# Texas ★ Branch
## AMERICAN SOCIETY FOR MICROBIOLOGY

# 2022 FALL MEETING

## HOUSTON, TEXAS
## 11/10/2022 - 11/12/2022

**ASM Branches**
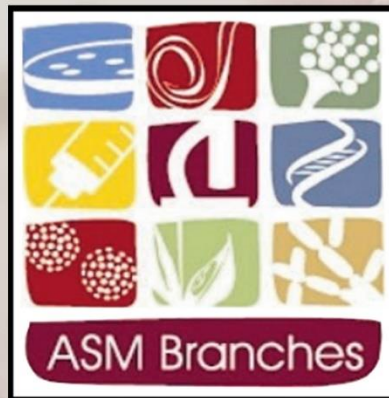
# Hosted by Rice University
## 6100 Main St, Houston, TX 77005

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# Past Texas Branch ASM Presidents

- James Stewart 1999-2001

- Karl Klose (University of Texas Health Science Center at San Antonio) 2001-2003

- Robert McLean (Texas State University at San Marcos) 2003-2005

- Heidi Kaplan (University of Texas Medical School at Houston) 2005-2007

- Poonam Gulati (University of Houston Downtown) 2007-2009

- Marvin Whiteley (University of Texas at Austin) 2009-2011

- Todd Primm (Sam Houston State University) 2011-2013

- Kendra Rumbaugh (Texas Tech University Health Sciences Center) 2013-2015

- Ali Azghani (University of Texas at Tyler) 2015-21017

- Lee Hughes (University of North Texas) 2017-2019

- Madhusudan Choudhary (Sam Houston State University) 2019-2021

# Meeting Venue

Thursday, November 10 – Saturday November 12
The meeting will begin Thursday late afternoon, and end Saturday at noon.
All student talks and posters will be on Friday. The banquet will be Friday evening.

**BIOSCIENCE RESEARCH COLLABORATIVE (BRC)**
6500 Main St, Houston, TX 77030
- If you stay at the Hilton Houston Plaza/Medical Center 6633 Travis Street, Houston, TX, 77030, you can walk to the conference.

**Parking**
- There is a $20 self-parking fee for the Hilton Houston Plaza/Medical Center.
- If you drive to the conference and need to park on site (at the BRC building where conference will be held), parking is $1/12 min, with a max of $12/day, for a single entry on the Rice University campus. If you leave and return later, you will pay the charge again.

