-soil, in which there was a reduction in microbial respiration, substrate-derived respiration, biomass, and carbon use efficiency. Overall, N-deposition reduced the microbial diversity (prokaryotes and eukaryotes) in both AM and ECM-soil. Results from qSIP measurement showed that fertilization reduced the activity in Proteobacteria (growth) and Nitrospirae (growth and C-assimilation). AM-soil community showed a general reduction in growth and C-assimilation under fertilization. However, ECM-soil community were more variable in response to fertilization. Our findings suggest that AM-soil community maybe more effected by N-deposition than ECM-soil community. The reduction of microbial biodiversity, carbon use efficiency and increase in priming under fertilization suggest that N-fertilization may reduce soil carbon stock in AM than in ECM ecosystem.

Iron Bioremediation in Passive Remediation Systems Treating Acidic Abandoned Mine Drainage
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In Pennsylvania, 5,000 km of streams are polluted by abandoned mine drainage (AMD). Passive remediation of AMD can be carried out in a system composed of settling ponds, vertical flow ponds, limestone beds, and wetlands designed to increase the pH and precipitate metals onsite, preventing metals from entering the watershed. High levels of iron present in AMD can cause staining, damage water infrastructure, is associated with an increased risk of infection, may lead to cancer, and can be toxic. Since passive remediation systems are open to the environment, they are naturally colonized by native microbes that can impact the remediation within these systems. Bioremediation of iron can be increased by microbial oxidation of Fe(II) to Fe(III) causing it to precipitate from solution and remain within the treatment system. In acidic AMD, iron oxidation is mainly mediated by microbes. Within the acidic Boyce Park passive remediation system, we have isolated *Paraburkholderia sp.* AV18 that contributes to iron bioremediation through nitrate-dependent iron-oxidation (NDFO). Whole genome sequencing identified potential genes in AV18 that may be involved in iron oxidation (cytochrome c4 *cc4*) or nitrogen reduction (nitrate reductase *napA*). Novel primers have been developed for *cc4* and *napA* and the PCR product confirmed through amplicon size and sequencing. Determining the microbial mechanism used in NDFO and the genes involved will allow us to determine the effectiveness of iron bioremediation, by the abundance and expression of these genes, and allow for the development of bioindicators to address the effectiveness of the passive remediation system

Isolation of natural products from soil samples in Pennsylvania identifies two compounds with potent antibacterial activity against Bacillus anthracis
Authors: **Annalee Schmidt**, John Alumasa

Deadly bacterial infections such as anthrax continue to pose a significant threat to human health worldwide. This disease is caused by Bacillus anthracis, which is classified by the CDC as a Tier 1 biological agent due to its ability to form spores that are resistant to severe environmental stress conditions, including antibiotics. Identifying new antibacterial agents against this pathogen is therefore crucial for combatting anthrax infections. In this research, crude soil extracts from an Amish field in Jersey Shore, Pennsylvania were purified using various chromatography methods resulting in seven natural products, which were assessed for their antimicrobial properties. Minimum inhibitory and bactericidal assays revealed two compounds, AMS002 and AMS003, that inhibited growth and killed B. anthracis cells at 0.8 mg/ml and 0.2 mg/ml, respectively. Both compounds inhibited greater than 80% of translation relative to the control samples in cell-based and in-vitro fluorescence or luminescence reporter assays, indicating that they may be targeting the bacterial protein synthesis pathway as their primary mode of action. Nuclear magnetic resonance and mass spectroscopy analyses were used to confirm the structures and molecular masses of AMS002 and AMS003, suggesting potential novel discoveries. To identify genes associated with the antibacterial activity of these two compounds, resistant mutants of B. anthracis were generated through serial passaging to induce drug stress. Data from ongoing whole genome sequencing analysis will be used to localize mutations that are associated with the observed resistance and help distinguish which component(s) of the protein synthesis pathway are targeted by the isolated natural products. This discovery is important due to the resistant nature of B. anthracis spores and their potential use as a weapon of bioterrorism to cause widespread Anthrax infections.

Herbs and Spices Modulate Gut Bacterial Composition in Adults at Risk for CVD: Results of a Prespecified Exploratory Analysis from a Randomized, Crossover, Controlled-Feeding Study
Samantha Anderson

The effect of culinary doses of herbs and spices consumed as part of a well-defined dietary pattern on gut bacterial composition has not been previously studied. The aim of this prespecified exploratory analysis was to examine gut bacterial composition following an average American diet (carbohydrate: 50% kcal; protein: 17%; total fat: 33%; saturated fat: 11%) containing herbs and spices at 0.5, 3.3, and 6.6 g.d–1.2100 kcal–1 [low-, moderate-, and high-spice diets, respectively (LSD, MSD, and HSD)] in adults at risk for CVD. Fifty-four adults (57% female; SD age: 45 $\pm$ 11 y; BMI: 29.8 $\pm$ 2.9 kg/m2; waist circumference: 102.8 $\pm$ 7.1 cm) were included in this 3-period, randomized, crossover, controlled-feeding study. Each diet was provided for 4 wk with a minimum 2-wk washout period. At baseline and the end of each diet period, participants provided a fecal sample for 16S rRNA gene (V4 region) sequencing. QIIME2 was used for data filtration, sequence clustering, taxonomy assignment, and statistical analysis. Alpha Diversity assessed by the observed features metric (P = 0.046) was significantly greater following the MSD as compared with the LSD; no other between-diet differences in α diversity were detected. Differences in β diversity were not observed between the diets (P = 0.45). Compared with baseline, β diversity differed following all diets (P < .02). Enrichment of the Ruminococcaceae family was observed following the HSD as compared with the MSD (relative abundance = 22.14%, linear discriminant analysis = 4.22, P = 0.03) and the LSD (relative abundance = 24.90%, linear discriminant analysis = 4.47, P = 0.004). The addition of herbs and spices to an average American diet induced shifts in gut bacterial composition after 4 wk in adults at risk for CVD. The metabolic implications of these changes merit further investigation.

Going Nutty: Are Walnuts Beneficial for CV Health?
Jillian Leister

Background: It is unclear whether the favorable effects of walnuts on the gut microbiota are attributable to the fatty acids, including α-linolenic acid (ALA), and/or the bioactive compounds and fiber. Objective: This study examined between-diet gut bacterial differences in individuals at increased cardiovascular risk following diets that replace SFAs with walnuts or vegetable oils. Methods: Forty-two adults at cardiovascular risk were included in a randomized, crossover, controlled-feeding trial that provided a 2-wk standard Western diet (SWD) run-in and three 6-wk isocaloric study diets: a diet containing whole walnuts (WD; 57–99 g/d walnuts; 2.7% ALA), a fatty acid–matched diet devoid of walnuts (walnut fatty acid–matched diet; WFMD; 2.6% ALA), and a diet replacing ALA with oleic acid without walnuts (oleic acid replaces ALA diet; ORAD; 0.4% ALA). Fecal samples were collected following the run-in and study diets to assess gut microbiota with metatranscriptomic sequencing. Results: All three diet plans displayed enrichment of *Anaerobutyricum* (WD p= 1.42E-07, WFMD p= 2.78E-04, ORAD p=7.29E-05), *Gordonibacter* (WD p=8.20E-07, WFMD p=1.5E-04, ORAD p=1.04E-06), *Actinobacteria* (WD p=3.18E-05, WFMD p=1.5E-04, ORAD p=4.75E-05), and *Firmicutes* (WD p=0.013, WFMD p=0.011, ORAD p=0.002). Following the run-in diet, the phyla *Bacteroidetes* and *Proteobacteria* were both enriched. Conclusions: The genus *Gordonibacter* was similarly enriched in both WD and WFMD, likely due to similar fatty acid composition. *Gordonibacter* enrichment and its inverse association with cardiovascular risk factors suggests that the gut microbiota may contribute to the health benefits of walnut consumption in adults at cardiovascular risk.

Comparative meta-omics for identifying pathogens associated with prosthetic joint infection

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Prosthetic joint infections (PJI) are economically and personally costly, and their incidence has been increasing in the United States. We compared 16S rRNA amplicon sequencing (16S), shotgun metagenomics (MG) and metatranscriptomics (MT) in identifying pathogens causing PJI in collaboration with the Rothman Institute. Synovial fluid (SF) samples were collected from 30 patients, including 10 patients undergoing revision arthroplasty for infection, 10 patients receiving revision for aseptic failure, and 10 patients undergoing primary total joint arthroplasty. Analysis revealed distinct microbial communities between primary, aseptic, and infected samples using MG, MT, (PERMANOVA $p = 0.001$), and 16S sequencing (PERMANOVA $p < 0.01$). MG and MT had higher concordance with culture (83%) compared to 0% concordance of 16S results. Supervised learning methods revealed MT datasets most clearly differentiated infected, primary, and aseptic sample groups. MT data also revealed an increased number of antibiotic resistance gene annotations, with improved concordance results compared to MG. These data suggest that a differential and underlying microbial ecology exists within uninfected and infected joints. This study represents the first application of RNA-based sequencing (MT) to detect pathogens associated with PJI *in situ*. Further work on larger cohorts will provide opportunities to employ deep learning approaches to improve accuracy, predictive power, and clinical utility, potentially in multiple disease states

Purification and characterization of the iron-sulfur cluster containing ArxB2 from *Alkalilimnicola ehrlichii*

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The respiratory arsenite oxidase Arx is an anaerobic molybdoenzyme in the dimethyl sulfoxide reductase (DMSOR) family. The Arx operon encodes four protein subunits, the catalytic ArxA, the 4Fe-4S cluster containing ArxB and ArxB2, and a membrane anchoring ArxC. The function of ArxB2 has been hypothesized to influence the directionality of this enzyme as an oxidase instead of a reductase, but little is known about its structure or redox potential. Thus the goal of this work is to overexpress ArxB2 in *E.coli*, and characterize it (i.e., absorption spectrum oxidized vs reduced, quantification of iron-sulfur clusters). The *arxB2* gene with additional flanking restriction enzyme cut sites SalI and BamHI was PCR amplified from *Alkalilimnicola ehrlichii* strain MLHE-1 using Q5 polymerase. The *arxB2* PCR product was treated with dATPs and TAQ to generate A overhangs, then was ligated to pCR-4 TOPO vector and transformed into chemically competent *E. coli* TOP10 cells. Both pRSF-Duet vector and pCR-4 TOPO vector containing *arxB2* were digested with SalI and BamHI, then *arxB2* and linear pRSF-Duet were ligated together. pRSF-Duet with *arxB2* was transformed into chemically competent *E. coli* BL21 DE3 cells. After 3 hours growth in TB media at 37°C expression of *arxB2* was induced with 1mM IPTG . Cells were grown for an additional 3 hours at 37°C before being collected by centrifugation. The cells were lysed by French Press and the pellet, rich with protein in inclusion bodies, was collected by centrifugation. The pellet was washed several times and then dissolved in buffer containing 8M urea. Denatured ArxB2 was then purified with cobalt resin in metal affinity chromatography. The next steps will involve refolding the enzyme and reconstitution of the iron-sulfur clusters under anaerobic conditions. Phylogenetic analysis of ArxB2 was done using Raxml, with B and B2 subunits from other molybdoenzymes in the DMSOR family.

Import of a unique host-derived sugar *N*-acetylglucosamine 1-P by rickettsiae for biosynthesis of cell envelope glycoconjugates

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The global impact of rickettsial diseases is highlighted by historical records, reemergence of fatal arthropod-borne rickettsioses, and emergence of new pathogens. Our recent reconstruction of the *Rickettsia* metabolic and transport network identified 51 host-acquired metabolites needed to compensate for degraded biosynthesis pathways in these obligate intracellular microbes. The resultant host-dependent metabolic parasitism likely contributes to rickettsial virulence. Without glycolysis and the pentose phosphate pathway, peptidoglycan (PGN) and lipopolysaccharide (LPS) must be synthesized using host sugars. *N*-acetylglucosamine 1-P (*N*AG-1-P), a precursor of both PGN and lipid A disaccharide backbones (as well as other LPS sugars), is usually synthesized by bacteria using the bifunctional enzyme GlmU. Curiously, *Rickettsia* spp. contain a truncated GlmU enzyme with only the uridyltransferase domain, indicating rickettsiae do not synthesize GlcN-1-P. We speculate that rickettsiae import host *N*AG-1-P and convert it to UDP-*N*AG using a streamlined enzyme (GlmU_N) tailored to eukaryotic metabolism. Specifically, we hypothesize that rickettsiae utilize host-derived *N*AG-1-P for biosynthesis of cell envelope components and that such metabolite thievery from the host amino sugar biosynthesis pathway is essential for rickettsial intracellular replication and survival. To test our hypothesis, we are characterizing rickettsial uptake and metabolism of host *N*AG-1-P by monitoring bioorthogonal sugars incorporated into LPS. Preliminary data indicate that rickettsial growth is sensitive to limiting and excess *N*AG-1-P. We have isolated and characterized PGN, lipid A and core and O-antigen polysaccharide (COPS) from rickettsiae to trace azidosugar incorporation into cell envelope glycoconjugates. Our data revealed divergent lipid A structures for different *Rickettsia* species. Furthermore, we have identified novel sugars within the Rickettsia COPS. Collectively, our work will illuminate a novel rickettsial trait (GlmU_N and a unique *N*AG-1-P transport system) that stands as a promising target for therapeutics aimed at combatting fatal rickettsioses and the impact (pathogenesis) of *N*AG-1-P pilfering on host cell metabolism.

Mucosal Antibodies in Mild and Asymptomatic COVID-19

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SARS-CoV-2, the causative agent of COVID-19, has claimed more than 15 million lives worldwide, yet many healthy individuals remain disease free. To date, systemic vaccines containing the viral spike protein have effectively reduced disease fatality, but they have failed to block virus transmission, as breakthrough infections are a common occurrence. In the current study, we looked for the presence of antibodies against SARS-CoV-2 proteins in the saliva of “healthy” individuals at WVU to characterize the “protective” immune response against this virus. Some of these individuals had established exposure to the virus, with few or no disease symptoms, and some were vaccinated prior to saliva collection. We find that saliva from most of our subjects contained primarily IgA antibodies against the RBD domain of the spike protein and viral nucleocapsid (N). Such antibodies were absent in saliva collected prior to November 2019, suggesting recent exposure to the virus in our subjects, irrespective of RT-PCR test results and vaccination status. They also demonstrate that vaccination fails to induce/boost virus specific mucosal IgA and may therefore be unable to protect against virus transmission, a likely reason for breakthrough infection.

Defining the Mechanism by which *Mycobacterium tuberculosis* Inhibits TRAP-positive Syncytial Formation in RAW 264.7 Cells

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Osteoarticular tuberculosis (OAT) results from an extrapulmonary *Mycobacterium tuberculosis* (Mtb) infection. Healthy bones stem from the actions of two cell types: osteoclasts, which resorb bone, and osteoblasts, which create new bone. OAT is characterized by increased bone resorption due to enhanced osteoclastogenesis, resulting in bone erosion, joint swelling, and joint effusion. In osteoclastogenesis, monocytes form tartrate-resistant acid phosphatase (TRAP)-positive syncytia. Our preliminary data show that the coincubation of RAW 264.7 cells with Mtb extract results in the formation of TRAP-negative syncytia. To define the mechanisms by which Mtb extract inhibits the formation of TRAP-positive cells, we attempted to measure TRAP expression by qPCR and western blot in RAW 264.7 cells following coincubation with Mtb. Unexpected results revealed a decrease in total protein and RNA isolated from Mtb wells in comparison to RANKL and media-only control wells. To determine if decreased protein and RNA expression were a result of cell death, apoptosis and necrosis were assessed using FITC Annexin V and PI staining. Future experiments will determine the Mtb-dependent mechanisms by which TRAP expression is decreased in RAW 264.7 cells.

Exploration of new pertussis booster formulations with the BECC438b adjuvant

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With the knowledge that the protection afforded by acellular pertussis formulations is waning, there has been an effort to develop improved vaccine formulations. Options to improve the vaccines involve the inclusion of different antigens, the utilization of different adjuvants, and administration via different routes. While intramuscular (i.m.) vaccination provides a robust systemic immune response, intranasal (i.n.) vaccination induces a localized immune response within the nasal cavity. In the case of a pertussis infection, i.n. vaccination results in an immune response that is similar to natural infection, which provides the longest known duration of protection. Further, current acellular formulations utilize the alum adjuvant, which is thought to play a role in the waning immune response. With this experiment, we aimed to implement a new adjuvant, BECC438b – a TLR4 agonist – into both i.m. and i.n. acellular formulations to determine its ability to protect against pertussis infection in mice. We observed that DTaP + BECC438b reduced bacterial burden within the lung and trachea over DTaP alone for both i.m. and i.n. administration. Further, i.n. administration of DTaP + BECC438b induced a Th1 polarized immune response, while i.m. vaccination, regardless of formulation, only polarized toward a Th2 immune response, which does not afford long-term protection against pertussis. RNAseq analysis demonstrated the ability of BECC438b to activate completely different biological pathways when compared to what was seen in mice vaccinated with DTaP alone. It is of note that i.n. administration of DTaP + BECC438b activated a multitude of pathways associated with the immune system, data which is supported by i.n. vaccination having a higher total count of immunoglobulin genes. Overall, this data supports the use of the BECC438b adjuvant, with further benefits seen from i.n. vaccination in regard to the activation of immune pathways and the induction of immunoglobulin genes.

Quantification of Minimum Biofilm Eradication Concentration (MBEC) of Clinical Prosthetic Joint Infection Isolates of *Staphylococcus aureus*

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Prosthetic Joint Infection (PJIs) treatment involves the delivery of systemic and local antimicrobials. Dosing of these agents is guided by the Minimum Inhibitory Concentration (MIC) of identified pathogens, determined using planktonic bacteria. However, most PJIs are caused by bacteria within biofilm, which can have up to a 50,000-fold increase in the antimicrobial susceptibility when calculated using the Minimum Biofilm Eradication Concentration (MBEC). The purpose of this study is to quantify the MBEC of *Staphylococcus aureus* biofilms from clinical isolates and compare to the reported MIC. Our hypothesis is that the MBEC will be higher than the MIC. With IRB approval (#2203550953) clinical isolates of S. aureus from revision arthroplasty cases collected over two years were identified from the clinical microbiology registry. The MIC of each isolate was determined using the Vitek 2 Susceptibility Panel. To quantify the MBEC, four clinically relevant antimicrobials were tested: Daptomycin, Doxycycline, Oxacillin, and Vancomycin. Inoculums of each isolate were standardized and introduced into a 96-well MBEC Assay device, in which pegs remain immersed in the inoculum to form a biofilm. Pegs were then submerged in the antibiotics challenge plate for 20h. Finally, the remaining biofilm was determined via sonication, spot plating, and CFU quantification. Concentrations at which no viable bacteria remained were identified as the MBEC. At this time, testing is complete on two isolates. The MIC values for both isolates were: Daptomycin (0.25 µg/mL), Doxycycline (<0.5 µg/mL), Vancomycin (1 ug/mL) and Oxacillin(≤0.5 µg/mL). The MBEC ranges varied by samples with ranges for each antimicrobial and specimen listed: Daptomycin (>15,000µg/mL), Doxycycline (10-100 µg/mL), Vancomycin (2500-5000 µg/mL) Oxacillin (>27,000). The Minimum Biofilm Eradication Concentration (MBEC) for these clinical isolates was substantially higher than the Minimum Inhibitory Concentration (MIC) for each antibiotic tested. Continued evaluation of the discrepancy between these values should be explored.

Efficacy of intra-articular versus systemic vancomycin and Mitochondrial Response to In Vivo Prosthetic Joint Infection

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Disclosures: Nour Bouji (N), Ethan Meadows (N), John Hollander (N), Elizabeth Stewart (N), PI: Matthew Dietz (Guidepoint Consulting, Heraeus Medical, Peptilogics) Introduction: A primary cause of orthopaedic morbidity and mortality is prosthetic joint infection (PJI). Difficulties to treat PJI derive from microorganisms forming biofilms on necrotic tissue and alloplastic implants making systemic antibiotics outcomes unsatisfactory. Increasing evidence has demonstrated the importance of reaching a higher antibiotic concentration levels that are achieved with current systemic delivery techniques. This study investigates a comparison between systemic and intra-articular vancomycin administration for the optimal infection eradication, considering the potential for toxicity that might arise impacting the mitochondria. Methods: Using an established prosthetic implant associated *in vivo* model inoculated with a standardized quantity of methicillin sensitive *Staphylococcus aureus* (MSSA) *(*ATCC 25923), two groups were assessed evaluating treatment in these models comparing intraperitoneal (IP) (n=6) versus intra-articular (IA) (n=6) vancomycin treatment for 10 days. At postoperative day 20, tissue bacterial burden was quantified, and overall mitochondrial function and health were assessed using mitochondrial coupling assays in isolated mitochondria to measure oxygen consumption rate (OCR) and extracellular acidification rate (ECAR) in each group. Electron flow was also measured through the mitochondrial ETC (Electron Transport Chain) complexes to reflect their activity in each group. Standard statistical analysis was performed, and data were compared by unpaired two-tailed t-test. Results: The infected joint model receiving IP vancomycin revealed an average of $3x10^7$ CFU/g versus IA vancomycin revealed $8x10^5$ CFU/g ($p < 0.0001$). In the IA vancomycin treatment group, the OCR was higher when compared to the IP vancomycin treatment group ($P=0.02$). The evaluation of complex I, III, IV, and V activity demonstrated no difference (all groups $p > 0.1$) between the groups. Conclusion: Vancomycin administration has a higher rate of bacterial eradication; therefore, it is considered a potential alternative to systemic administration for PJI control due to its efficacy and its ability to reduce the impact of infection on mitochondrial function.