# Friday Evening Banquet

## **Glasscock School of Continuing Studies**, First floor Commons

6100 Main St, Houston, TX 77005
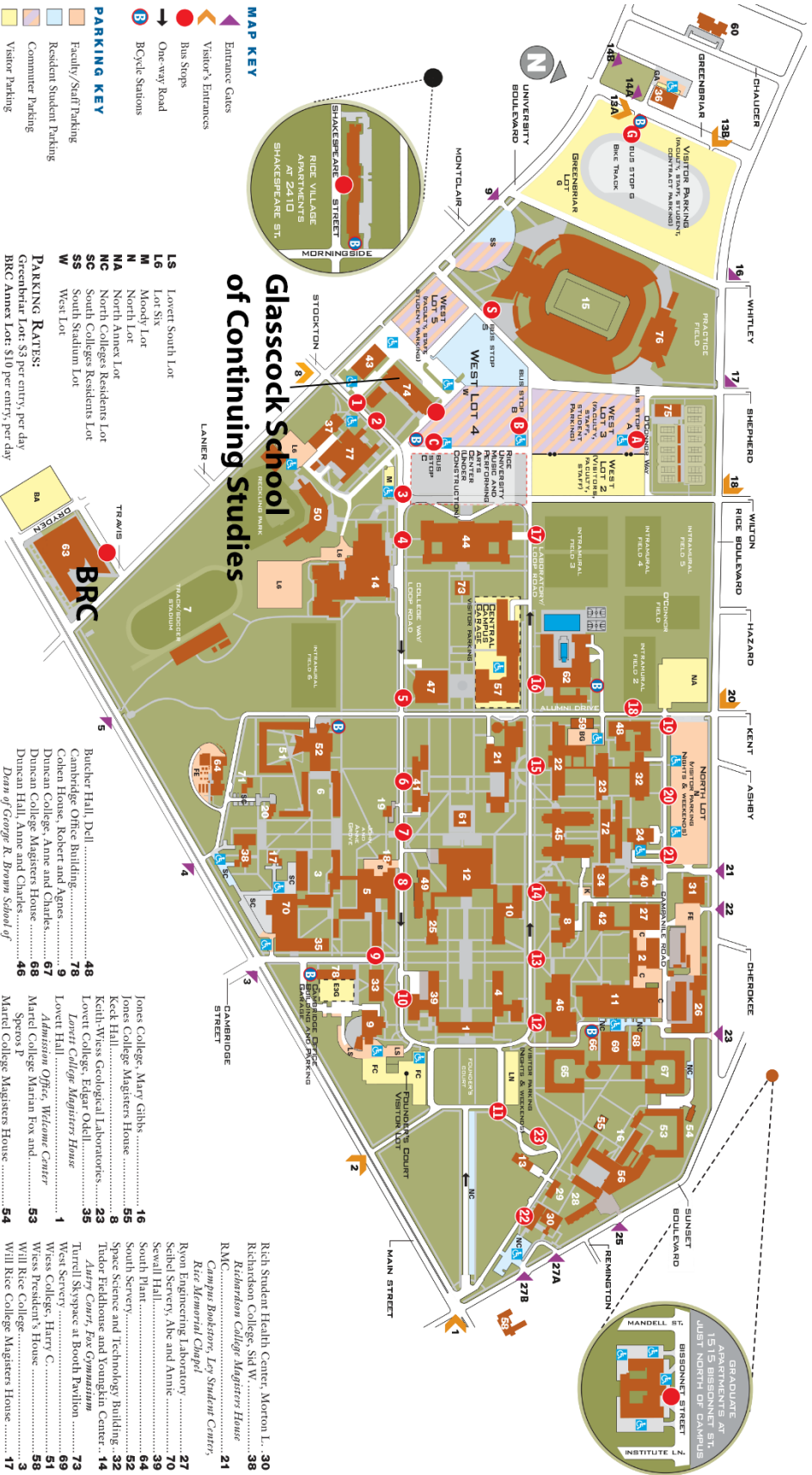


**Directions:**

**Walking (Recommended, 8 min walk, 0.4 miles)**

- Upon leaving the BRC, turn onto University Drive. (Perpendicular to Travis St, which runs between the hotel and the conference site).
- Walk down University away from the fraffic light for 3 blocks to Stockton St. (There is a traffic light at University and Stockton.)
- Turn right onto Stockton and walk past the Rice Police Department.
- The Glasscock Building is the SECOND building on the left, with the Cabbage sculptures on the lawn.

**Driving (Parking is $1/10 minues, $12/day maximum for either lot)**

- *Very limited parking* is available at the Moody Lot (~25 spaces total, some may be occupied). This lot is across the street from the Glasscock Building and is indicated by an 'M" above.
- Additional parking is available at West Lot 2 (see map on the next page), which is 0.3 miles from the Glasscock Building.

# RICE UNIVERSITY CAMPUS MAP

## Glasscock School of Continuing Studies

**BRC**

SHAKESPEARE APARTMENTS AT 2410 SHAKESPEARE ST.

GRADUATE APARTMENTS AT 1515 BISSONNET ST., JUST NORTH OF CAMPUS

**MAP KEY**
- ▲ Entrance Gates
- ▲ Visitor's Entrances
- Bus Stops
- One-way Road
- BCycle Stations
- B BCycle Stations

**PARKING KEY**
- Faculty/Staff Parking
- Resident Student Parking
- Commuter Parking
- Visitor Parking
- Accessible Parking

LS  Lovett South Lot
L6  Lot Six
M   Moody Lot
N   North Lot
NA  North Annex Lot
NC  North Colleges Lot
SC  South Colleges Residents Lot
SS  South Stadium Lot
W   West Lot

**PARKING RATES:**
Greenbriar Lot: $3 per entry, per day
BRC Annex Lot: $10 per entry, per day
All Other Visitor Parking Facilities: $1 each 10 minutes, $12 daily maximum

**PAYMENT METHODS:**
BioScience Research Collaborative Garage and Central Campus Garage: cash or credit card
All Other Visitor Parking Facilities: credit card only; used to enter and exit