Empirical genomic methods for tracking plasmid spread among healthcare-associated bacteria

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Background: Healthcare-associated bacterial pathogens frequently carry plasmids that contribute to antibiotic resistance and virulence. The horizontal transfer of plasmids in healthcare settings has been previously documented, but genomic and epidemiologic methods to study this phenomenon remain underdeveloped. The objectives of this study were to develop a method to systematically resolve and analyze plasmids circulating in a single hospital, and to identify epidemiologic links that indicated likely horizontal plasmid transfer. Methods: We derived empirical thresholds of plasmid sequence similarity from comparisons of plasmids carried by bacterial isolates infecting individual patients over time or involved in hospital outbreaks. We then applied those metrics to perform a systematic screen of 3,074 genomes of nosocomial bacterial isolates from a single hospital for the presence of 89 plasmids. We also collected and reviewed data from electronic health records for evidence of geotemporal associations between patients infected with bacteria encoding plasmids of interest. Findings: Our analyses determined that 95% of analyzed genomes maintained roughly 95% of their plasmid genetic content at a nucleotide identity at least 99.985%. Applying these similarity thresholds to identify horizontal plasmid transfer identified 45 plasmids circulating among clinical isolates. Ten plasmids met criteria for geotemporal links associated with horizontal transfer. Several plasmids with shared backbones also encoded different additional mobile genetic element content, which were variably present among the sampled clinical isolate genomes. Interpretation: The horizontal transfer of plasmids among nosocomial bacterial pathogens is frequent within hospitals and can be monitored with whole genome sequencing and comparative genomics approaches. These approaches should incorporate both nucleotide identity and gene content preservation to study the dynamics of plasmid transfer in the hospital. Funding: This research was supported by the National Institute of Allergy and Infectious Disease (NIAID) and the University of Pittsburgh School of Medicine.

Poster session abstracts:

***Caenorhabditis elegans* as a Useful Model for Studying Duchenne Muscular Dystrophy**
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Duchenne Muscular Dystrophy (DMD) is an X-linked recessive disorder that causes a gradual loss of muscle function that affects everyday movements and activities and occurs in childhood. Animal models are used to investigate both the disease and the treatments. *Caenorhabditis elegans (C.elegans)* is a tiny, free-living nematode found worldwide that is useful for modeling human diseases because of the easy manipulation and cultivation in the lab, as well as a rapid life and reproductive cycles. This soil-dwelling creature contains the dystrophin protein in their muscles, making them ideal for studying DMD when there is a mutation in that gene. For instance, we are testing Ginseng to determine the effects of pure ginsenoside's active ingredient on the lifespan and muscle performance of our model organism. Ginseng is a type of plant. The root of Ginseng is helpful for treatments worldwide, such as in Asia to North America. Prednisone, one of the treatments in question is a corticosteroid and a prescription drug that most doctors prescribe to patients as their treatment plan for conditions such as arthritis, asthma, muscular dystrophy, and lupus. This study aims to provide an alternative treatment for Duchenne Muscular Dystrophy through Ginseng. We will be checking the following phenotypes development, thrashing and chemotaxis for any significant changes.

Effects of UV light exposure time and temperature on efficiency of PET plastic biodegradation using *E. coli* transformed with genes PETase and MHETase
Maya Scarpaci and Michelle M. Valkanas

Polyethylene terephthalate (PET) plastic is one of the most produced and polluted plastics on Earth, and can cause environmental harm when left in uncontrolled conditions as it breaks down into microplastics that contaminate soils and waterways. The bacterial strain *Ideonella sakaiensis* was first discovered in Japan with PET plastic degrading properties, which were linked to the genes PETase and MHETase (named after the enzyme they encode). However, this bacteria grows in harsh conditions and can be hard to utilize realistically. PET degradation is a slow process encompassing many stages making it difficult to predict how degradation would look in real world examples. It is well known that exposure to UV light partially degrades PET plastics and, as such, was used to examine the rate of degradation in partially broken-down PET. Plasmids pET21(+)-Is-PETase and pCJ136 were used in the experiment as they carried the PET degrading genes PETase and MHETase, respectively. Both plasmids however, contained the same antibiotic resistance gene to ampicillin. To combat this, plasmid pET21(+)-Is-PETase was modified to insert a kanamycin resistance gene in the area of the ampicillin resistance gene, therefore inactivating it and allowing for antibiotic bacterial selection. After transformation of the PETase and MHETase genes into *E. coli,* the bacteria were grown on PET plastic exposed to UV rays in the form of either sunlight or artificial UV light for varied amounts of time. It was hypothesized that plastic with the most UV exposure prior to the biodegradation process yields the highest rate and efficacy of plastic degradation using genetically modified *E.coli*. With the continuing accumulation of plastic waste in the environment, this method of PET plastic degradation is a hopeful, environmentally healthy and viable solution of managing our plastic waste, in order to further preserve the integrity and sustainability of the ecosystem around us.

Isolation and Characterization of Vibriophage Originating from Chesapeake Bay
Natalie Giacobe & Dr. Gregory Broussard
Bacteriophages have been suggested as an alternative therapy to unwanted bacterial pathogens. Specifically in marine environments, *Vibrio harveyi* is pathogenic to marine fish and invertebrates. Vibriophages, phages that infect *Vibrio* species, were isolated from oysters originating from Chesapeake Bay using the host bacteria, *V. harveyi*. Genome sequencing, annotation, and comparison was completed for all isolates revealing high similarity among most phages. Additionally, a host range assay was completed to determine what *Vibrio* species isolates can infect. Clear plaque mutants were purified from one phage isolate, Bennett. Clear plaque mutants suggest a higher frequency of infection lysis and lower frequency of lysogeny. Sequencing of Bennett clear plaque mutants is in process to determine lytic and lysogenic gene switches.

The Impacts of Hydraulic Fracturing on Microbial Communities in Stream Ecosystems in Northern Pennsylvania Using Metatranscriptomic Data

Cameron Trowbridge, The Nandar Su, Tai Pham, Regina Lamendella, Jeremy Chen See

Fracking allows natural gas to be removed from rock formations. From 2014 – 2050, the global usage of natural gas is predicted to rise from 90.8 to 218.2 quadrillion BTUs. In the summers of 2019 and 2020, water and sediment samples were collected from streams in northern Pennsylvania. For each sample, water chemistry and biomass data were collected, and DNA and RNA were extracted, amplified, and sequenced. 16S and Metatranscriptomics (MT) data were collected after sequencing. Samples were filtered into the following groups, all water samples, all sediment samples, upstream and downstream water samples, and upstream and downstream sediment samples. When comparing the beta diversities of UOG+ and UOG- samples, the beta diversities are significantly different for water and sediment samples in 16S and MT data. Active bacteria in the order Burkholderiales, an indicator taxon for fracking activity, were found to be more enriched in water and sediment samples that were near streams near a well pad (UOG+) than streams not near a well pad (UOG-). ARGs enriched in the Burkholderiales order include those associated with aminoglycosides, macrolides, and tetracyclines. Many fungal taxa were discovered to be contributing towards tyrosinase gene expression while cyanobacteria were one of the main contributors towards the expression of photosystem genes. Streams being exposed to fracking fluids can cause antibiotic resistance to develop in bacteria in stream ecosystems, the potential degradation of biocides which can have effects on the environment, and oxidative stress which can select for bacteria more genetically equipped to effectively handle oxidative stress. Overall, fracking can have many effects on microbial communities and the chemical properties of stream ecosystems which can negatively impact stream health and the environment.

pH influence on sulfate reducing bacteria growth in passive remediation systems treating abandoned mine drainage

Natalie Lamagna, Dr. Nancy Trun

Pollution resulting from abandoned mine drainage (AMD) is widespread across the nation and impacts thousands of miles of watersheds in West Virginia and Pennsylvania alone. AMD contaminants pose hazards to ecosystems and to human health by lowering the pH and by suspending toxic heavy metals in water. Microorganisms play a major role in the formation of AMD contaminants and ultimately determine their fate and removal. Specifically, sulfate reducing bacteria (SRB) are known to remove metals and sulfur species from mine drainage through the formation of metal sulfide precipitates and have been documented to raise the alkalinity of water. Therefore, it is necessary to understand the environmental conditions in which these microorganisms survive. It was hypothesized that SRB growth would be optimized in neutral pH, with decreased growth in acidic water. Slurry samples were collected from two circumneutral AMD passive remediation systems containing a pH above 7 and one acidic system with a pH below 5. Samples were diluted and plated onto a sulfide indole motility (SIM) medium, which consisted of an overlaying top agar to promote anaerobic conditions for growth of facultative anaerobes. Results indicate high numbers of facultative SRB in samples from the circumneutral remediation systems, while little to no growth was found in the acidic samples.

Temporal Transcriptomics of Gut E. coli in *C. elegans* Models of Aging
Sarah Cook

Host-bacterial interactions over the course of aging are understudied due to complexities of the human microbiome and challenges of collecting samples that span a lifetime. To investigate the role of host-microbial interactions in aging, we performed transcriptomics using wild type *C. elegans* (N2) and three long-lived mutants (daf-2, eat-2, and asm-3) fed *E. coli OP50* and sampled at days 5, 7.5, and 10 of adulthood. We found host age is a better predictor of the *E. coli* expression profiles as compared to host genotype. Specifically, age was significantly associated with clustering (PERMANOVA, p=0.001) and variation (Adonis, p=0.001, R2=11.5%) among samples whereas host genotype was not (PERMANOVA, p>0.05 and Adonis, p>0.05, R2=5.9%). Differential analysis of the *E. coli* transcriptome yielded 22 KEGG pathways and 100 KEGG genes enriched when samples were grouped by time point (LDA≥2, p≤0.05), including several involved in biofilm formation, amino acid utilization, and membrane transport. Co-expression analysis of host and bacterial genes yielded six modules of *C. elegans* genes that were co-expressed with one bacterial regulator gene over time. The three most statistically significantly bacterial regulators included genes relating to extracellular signaling, lipopolysaccharide production, and thiamine biosynthesis. Understanding changes to the microbial transcriptome over time is an important step towards elucidating host-microbial interactions and their potential relationship to aging. Co-expression analysis further revealed interactions between *E. coli* and *C. elegans* that occured over time, raising the possibility of similar interactions occurring between humans and their microbes.

Optimization of bioluminescent *E. coli (O1:K1:H7)* to evaluate bacterial translocation in a mouse model of stroke
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It is estimated that 30% of stroke patients develop an infection post-stroke, with the most common infections being urinary tract infections and post-stroke pneumonia. One potential mechanism of post-stroke infection is bacterial translocation, specifically the translocation of gastrointestinal bacteria into the circulation and to other organs. A commonly accepted way to evaluate bacterial translocation is by administering a bioluminescent bacterium and performing whole body imaging at various timepoints post-administration. *In vivo* bioluminescence of bacteria in mice can be detected and quantified non-invasively by the IVIS Spectrum CT instrument. The goal of this study is to optimize parameters for administration of bioluminescent *E. coli (O1:K1:H7)* to use as a tool to monitor bacterial translocation in mice post-stroke. First, a growth curve was created to track bacterial growth and to determine the concentration of the bacteria. Next, mice will be oral gavaged with various doses of *E. coli (O1:K1:H7)* to determine the optimal concentration for imaging. In addition, images will be taken at various timepoints post administration to optimize bacterial imaging parameters as well as the overall timeframe of translocation to other organs. Once this bacterium is optimized in mice we will move on to validating it within the photothrombotic stroke model followed by comparison of translocation mechanisms between wild type and knockout mouse strains. Overall, these studies will provide us with a powerful tool to improve our understanding of the mechanisms that regulate bacterial translocation post-stroke.

Effects of Novel Compound (ELP-004) on Multiple Myeloma Cell Proliferation and Associated Bone Degradation

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Multiple myeloma (MM) plasma cells cause bone erosion in patients by secreting factors that activate osteoclasts, cells responsible for bone degradation. Our lab has demonstrated that a novel small molecule (ELP-004) reduces proliferation of osteoclasts and prevents bone loss in mouse models of arthritis. We hypothesize that ELP-004 also inhibits proliferation in MM cell lines and reduces bone loss in MM mouse models. Here, MM cells were treated with increasing concentrations of ELP-004 to assess cell proliferation and apoptosis. In human (MM.1S and U266) and mouse (MOPC-315) cell lines, ELP-004 inhibited proliferation and increased apoptosis in a concentration-dependent manner. When used in combination with bortezomib, a drug used to treat MM patients, ELP-004 increased apoptosis as compared to bortezomib alone in U266 and MM.1S cells. To test whether ELP-004 reduces the bone erosion associated with MM, U266 cells were engrafted into NOG mice and ELP-004 was administered for ~6 weeks. Preliminary microCT analysis of the paws, femurs, and spines suggests that U266 cells have successfully engrafted, causing disease. Future studies are planned to engraft U266 cells into immunocompromised NSG mice as a model of MM. These data suggest that ELP-004 may be a promising drug to treat bone degradation in MM patients, increasing their quality of life and decreasing the burden of symptoms.

Addition of BECC-438 adjuvant to veterinary Vanguard vaccine increases antibody titers against Lyme disease.

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Borrelia burgdorferi is the main causative agent of Lyme disease in North America. Transmitted to humans via infected Ixodes deer ticks, this pathogen is predominantly found in the Northeastern United States. According to the CDC, there are approximately 400,000 reported cases each year, with numbers rising increasingly due to reforestation and suburban development. Although treatable when caught early, Lyme disease still results in life-changing health complications including chronic disease. As such, vaccinations are important for reducing associated mortality and morbidity. There are no licensed human vaccines against Lyme, but there are vaccines approved for veterinary use. These vaccines can be studied as a model for characterizing immune response to aid in the development of human vaccines against Lyme. One approved veterinary vaccine is Vanguard, a recombinant acellular vaccine composed of two outer surface proteins of *B. burgdorferi*: OspA and a chimeric OspC. In our murine vaccination and challenge model, Vanguard demonstrated short term efficacy and immunity wanes over time. The goal of this study is to determine if the addition of adjuvants to Vanguard would enhance immunogenicity and long term immunity. The adjuvants used were SWE, a squalene-water emulsion, and Bacterial Enzymatic Combinatorial Chemistry (BECC). C3H mice were vaccinated with either Vanguard + SWE, Vanguard + BECC-438, or Vanguard alone, boosted 21 days later and challenged with a GFP+ *B. burgdorferi* strain 35 days post prime vaccination. Immunogenicity of the vaccines were evaluated over time via ELISA. Increased IgG and IgM serum titers were found in the Vanguard + BECC-438 group in comparison to Vanguard alone. Two weeks after the challenge, bacterial burden was determined but no differences were observed. These results suggest that the addition of an adjuvant could improve the Vanguard vaccine, but further studies will be required to investigate the effect that BECC-438 has on the humoral and cellular immunological responses.

***Helicobacter pylori* evades the inflammatory response within the mouse gastric epithelium**

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Gastric cancer is the 5th most diagnosed cancer in the world. Infection by the bacteria *Helicobacter pylori* is associated with approximately 90% of gastric cancer cases. *H pylori* induces chronic gastric inflammation, which damages the stomach and fosters carcinogenesis. *Helicobacter felis* infection in mice is often used to study gastric carcinogenesis as the inflammation closely reflects *H pylori*-induced inflammation in humans. This research aims to identify the differences in the immune responses between *H felis* and *H pylori*-infected mice and their effects on the gastric epithelium. After two months of infection in mice, *H felis* induced widespread inflammation throughout the gastric corpus. In contrast, *H pylori*-induced inflammation was largely restricted to the gastric pylorus. The increased inflammation induced by *H felis* was associated with significant remodeling of the gastric glands and metaplasia development. Flow cytometry revealed more immune T cell infiltration associated with *H felis* infection, and PCR demonstrated that *H felis* induced a Th1-biased immune response compared to *H pylori*, which is associated with increased gastric cancer risk in humans.

The presence of RND efflux pumps in abandoned mine drainage can lead to antibiotic resistance

Bryce Lincoski and Michelle M. Valkanas

Abandoned mine drainage (AMD) is the result of outflow of acidic water and heavy metals from mining. Pennsylvania dominated the coal industry in the 1900's becoming one of the top producers of coal. Now, we are seeing the impacts. Not only is this very harmful to our environment but treating AMD can be costly. Passive Remediation Systems were built to combat AMD as it is inexpensive and requires little maintenance. Bacteria that live in these harsh conditions have found a way to survive such conditions. Resistance- nodulation -division (RND) is a type of efflux pump found in bacteria. RND affectively pumps out the harmful substances, such as metals, that the bacteria may encounter while in these systems. The presence and upregulation of RND efflux pumps allows the bacteria to survive in extreme environments. The bacteria pass through the remediation system and flow back into streams that are used as drinking water sources. The bacteria often use the same mechanism/ pumps when encountered with antibiotics. This research investigated the presence of RND efflux pumps in Lowber Passive Remediation System. Enrichment studies were performed to look for isolates utilizing RND efflux pumps and inhibition growth studies were performed to identify the impacts of RND efflux pumps on growth. It was hypothesized that the bacteria in these systems used RND efflux pumps in order to survive their environment causing them to become resitant to antibiotics. The application and knowledge of RND efflux pumps in AMD systems and downstream water systems can help us identify sources of antibiotic resistance before it becomes larger problem.

Comparison of 16S rRNA, rpoB, AutoMLST, and GTDB Trees in Evaluating the Position of Novel *Pedobacter* Species, as well as Known *Pedobacter* Species, in a Potential Reclassification

Lilly Saar and Jeffery Newman
Lycoming College

This analysis is a portion of a larger project on the reclassification of the *Pedobacter* genus of bacteria. This involves creating a new genus for the pink *Pedobacter* species, as well as incorporating several species previously classified in the genus *Pedobacter* into the existing genus *Nubsella*. Phylogenetic trees were constructed using the genomes of novel bacterial species isolated from a freshwater creek in Lycoming County, as well as close relatives in order to visualize the relationships among current members of the *Pedobacter* genus. Trees were created using the 16S rRNA gene, the rpoB gene, a set of 77 concatenated proteins (AutoMLST=Automatic Multi-Locus Sequence Typing) and a set of 120 proteins (GTDB=Genome Taxonomy Database). The different trees are compared in the context of their ease of construction, resolution, timeliness, and other factors to determine which tree would be the most appropriate to accurately illustrate the evolutionary relationships among the organisms.

Screening endocrine disruptors for their impact on glucocorticoid signaling and inflammatory disease
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Glucocorticoids are steroid hormones that suppress inflammation and promote resolution. Disruption of glucocorticoid signaling is associated with inflammatory and autoimmune diseases. Endocrine-disrupting compounds (EDCs) are chemical substances pervasive in the environment. The effects of EDCs on sex steroids have been established, but their effects on glucocorticoids are unknown. In this study, we investigated the effect of EDCs on glucocorticoid signaling. We utilized a glucocorticoid-responsive luciferase assay to screen chemicals for their ability to alter glucocorticoid transcriptional activity. In addition, we assessed if candidate EDCs disrupted the induction of GR-regulated genes. We found that the pesticide Metolachlor partially blocked GR signaling. We assessed the effects of Metolachlor on GR ex vivo suppression of LPS-induced inflammation using macrophages. Metolachlor significantly blocked glucocorticoid regulation of *Tnf.* Finally, mice treated with Metolachlor via drinking water exhibited a mild reduction in glucocorticoid-regulated gene expression and developed gastric inflammation. These studies suggest that Metolachlor elicits glucocorticoid resistance and may increase susceptibility to inflammatory diseases.