**PARKING LOTS:**
B    Baker College—Housing & Dining Lot
BA   BioScience Research Collaborative (BRC) Annex
BG   Biology–Geology Lot
BRC  BioScience Research Collaborative Garage
C    Campanile Lot
CG   Central Campus Garage
E3G  Entrance 3 Garage
FC   Founder's Court Visitor Lot
FE   Facilities, Engineering and Planning Lot
G    Greenbriar Lot
GA   Greenbriar Annex
K    Keck Lot
LN   Lovett North Lot

# Meeting Sponsors

# Sponsor Information

**Stemcell technologies**
https://www.stemcell.com/
**Ty Fletcher-Beals**
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**BRUKER Technologies;**
https://www.bruker.com/en.html
**Michael (Mike) Baker**
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**Wiley Publisher**
https://www.wiley.com/en-us
**Laura Owen**
lowens@wiley.com

# ACKNOWLEDGEMENTS

# Opening Keynote Speaker

## Dr. Danielle Garsin's Bio

**Dr. Garsin** is a professor in the McGovern Medical School Department of Microbiology and Molecular Genetics at the University of Texas Health Science Center at Houston. Dr. Garsin came to UTHealth as an assistant professor in 2004 following a postdoctoral fellowship at Massachusetts General Hospital/Harvard Medical School. She earned her Ph.D. in Biochemistry at Harvard University and her B.S. in Biological Sciences at Cornell University.

Dr. Garsin is interested in microbial pathogenesis, gene regulation, host-microbe, and microbe-microbe interactions. Her studies are centered on the biology of human bacterial pathogens such as *Enterococcus faecalis.* One NIH-funded research focus is on the roles and regulation of ethanolamine utilization. Another is on the biology of the immune responses elicited in the model host *Caenorhabditis elegans.* Finally, Dr. Garsin studies the interactions between *E. faecalis* and the human fungal pathogen, *Candida albicans.* She and her collaborators discovered that the microbes inhibit each other's virulence leading to the identification of compounds with potential for anti-infective therapeutic development.

Dr. Garsin has received many commendations for excellence in research and education. In 2004, she received an Ellison Medical Foundation New Scholar Award in Global Infectious Disease. In 2008, she was awarded a UT Young Investigator award. She was the recipient of the Dean's Teaching Excellence Award in multiple years. Finally, Dr. Garsin was elected as a Fellow to the American Academy for Microbiology in 2019. She served as a permanent member of the Prokaryotic Cell and Molecular Biology (PCMB) NIH review group and is currently a permanent member of Innate Immunity and Inflammation (III). Dr. Garsin is also currently an associate editor of *PLOS Genetics* and on the editorial board of *mBio*.

# Closing Keynote Speaker

# ASM Distinguished Lecturer

## Dr. Miriam Braunstein's Bio

**Dr. Miriam Braunstein** is a Professor of Microbiology and Immunology at the University of North Carolina School of Medicine. She received her Ph.D. from Princeton University, where she carried out graduate research with Dr. James Broach on the Sir2 protein of *Saccharomyces cerevisiae*, histone deacetylation, and transcriptional silencing. After completing her Ph.D. degree, she became a Life Sciences Research Foundation (LSRF) Fellow working at the Albert Einstein College of Medicine with Dr. William Jacobs Jr. It was during her postdoctoral training that she began studying *Mycobacterium tuberculosis*, the bacterial pathogen responsible for tuberculosis. Dr. Braunstein's laboratory studies the basic biology and pathogenic mechanisms of mycobacterial pathogens, including *M. tuberculosis* and nontuberculous mycobacteria. Her research includes mechanistic studies of mycobacterial protein secretion pathways and of secreted effector proteins that promote mycobacterial survival in macrophages. Her laboratory also collaborates on translational projects to develop new therapies for mycobacterial disease that include exploring the potential to use bacteriophage to treat mycobacterial disease. Dr. Braunstein is the recipient of a Burroughs Wellcome Fund Investigators in the Pathogenesis of Infectious Disease Award and a Fellow of the American Academy of Microbiology.