Investigating Antiviral Defense Mechanisms in *Pedobacter* Organisms
Isabella Adair Trotter

Clustered Regularly Interspaced Short Palindromic Repeats (CRISPR) systems are a group of highly variable prokaryotic adaptive immune mechanisms. They incorporate spacer sequences that align with invading viral and exogenous DNA to defend against phage infection (Pourcel et al. 2005). A CRISPR locus is composed of a leader sequence, spacers and repeats of similar lengths, and Cas proteins (Barrangou et al. 2007). CRISPR loci use highly conserved cas1 proteins, leader sequences, and incorporate spacer sequences in a directional manner (Karginov & Hannon 2010). These elements of CRISPR may be characteristics of closely related organisms and phylogenetically distinguish genera. Restriction-modification (RM) systems utilize endonuclease and methyltransferase pairs to recognize and cleave specific sequences in phage DNA (Bunjnicki 2001). RM systems are variable and have a traceable phylogenetic history (Bunjnicki 2001). This research seeks to characterize understudied *Pedobacter* organisms by investigating their antiviral defense systems present and identifying phages that target them. The genus *Pedobacter* is composed of organisms with genetic diversity that exceeds the bounds of a singular genus. By examining the distribution of CRISPR and different RM systems within the genus *Pedobacter*, we will determine whether the distribution of correlates with the proposed division into several smaller genera.

Genomic Analysis of the *Pedobacter* Genus
James Johnson*, Dr. Jeffrey Newman

The genus *Pedobacter* (family *Sphingobacteriaceae* in the phylum *Bacteroidota*) includes a very diverse group of organisms, many of which differ phenotypically from the type species of the genus, *Pedobacter heparinus*, The purpose of this study was to evaluate the hypothesis that *Pedobacter* should be split into several genera and if so, to designate type species of these new genera. In order to group organisms into the different genera, we used Average Amino Acid Identity (AAI), the Genome Taxonomy Database (GTDB) Tree, and Rapid Annotation using Subsystems Technology (RAST). Clustering based on the GTDB tree, which is based on alignment of 120 concatenated protein sequences from all genomes in NCBI RefSeq, divided the current *Pedobacter* genus into ten genus-level clades. RAST was used to perform pairwise sequence-based comparisons, which allowed us to calculate AAI values, and generate Venn Diagrams of shared and unique genes. The AAI values of 70% and above were within genus-level clades; and AAI values below 70% were indicative of different genera with only a few exceptions. The Venn Diagrams identified the unique genes present in members of the proposed new genera, which are being used to predict distinguishing phenotypes that can be tested in the laboratory.

The Effect of Efflux Pumps and Oxidative Stress on *Neisseria gonorrhoeae* Susceptibility to Resazomycins

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The increasing prevalence of multidrug-resistant *Neisseria gonorrhoeae* strains underscores the need for novel antimicrobials against this pathogen. Resazomycins, derivatives of resazurin (Rz), have shown robust antimicrobial activity against *N. gonorrhoeae* (*Ng*) *in vitro*. In vivo, however, resazomycins exhibit limited efficacy in a mouse model of gonorrhea. Previous experiments have shown that *N. gonorrhoeae* is more resistant to resazurin at oxygen levels comparable to those seen in host tissue (2%). We hypothesized this difference in susceptibility at low oxygen compared to atmospheric oxygen (~20%) was due to altered activity of multi-drug efflux pumps. To test this, we screened a selection of *N. gonorrhoeae* mutants that do not express or overexpress either the MtrCDE or NorM efflux pumps for Rz susceptibility. Overexpression of MtrCDE resulted in increased resistance to Rz at both 2% and ~20% oxygen suggesting resazurin may be a substrate of this efflux pump. Loss of expression of either MtrCDE or NorM had no effect on the increased resistance of *N. gonorrhoeae* to resazurin at low oxygen. We next sought to determine whether the increased susceptibility of Rz at 20% oxygen is due to oxidative stress. To test this, we tested the susceptibility of *N. gonorrhoeae* to Rz in the presence and absence of the antioxidants, cysteine hydrochloride (cysteine HCl) and Glutathione at 20% oxygen. When cultured with cysteine HCl, *N. gonorrhoeae* has a higher Rz MIC at 20% oxygen. The same was seen when glutathione was used but with less effectively than cysteine HCl. Here, we have shown oxygen concentration affects *N. gonorrhoeae* susceptibility to Rz likely due to increased oxidative stress.

The Effects of Gingerol on Duchenne Muscular Dystrophy and Fertility as Modeled in *C. elegans*

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Duchenne Muscular Dystrophy (DMD) is the most common form of muscular dystrophy; the gene affected encodes dystrophin, a protein that links actin filaments to other proteins in the muscular cell membrane for stability. *Caenorhabditis elegans* (*C. elegans)* is a common model organism for human diseases due to its entire genome being fully discovered and its short lifespan (~3 weeks) is ideal for undergraduate research. Currently, Muscular Dystrophy has been modeled in *C. elegans* and a variety of work has been completed including understanding the disease and testing different treatments. Gingerol is a compound found in ginger that is responsible for its pungent scent and spice. It is commonly used for medicinal purposes. There are significant research gaps with studies using gingerol as a treatment and its general effect on *C. elegans*, and none utilizing the DMD model. This study hopes to shed light on the potential use of Gingerol as a treatment for DMD in worms. It will focus on determining if phenotypes (eggs laid, muscle movement and development) changes upon treatment with Gingerol in both wild type and DMD animals.

Effect of Erythromycin on Virulence Gene Expression in Macrolide Resistant M92-type Group A *Streptococcus*

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Group A *Streptococcus* (GAS) is the causative agent of diverse human diseases, including superficial as well as invasive and systemic infections, and autoimmune post-infectious sequelae. Macrolides and clindamycin are used to treat invasive (iGAS) infections, such as flesh-eating disease and toxic shock syndrome. Both antimicrobials bind to 50S ribosome subunit and inhibit translation. We previously identified *emm92*-type isolates as a main cause of iGAS infections at J. W. Ruby Memorial Hospital clinical laboratory, which predominately affected adults with injection drug use history. Those isolates were uniformly resistant to macrolides and clindamycin. Here, we *hypothesized* that treatment of resistant *emm92* iGAS infection with macrolide-erythromycin affects translation-coupled bacterial transcriptome. Using an RNAseq approach, we investigated transcriptomes of *emm92* iGAS grown in cultures with or without erythromycin. Intriguingly, we observed increased expression of several key GAS virulence factors following antibiotic treatment that included genes encoding hemolysins *sls* and *slo*, capsule operon *hasA-C*, IgG-cleaving protease *ideS*, and superantigen *speJ*. The RNAseq data were further verified by RT-qPCR with gene-specific primers. Our data suggest that macrolide resistant iGAS show increased virulence upon antibiotic treatment, which paves the way for *in vivo* studies using animal models.

Determining Gene Function of *FYV8* and *ECM13* in *Saccharomyces cerevisiae*

Claire Magill and Dr. Jill B. Keeney, Juniata College

Despite *Saccharomyces cerevisiae* being an intensely studied model organism, there remain thousands of genes of unknown function. The Yeast ORFan Gene Project is an undergraduate and faculty research project to determine molecular functions of genes with unknown functions. I am studying *FYV8* (YGR196C), a protein of unknown function required for survival upon exposure to K1 killer toxin. K1 killer toxin is a protein secreted by *Saccharomyces cerevisiae* that kills sensitive yeast strains. I am also studying *ECM13* (YBL043W), a protein of unknown function induced by treatment with 8-methoxypsoralen and UVA irradiation that is thought to be involved in cell wall biosynthesis. Bioinformatics investigations reveal *FYV8* may be involved in a protein pathway that degrades killer toxins and that *ECM13* may be a nuclear protein. In an attempt to determine the function of *FYV8*, I created a deletion strain and compared growth to a wild type strain. Given that killer toxins interact with receptors in the cell wall, I tested the *FYV8* deletion and wild type strains on media that impact cell wall integrity. The *FYV8* deletion strain shows sensitivity to Calcofluor White. *ECM13* has an annotated phenotype for Calcofluor White sensitivity, so I made a double deletion strain of *FYV8* and *ECM13*. The double deletion strain is sensitive to Calcofluor White. Future experiments include testing the two single deletion strains and the double deletion strain on other media that disrupts cell wall organization, such as Congo Red and SDS, as well as attempting a K1 Killer Lawn Assay to confirm the killer toxin phenotype.

16S rRNA analysis of Drosophila to model gut microbiome of Parkinson's patients

Konner Foor, **Shveta Kalathur**m, Dr. Regina Lamdendlla, Juniata College: Department of Biology

Parkinson's Disease (PD) affects many individuals across the world. Finding a way to characterize the gut microbiome of affected individuals would go a long way in the rapid identification and treatment of Parkinson's symptoms. By studying the gut microbiome of Drosophila that have been bred to display Parkinson's symptoms we can identify differences in the gut microbiome between healthy wild type and glucosidase, beta acid 1 (GBA) deletion flies. So far through 16S rRNA, Illumina miseq sequencing a great difference in both alpha and beta diversity has been observed. This along with the different taxa that were being enriched between the mutant and wild-type flies. The current research supports that there is a significant difference between the gut microbiome of a mutant fly and a wild-type fly. It was also found that in GBA deletion flies, there was a distinct difference in the expression of glial cells when compared to wild-type flies. The findings of this study support that there are differences between the genotypes sequenced from the gut microbiomes. Further research will help to explain how microbes affect phenotypes. What we hope to characterize with future study is a way to quickly detect and identify a healthy gut microbiome when compared to a Parkinson's patient's gut microbiome.

Borrelia burgdorferi's Gene Expression Variance regarding N-acetylglucosamine (NAG/GlcNAc) Acquisition

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Lyme disease is the most common vector borne disease in the United States. It can present as both acute and/or chronic infections which can progress into multisystemic debilitating manifestations including neurologic, cardiac, rheumatologic, and integumentary pathologies. The spirochete *Borrelia burgdorferi*, the etiological agent of Lyme disease, is transmitted to mammals by the black-legged tick *Ixodes scapularis*. *B. burgdorferi* is characterized by a reduced linear genome with more than 20 circular/linear plasmids and lacks complete pathways for the production of required nutrients. Notably, *B. burgdorferi* lacks several genes required to synthesize N-acetylglucosamine (NAG, or GlcNAc), an important precursor of peptidoglycan and required for the bacterial cell wall. In mammals, NAG is readily present in the extracellular matrix and is thought to be imported by *B. burgdorferi* through an EII element of the glucose phosphotransferase system (PTS). In arthropods, NAG is present as the monomer of the abundant polysaccharide, chitin. The *B. burgdorferi* plasmid cp26 encodes a putative additional PTS (*chbABC*) thought to recognize chitobiose (the dimer subunit of chitin). Previous work using cultured *B. burgdorferi* has demonstrated a temperature-dependent switch in the expression of these two systems, with *chbABC* up-regulated at 27°C (arthropod-like) and EII at 37°C (mammalian-like). Further, we establish a laboratory infection model in order to study the expression patterns of these NAG import systems *in vivo* in both infected ticks and mice.

Deciphering the mechanisms of virulence and antibiotic resistance in emerging infection with invasive Group A *Streptococcus*

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Background: A growing number of skin and soft tissue (SSTI) infections are attributable to invasive group A *Streptococcus* (iGAS) in the United States. Ongoing CDC population-based surveillance in the U.S. and our recent studies in WV confirm that iGAS infections primarily affect mid-aged adults, particularly those with a history of intravenous drug use. These infections are caused predominately by newly emerging iGAS isolates of *emm*-type 92 that carry the *ermT* methylase gene, which encodes the MLS_B resistance phenotype. The *ermT* gene imparts cross-resistance to erythromycin and clindamycin, thus limiting the effectiveness of SSTI therapy. Importantly, the pathophysiology of *emm92*-type iGAS infections has not been studied in animal models. Objectives: Our research *objectives* were to elucidate mechanisms of MLS_B resistance and to establish a murine model of SSTI for *emm92* iGAS. Methods and Results: MLS_B resistance to clindamycin is either constitutive ($cMLS_B$) or inducible ($iMLS_B$). Analysis of *ermT* promoter regions identified sequence polymorphisms in isolates with $cMLS_B$ resistance to clindamycin, which comprised 11% of the *emm92*-type isolates. Biofilm formation also potentiates antibiotic resistance and virulence in bacteria. *In vitro,* 24-hour biofilm formation by *emm92* iGAS was comparable with the biofilm formed by *emm3* iGAS, as quantified spectrophotometrically following crystal violet staining. Subcutaneous injection of *emm92* into hairless immunocompetent SKH1 mice produced increased skin pathology in a dose dependent-manner. Two-photon fluorescence microscopy, performed on skin lesions excised from mice infected with a GFP-expressing *emm92* isolate, showed bacterial microcolonies co-localized with the TRITC-conA stained glycocalyx. Conclusions: Rapid emergence of antibiotic-resistant *emm92* iGAS across the U.S. emphasizes the need for further investigation. Here we determined that insertions in the *ermT* promoter sequence facilitate constitutive gene expression and clindamycin resistance. Establishment of a murine model for *emm92* iGAS infections will allow characterization of this increasingly important *emm* type of GAS to aid pre-clinical studies.

***Caenorhabditis elegans* as a Useful Model for Studying Duchenne Muscular Dystrophy**
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Duchenne Muscular Dystrophy (DMD) is an X-linked recessive disorder that causes a gradual loss of muscle function that affects everyday movements and activities and occurs in childhood. Animal models are used to investigate both the disease and the treatments. *Caenorhabditis elegans (C.elegans)* is a tiny, free-living nematode found worldwide that is useful for modeling human diseases because of the easy manipulation and cultivation in the lab, as well as a rapid life and reproductive cycles. This soil-dwelling creature contains the dystrophin protein in their muscles, making them ideal for studying DMD when there is a mutation in that gene. For instance, we are testing Ginseng to determine the effects of pure ginsenoside's active ingredient on the lifespan and muscle performance of our model organism. Ginseng is a type of plant. The root of Ginseng is helpful for treatments worldwide, such as in Asia to North America. Prednisone, one of the treatments in question is a corticosteroid and a prescription drug that most doctors prescribe to patients as their treatment plan for conditions such as arthritis, asthma, muscular dystrophy, and lupus. This study aims to provide an alternative treatment for Duchenne Muscular Dystrophy through Ginseng. We will be checking the following phenotypes development, thrashing and chemotaxis for any significant changes.

Glucocorticoids moderate T cell activation during *Helicobacter* infection
Sara R. Druffner, Maeve T. Morris, Jordan P. Pascoe, Benjamin C. Duncan Jonathan T. Busada

Gastric cancer is the world's fourth leading cause of cancer deaths, and almost all gastric cancer cases are associated with *Helicobacter pylori* infection. Infection with this bacterium causes chronic, T cell-driven inflammation within the gastric mucosa that leads to the development of a pre-neoplastic condition known as spasmolytic polypeptide-expressing metaplasia (SPEM). In this study, we investigated how glucocorticoids regulate T cell responsiveness to *Helicobacter* infection and how these cells contributed to the development of gastric pathologies. We adrenalectomized (ADX) mice to remove endogenous glucocorticoids and infected them with *H felis* within a week of surgery. Two months post-infection, flow cytometry revealed that ADX-infected mice had significantly more T cell infiltration and a greater proportion of activated T cells. Quantitative RT-PCR for characteristic Th1 genes (*Tnf* and *Ifng*) and Th2 gene, *Il13,* indicate that ADX-infected mice have a more Th1 skewed phenotype while intact-infected mice have a more Th2 skewed response. Our results suggest that glucocorticoid signaling influences the T cell phenotype in response to *H felis* infection.

Acid Mine Drainage, Passive Systems, and Their Effects on Microbial Communities
Alivia R. Yauger and Michelle M. Valkanas

Acid Mine Drainage (AMD) is a wide-spread surface water pollutant that occurs when water travels over/through sulfur-harboring elements. This pollution can form extremely acidic solutions, and the majority of AMD in Pennsylvania comes from abandoned coal mines near water sources. AMD causes economically concerning outcomes that impact drinking water, wildlife health and their ability to reproduce, and erosion that is irreversible. One way to treat this issue is through passive remediation systems which consists of a series of ponds that contain heavy metal oxidizing bacteria; this helps to remove heavy metals and raises the pH of the water. However, within these systems are also reducing bacteria that work against the remediation efforts, reducing (solubilizing) the metals back into the water. Samples from Middle Branch Passive Remediation System (Elk County) were collected, and bacteria were isolated to test their ability to reduce heavy metals, specifically manganese reducing bacteria. Of the isolates screened, four (CF3R, CF7R, CF8R, and CF6A) had previously showed a potential to reduce manganese. This work looks to further characterize these isolates and validate their ability to reduce manganese. Having bacteria that reduces manganese is counter productive in a passive remediation system, knowing the properties of these manganese reducing bacteria can be used to optimize the treatment of heavy metals in passive remediation systems.

Functional Characterization of UBP11 in Saccharomyces Cerevisiae By Inducing Environmental Stresses of UV Light Exposure, Varying Temperatures, NaCl and Ethanol Concentrations.

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Mount Aloysius College

Saccharomyces cerevisiae utilizes ubiquitination to temporally and spatially control protein and various other nonproteolytic functions. Ubiquitination is a form of post-translational modification in which the ubiquitin-protein is covalently attached to a substrate protein. The UBP11 gene in *S. cerevisiae* is known to be involved in ubiquitination, by acting in the deubiquitination of proteins. The gene is thought to cleave or remove ubiquitin from protein targets. The UBP 11 gene has orthologs in many eukaryotic organisms, including humans. Although the molecular function of *upb11* is known, its biological process and cellular component are unknown. Previous research and bioinformatic analysis has indicated its involvement in response to stress. Here, we analyze *S. cerevisiae ubp11* mutants morphological and growth responses to stress by performing the following environmental stress inducing assays: exposure to UV light (DNA damage), extreme temperatures, and NaCl and sorbitol. It is predicted that the *ubp11* mutants will have a similar CFU and cell budding count to UV light and extreme temperatures. The mutant will have a faster growth rate in NaCl and sorbitol compared to the wild-type strain.

A program to retrieve information from NCBI using assembly accessions in a Newick-formatted phylogenetic tree.

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The Genome Taxonomy Database has created a phylogenomic tree based on an alignment of 120 concatenated protein sequences derived from over 300,000 Bacterial genomes, including many Metagenome Assembled Genomes (MAGs). This massive tree can be downloaded and searched for genomes of interest, and subtrees of desired clades can be produced and saved a Newick files, however the labels at the tips of the branches are assembly accessions (GCF or GCA), which are not very meaningful to a viewer. In past years, students from our lab have manually looked up the organisms' names using the accessions, but this we very tedious and time consuming. In this poster, we describe programs initially coded in python and then java that take a tree in the form of a .nwk, .tree, or .txt file, isolates the accessions inside and searches each accession within the NCBI assembly database. The name, strain, and total sequence length is then isolated from the webpage, and the accession within the original file is supplemented with the newly found name and strain designation for inclusion in the Newick file as branch labels. The program can fully process 0.5 accessions per second on average, making the time that it takes to construct relatively large trees only minutes instead of hours/days. In addition, other desired information such as genome size or GC% in the genome can be retrieved from the NCBI assembly database and presented as a spreadsheet in the same order as they are listed in the tree. The advantage of this is that it allows evolutionary patterns in genome size or GC% to be detected more easily.

The effect of TiO2 on the gut microbiome.

Evan Thomas and Regina Lamendella

Titanium Dioxide (TiO_2) is a food additive used in processed foods as a whitening agent. This food additive which was banned in multiple countries across the EU is hypothesized to be having adverse effects on the gut microbiome. Using High-throughput sequencing we aim to investigate the bacterial profiles of the gut microbiota by using 16S rRNA gene sequencing. A comparison of high and low exposure (n= 80 people, n=240 samples) groups will be performed to investigate potential impacts on the gut microbiota. Additionally, we aim to perform a metatranscriptomic analysis on the samples from extracted RNA. This data will show which microbes and metabolic pathways are activated by looking at their gene expression. All these data integrated with human metadata will help us determine the potential effects of TiO_2 on the gut microbiome and human health.

Determining Gene Function of *YAL034C* and *YOR338W* in *Saccharomyces cerevisiae*
Hailey Hendricks and Dr. Jill B. Keeney

The yeast, *Saccharomyces cerevisiae*, is widely studied, yet the function of nearly 10% of genes is unknown; my research focuses on these genes of unknown function and strives to determine function through a series of biological processes. I study *YAL034C* and *YOR338W,* which are homologs of one another*. YAL034C* is a non-essential protein of unknown function with gene ontology terms of molecular function, biological process, and cellular component annotated as unknown. *YOR338W* is a putative protein of unknown function with gene ontology terms of molecular function and cellular component annotated as unknown and a biological process annotated for ascospore formation. A series of bioinformatic modules revealed that both *YAL034C* and *YOR338W* may be localized to the nucleus. In an attempt to determine the function of *YAL034C*, I deleted the *YAL034C* ORF to study phenotypic differences from wild type in specific growth conditions. Deletion does not impact growth rate. Wildtype and deletion strains were treated with 2-deoxy-D-glucose, a molecule in which the 2-hydroxyl group is replaced with hydrogen, blocking further metabolism. Results showed that 2-deoxy-D-glucose had no effect on *YAL034C*. A double deletion with *YAL034C* and *YOR338W* was successfully constructed. Future experiments include the testing of the *YAL034C* deletion and the double deletion on YPD plates supplemented with copper sulfate. I previously found that *YOR338W* expressed sensitivity to copper sulfate. I have reason to believe that *YAL034C* may express some sensitivity to copper sulfate as well given its relationship to *YOR338W*.

16s rRNA Analyses of Microbial Communities at Tytoona Cave, Pennsylvania, USA
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Caves are unique subterranean ecosystems formed over evolutionary time that harbor a variety of both obligate and facultative organisms. Microbial communities within caves may often be limited in diversity than their exterior counterparts, but the microbes present within caves contribute to the formation of caves and overall ecosystem health through their metabolic pathways through various geochemical and nutrient cycling processes. Additionally, microbial communities within caves may represent a novel approach to modern medicinal advancements. Tytoona Cave in Pennsylvania, USA, is understudied and represents an exceptional study area for bacterial communities based on regional geology. To determine what bacteria were present at Tytoona Cave, we sampled interior cave sites (n = 9) and exterior cave sites (n = 9) for a total of 18 samples across the two locations. We extracted RNA from filtered water samples, targeted the 16s rRNA gene for sequencing, and analyzed reads through the QIIME2 bioinformatic pipeline to determine community assemblages at Tytoona Cave. ~145,000 reads were obtained through quality filtering and decontamination. Four major bacterial phyla (Proteobacteria, Bacteroidota, Actinobacteriota, and Verrucomicrobiota) were determined to represent 82.5% of the microbial diversity across both exterior and interior locations. Significant differences (p = 0.02) in beta diversity were determined from exterior to interior locations. No differences were determined through alpha diversity analyses (p > 0.05). Significant correlations (p < 0.05) were found between total dissolved solids and alpha values. LeFSe generated data showed significantly enriched species in both exterior and interior locations. The results offer insight about the microbiomes present at Tytoona Cave and their predictive metabolic functions. Determining microbial communities present at Tytoona Cave and monitoring these communities provides additional support for the conservation of the cave and surrounding habitat.

Amino acid mimicry: Insights into glyphosate transport and mitochondrial toxicity

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Glyphosate, the most widely used pesticide in the world, directly inhibits the shikimate pathway. Even though humans lack this pathway, a number of reports have indicated various toxic effects, suggesting that glyphosate targets other pathways, too. In *Saccharomyces cerevisiae*, deletion of Dip5, a membrane glutamate/aspartate transporter, confers resistance to glyphosate, suggesting that despite its classification as a glycine analogue glyphosate resembles the size and charge distribution of glutamate and enters the cell through Dip5. Additionally, previous work from our group demonstrated that glyphosate changes expression of mitochondrial associated genes. Therefore, we tested if mitochondrial glutamate transporters are able to import glyphosate by determining if their deletion confers resistance, similar to *dip5* knockouts. Here we show that loss of two mitochondrial glutamate transporters in *S. cerevisiae* confers resistance to glyphosate. We further establish a dose equivalence between pure glyphosate and a commercial formulation, indicating that commercial formulations are more potent, perhaps due to increasing permeability through surfactants. We then examine the effects on nutrient availability on glyphosate and commercial formulation toxicity. This further corroborates the hypothesis that the structural similarity of glyphosate to glutamate allows it to utilize glutamate permeases to enter the mitochondria. This evidence also indicates that glyphosate likely has mitochondrial off-targets, potentially suggesting mechanisms for toxicity in organisms that rely on their diets for aromatic amino acid intake. We anticipate our results to be a starting point for more in-depth analysis of the effects of glyphosate on mitochondrial function, as well as further work on confirming the mechanisms of intracellular and intramitochondrial glyphosate transport.

Anti-inflammatory and anti-microbial activity of recombinant alkaline phosphatase (recAP) in a mouse model of sepsis

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Sepsis is a severe systemic immune response to infection in the body. Multi-organ failure associated with this response contributes to a high morbidity and mortality among sepsis patients. Currently, there are no FDA-approved therapeutics to treat sepsis other than supportive therapies. Alkaline phosphatase (AP) is an enzyme present throughout the body and is known to have anti-inflammatory properties, which lead us to hypothesize that it will exhibit similar systemic activity in sepsis. Recombinant alkaline phosphatase (recAP, AM-Pharma) is a drug currently in Phase III clinical trials to treat sepsis-associated acute kidney (SA-AKI.) and has been proven to reduce mortality by over 40% in these patients. RecAP is a chimeric protein which has the stability domain of placental alkaline phosphatase and the catalytic domain of intestinal alkaline phosphatase. The goal of this pilot study is to test the efficacy of recAP on survival, bacterial load, sepsis severity scores, and inflammation in a cecal ligation and puncture (CLP) model of sepsis. CLP was induced in male and female C57BL/6 age matched mice between 6 and 8 months old. Following CLP, mice were injected intraperitoneally with recAP (1.6mg/kg) at 3 hours post-surgery followed by daily injections for four days; controls were injected with a vehicle. The modified murine sepsis score (MMSS) was used to assess morbidity and sepsis severity and was recorded daily, while organs and fecal matter were harvested for assessment of bacterial load and inflammation. Results showed that recAP treatment decreased day 1 MMSS scores. Bacterial load within the ileum and liver were also altered following recAP treatment. Lastly, the proinflammatory cytokine interleukin-6 (IL-6) was decreased in the duodenum of mice treated with recAP. These results demonstrate a beneficial role for recAP in sepsis outcomes. Further experiments are ongoing to increase statistical power and evaluate additional intestinal and systemic endpoints.

Sex differences in 4-NQO-induced Head and Neck Squamous Cell Carcinoma Anti-Tumor Immunity

***Sabrina Siegan*[1]*, Quinn Hopen*[2]*, Carly Amato-Menker*[1,2]*, and Jennifer Franko*[1,2]

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Sex differences in head and neck squamous cell carcinoma (HNSCC) incidence/mortality rates exist. In West Virginia, the annual average-age adjusted incidence rate for oral cancer is 20.1%:7% (male-to-female). Potent anti-tumor immune responses play an important role in inhibiting HNSCC. However, it is not known if sex-specific anti-tumor immunity differentially influences tumor growth. To evaluate this, HNSCC was induced in male and female mice via 4-nitroquinoline-N-oxide (4-NQO) exposure. Splenic phenotypes were assessed by flow cytometry and draining lymph nodes were collected for gene expression analysis. As expected, male mice developed more tumors, that were larger in size and occurred at earlier time points. Consistent with a stronger anti-tumor response, higher percentages of CD4+ and CD8+ T cells were identified in the spleens of female mice. Future studies will evaluate the expression of immune activation markers in the spleen and lymph nodes, as well as tumor-infiltrating immune cell populations, in 4-NQO exposed mice. By delineating the mechanisms that contribute to HNSCC susceptibility, progression and immunotherapeutic efficacy, novel strategies may be developed to prevent and/or improve the treatment of HNSCC.

Characterization of Microbial Composition of Dental Aerosols

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Dental aerosols (DAs) can contain microbes capable of transmitting disease. Although larger aerosols generated during dental procedures have been evaluated, the microbial composition of smaller thoracic (<10 μm) and respirable (<4 μm) DAs, capable of reaching the lungs following inhalation, has not been thoroughly evaluated. Here we aim to develop a method to identify oral microbes in respirable and thoracic DAs, if present. To accomplish this, air sampling was performed prior to and during aerosol generating dental procedures using personal DataRAMs (pDR-1500, Thermo Scientific) to capture thoracic and respirable DAs onto 37mm PTFEfilters. Filters were then tested for microbial content. Strategy 1 - DNA was extracted directly from filters and PCR amplified (16s rRNA gene) to detect bacteria and prepare for metagenomic analysis. Strategy 2 – Filters were cultured in trypticase soy broth prior to DNA isolation. Adequate concentrations of DNA were not isolated from filters using Strategy 1 for detection. However, bacterial growth resulted in the isolation of high-quality DNA from the filter cultures using Strategy 2. Future studies will optimize Strategy 2 to detect oral microbes in DAs using quantitative real-time PCR and investigate the efficacy of commercial dental evacuation devices to minimize generation of potentially infectious DAs.

Characterizing Manganese Reducing Bacteria that Contaminate Abandoned Coal Mine Drainage Remediation

Kayla Brennan

Abandoned coal mine drainage (AMD) carries several contaminants including manganese, a toxic metal. This metal has been found in its solubilized form in certain locations throughout the Wingfield Pines neutral AMD passive remediation system. Solubilized manganese contributes to pollution as it can travel through the system into the watershed. Certain microbes increase manganese pollution by re-solubilizing manganese through reduction pathways. However, the specific site location, abundance, genera and the impact on AMD remediation of manganese reducing bacteria (MnRBs) at Wingfield Pines has not yet been determined. Each pond in the Wingfield Pines system was screened for the frequency of manganese reducing bacteria. Twelve bacterial isolates that preliminary show manganese reduction were purified and their genomes are being sequenced for identification. Current data shows that MnRBs are present throughout the entire system and a few strains are capable of resolubilizing amounts of manganese higher than what the EPA allows. To have a better understanding of the impact of manganese reducers, the exact amount of manganese re-solubilization at the remediation system will be determined.

Context matters: environmental microbiota of ice cream processing facilities affects the inhibitory performance of two lactic acid bacteria against *Listeria monocytogenes*

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Introduction: Lactic acid bacteria which produce compounds inhibitory to *Listeria monocytogenes* may complement cleaning and sanitizing procedures in dairy processing facilities; however, it remains unknown whether the environmental microbiota of a dairy processing facility affects the antilisterial activity of biological control agents. Purpose: The antilisterial activity of two lactic acid bacteria, previously tested in poultry processing facilities, was evaluated in the presence of environmental microbiota of three ice cream processing facilities (A, B, and C). Methods: Lactic acid bacteria were co-cultured in polypropylene tubes containing BHI for 3 days at 15°C with and without an 8-strain cocktail of *L. monocytogenes*, in the presence of the environmental microbiota collected from ice cream processing facilities. After incubation, the concentration of attached *L. monocytogenes* was quantified using the MPN method, and the attached biomass was characterized by sequencing of the 16S rRNA V4 gene region. Results: *L. monocytogenes* concentration increased compared to the positive control by 0.38±0.42 log MPN/ml in the treatments with the microbiota of Facility A, while it decreased by 0.99±0.59 and 2.54±0.45 log MPN/ml for all treatments when in the presence of the microbiota of facility B and C. The attached biomass had a high relative abundance of *Pseudomonas* and *Enterobacteriaceae* in facility A, of *Pseudomonas* in facility B, and of *Enterococcus* in facility C. Based on these results, we hypothesize that the presence of psychrotrophs, like *Pseudomonas*, may prevent adhesion of lactic acid bacteria to the surface reducing their inhibitory action against *L. monocytogenes*. Significance: Our study showed that the presence of certain bacteria can affect the attachment and inhibitory action of strains of lactic acid bacteria. Further work is being conducted to understand how *Pseudomonas* attach and may prevent inhibition of *L. monocytogenes* by lactic acid bacteria, and the optimal conditions for efficient inhibition of *L. monocytogenes* in dairy processing environments.

Role of FTL_0895 in *Francisella tularensis* Susceptibility to Resazurin

Claire Kelly, Emily Young, Jordan Gibson, Siena McGovern, Emma Beatty, Kendall Souder, Justin Rice, Ryan J. Percifield, Donald A. Primerano, Nicole Garrison, and Deanna M. Schmitt

Resistance to antibiotic treatments coupled with the decline in antibiotic discovery has resulted in a steady increase in deaths caused by once “curable” bacterial infections. Developing new drugs is crucial to prevent more loss of life in the future. We discovered the compound resazurin exhibits antimicrobial activity against gram-negative bacteria including *Francisella tularensis (Ft),* however, certain strains of *Ft* have developed resistance to resazurin. Whole genome sequencing of resazurin-resistant (Rzr) *Ft* LVS mutants revealed 93% of the strains contained mutations within the coding regions of FTL_0421, FTL_0895, and FTL_1504. The focus of my project was to explore the role of FTL_0895 in resazurin susceptibility. To confirm this gene plays a role in the reduced susceptibility of the Rzr strains to resazurin, we cloned the wild-type copy of FTL_0895 into the *Francisella* vector pABST which contains the robust *groE* promotor of *Ft.* The resulting plasmid was electroporated into Rzr1 and we tested the susceptibility of the complemented strain (Rzr1/pABST-FTL_0895) to resazurin. Both Rzr1 and Rzr1/pABST-FTL_0895 have the same minimal inhibitory concentration (MIC) of Rz suggesting expression of wild-type FTL_0895 in Rzr1 did not restore sensitivity to Rz. This data also suggests mutations in FTL_0895, FTL_0421, and FTL_1504 may be functioning synergistically to confer resistance to resazurin. In the future, we plan to complement back Rzr1 with wild-type copies of FTL_0895, FTL_0421, and FTL_1504 in different combinations and then test the Rz susceptibility of these strains to fully elucidate the contribution of FTL_0895 to the bactericidal action of resazurin.

Genomic comparisons between pink and non-pink *Pedobacter* species
Carter Branigan

The genus *Pedobacter* includes both pink and non-pink species, and the pink species are clustered into a distinct phylogenetic clade. The purpose of this study was to define differences in the sets of genes shared within each group that are not present in the other group. Full genome analysis using the Sequence-based comparison tool on the Rapid Annotation using Subsystems Technology (RAST) website identified orthologous genes for construction of Venn diagrams and calculation of average amino acid identity (AAI) values. Our custom Venn Diagram Tool identified 589 unique coding sequences present in the pink strains that were absent in the non-pink strains. There were 309 coding sequences shared by four representative non-pink strains that were absent in the representative pink strain. These coding sequence differences are currently being evaluated to identify connections to phenotypic differences. AAI Matrices show the extent of differences between the two clusters of bacteria and support the separation of the pink strains into the novel genus *Rosepedobacter* gen. nov from the existing genus *Pedobacter*.

Phenotypic diversity among *Vibrio fischeri* symbionts co-isolated from the light organ of *Euprymna scolopes* squid
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Animals depend on bacterial symbionts for normal physiology. Hosts often feature different strains of bacterial symbionts, but how such symbiont diversity arises is poorly understood and is important to predict ways to improve the resiliency of animal-bacterial symbioses in different environments. The mutualistic symbiosis between the Hawaiian bobtail squid, *Euprymna scolopes*, and the bioluminescent marine bacterium, *Vibrio fischeri,* was used as to model an animal-bacterial symbiosis that features strain diversity. Twelve natural isolates of *V. fischeri* were isolated from tissue homogenate derived from the light organ of a wild-caught adult squid. Whole-genome sequencing and multi-locus sequence typing revealed three distinct strains. The phenotypic diversity associated with these strains was determined by performing *in vitro* assays relating to biofilm formation, motility, growth, bioluminescence, and co-incubation. Additionally, to determine how effectively the three co-isolated strains could colonize animals, colonization assays were performed to identify the symbiotic dose that results in 50% of the exposed animals becoming colonized (SD_{50}). Using this concentration, squid colonization assays were performed to determine whether one strain affects the ability of other strains to colonize squid. This research revealed that there was phenotypic diversity as well as differences in SD_{50} among the three co-isolated symbionts. When inoculated together, the colonization frequency of one of the co-isolated strains was lowered, however, there was no effect on the colonization of the other two strains. In addition, despite this diversity, it appears that the original symbiosis can be reconstituted in juvenile animals. Taken together, these results contribute new insight to the assembly process involving multiple symbiotic strains, furthering the knowledge base of how multi-strain symbioses form.

The Effects of Gingerol on Duchenne Muscular Dystrophy and Fertility as Modeled in C. elegans
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Duchenne Muscular Dystrophy (DMD) is the most common form of muscular dystrophy; the gene affected encodes dystrophin, a protein that links actin filaments to other proteins in the muscular cell membrane for stability. Caenorhabditis elegans (C. elegans) is a common model organism for human diseases due to its entire genome being fully discovered and its short lifespan (~3 weeks) is ideal for undergraduate research. Currently, Muscular Dystrophy has been modeled in C. elegans and a variety of work has been completed including understanding the disease and testing different treatments. Gingerol is a compound found in ginger that is responsible for its pungent scent and spice. It is commonly used for medicinal purposes. There are significant research gaps with studies using gingerol as a treatment and its general effect on C. elegans, and none utilizing the DMD model. This study hopes to shed light on the potential use of Gingerol as a treatment for DMD in worms. It will focus on determining if phenotypes (eggs laid, muscle movement and development) changes upon treatment with Gingerol in both wild type and DMD animals.

Role of FTL_1504 in *Francisella tularensis* Susceptibility to Resazurin.

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Tularemia is a potentially fatal disease caused by the Category A bioterrorism agent *Francisella tularensis*. Aminoglycosides, fluoroquinolones, and tetracyclines can be used to treat tularemia; however, there is a high incidence of relapse and treatment failures when using these drugs. Furthermore, there is no tularemia vaccine licensed for use in the United States. Therefore, new antibiotics that target *F. tularensis* are being investigated. A novel family of resazurin-based antibiotics called resazomycins exhibit antimicrobial activity against *F. tularensis* and other Gram-negative pathogens including *Neisseria gonorrhoeae.* The mode of action of resazomycins has yet to be determined. To elucidate potential targets of resazurin (Rz), we screened for spontaneous Rz-resistant (Rzr) *F. tularensis* LVS mutants. Through the screen, 93% of all Rzr mutants sequenced contained mutations within the coding regions of FTL_0421, FTL_8095, and FTL_1504, which encodes the catalase KatG. To determine the role of FTL_1504 in Rz resistance, a wild-type copy of FTL_1504 was complemented into Rzr1. The Rz susceptibility of Rzr1 and the complemented Rzr1/pABST-FTL_1504 strain was unchanged suggesting the introduction of FTL_1504 into Rzr1 did not restore sensitivity to Rz. To further investigate the role of KatG in susceptibility to resazomycins, we evaluated catalase activity in select Rzr mutants and growth of these strains in the presence of hydrogen peroxide (H_2O_2). Rzr1 had reduced catalase activity compared to wild-type LVS. Rzr1 and Rzr8 had no observable growth in the presence of 0.1 mM H_2O_2 compared to LVS which grew exponentially. These data suggest resistance to resazurin is associated with an increased susceptibility to oxidative stress

The mature c-terminal domain of filamentous hemagglutinin provides protection against *B. pertussis* infection in the murine upper respiratory tract.

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The transition from whole cell (wP) to acellular pertussis vaccines (aPs) to protect against *Bordetella pertussis* has triggered an increase in pertussis (whooping cough) incidence despite wide-spread vaccine coverage. This can be attributed to fleeting immunological memory, the evolution of vaccine-escape strains, and the inability of aPs to prevent asymptomatic colonization and transmission of *B. pertussis*. The *B. pertussis* antigens in aPs include pertactin, fimbriae, filamentous hemagglutinin (FHA), and pertussis toxin (PT). Antibodies targeting these antigens are critical for protection against *B. pertussis,* and have been demonstrated to trigger opsonization, block bacterial adhesion, and neutralize toxin activity. However, antibodies generated following aP vaccination target diminished numbers of functional B cell epitopes due to chemical denaturation of antigens during vaccine formulation. The effects of denaturation on PT are well documented, but impacts on antigens such as FHA remain unclear. Additionally, the entire FHA molecule is included in aPs, yet most of the protein does not play a role in bacterial adhesion. To direct antibody responses toward non-denatured, functionally relevant portions of FHA we aimed to test the Mature C-terminal Domain (MCD) of FHA as a novel vaccine antigen. Protection provided by MCD was compared to several vaccine preparations including aP and native FHA. Using ELISA, we observed that both aP and wP vaccines lead to the induction of antibodies that recognize *B. pertussis,* native FHA, and MCD. Using a CD-1 murine model of *B. pertussis* vaccination and challenge, we also demonstrated that MCD is immunogenic and decreases bacterial burden in the murine airway, providing ~85% reduction in *B. pertussis* present in the nasal cavity. Overall, our data support the use of MCD as a vaccine antigen for protection against *B. pertussis* in mice. Ongoing studies focus on the selection of the adjuvants and route of administration to optimize vaccine efficacy.

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