## Dr. Braunstein's Personal Statement

I am passionate about training the next generation of scientists and in helping trainees of all levels achieve their career goals. I am the PI of an NSF-funded Summer Undergraduate Research Experience Program at the University of North Carolina. This program provides opportunities for students from groups underrepresented in the sciences or from schools with limited research capacity to work in UNC laboratories for an authentic summer research experience. I also founded the Southeastern Mycobacteria meeting, which provides students and postdoctoral trainees with opportunities to present their research and network with other scientists. I am an ASM member since 1996. I served as the ASM Division U (Mycobacteriology) Chair. I am currently a member of the ASM Journal of Bacteriology editorial board, a member of the ASM Press Committee, and an editor of the ASM Press Gram Positive Pathogens book published in 2019.

# Fall 2022 Texas Branch ASM Meeting

**Day 1, Nov 10**
**Location: BRC**
3:00 – 6:00 pm     **Registration + Appetizers** (pre-function space)
5:00 pm     **Opening /Welcome** (Auditorium): **Kelly Palmer**, UT Dallas; President, Texas
Branch ASM, & **Natasha Kirienko**, Rice University; Organizer
5:10 pm     Speaker Introducion: **Natasha Kirienko**, Rice University
5:15 – 6:15 pm     **Opening Keynote: Danielle Garsin**, UT Health
Infectious diseases discovery using the microbiovore *Caenorhabditis elegans*


**Day 2, Nov 11**
**Location: BRC**
**Vendor exhibit in the main hall & Event Space**
7:30 – 12:00 pm     **Registration**
7:30 – 8:20 am     **Breakfast (pre-function space)**
7:30 – 8:20 am     **Mentoring event (BRC 106): Careers in STEM**
Panelists: **Heer Mehta** (industry experience), **Daniel Kirienko** (research
administration), **Jennifer Walker** (academic faculty member), **Wesley Long**
(clinical research experience).

8:30-10:20 am     **Parallel sessions, faculty presentations**
**Medical Microbiology (Main Auditorium)**
Chair: **Jose L. Lopez-Ribot**, UTSA
Faculty Speakers:
8:35 – 9:00 am     **Jesus Romo**, UTSA
Characterizing the role of Candida species during gastrointestinal infection by *Clostridioides difficile*
9:00 – 9:25 am     **Dmitrios Kontoyiannis**, MD Anderson
COVID-19-associated mucormycosis
9:25-9:50 am     **Jennifer Walker**, UT Health
Investigating the recalcitrance of *S. aureus* isolates to prophylactic antibiotic treatment
9:55 – 10:20 am     **Natasha Kirienko**, Rice University
Know your enemy: characterization of acute virulence factors from *P. aeruginosa*.


**General Microbiology (Event Space)**
Chair: **Helene Andrews-Polymenis**, TAMU
Faculty Speakers:
8:35 – 9:00 am     **Andrea Mitchel**l, TAMU
Phospholipid transporters—why have three?
9:00 – 9:25 am     **Despoina Mavridou**, UT Austin
Cell envelope protein homeostasis underpins the evolution of antibiotic resistance
9:25 – 9:50 am     **Julian Hurdle**, TAMU
Decoding a cryptic mechanism of metronidazole resistance among globally disseminated

fluoroquinolone-resistant *Clostridioides difficile*

9:55 – 10:20 am    **Jolene Ramsey**, TAMU

    Timing your escape: mechanisms phages use to lyse their bacterial hosts


10:20 – 10:40 am    **Break**


10:40 – 12:10 pm    **Parallel sessions, trainee presentations**

**Medical Microbiology (Main Auditorium)**

    <u>S.E. Sulkin Award - Oral graduate student presentation award in Medical Microbiology</u>

Chair: **Nicole De Nisco**, UT Dallas

10:40 - 10:55 am    **Caroline Black**, Texas Tech University

    Mechanisms of altered antibiotic susceptibilities in a polymicrobial community

10:55 - 11:10 am    **Braden Shipman**, University of Texas at Dallas

    identification and characterization of chondroitin sulfate degradation and metabolism by uropathogenic *Proteus mirabilis*

11:10 - 11:25 am    **Brittany Shapiro**, TAMU Health Science Center

    *Borrelia burgdorferi* BosR binds small non-coding RNAs (sRNAs): implications for borrelial post-transcriptional gene regulation and pathogenesis

11:25 - 11:40 am    **Nowrosh Islam**, University of Texas at Arlington

    Peptidoglycan recycling promotes outer membrane integrity and carbapenem tolerance in *Acinetobacter baumannii*.

11:40 - 11:55 am    **Alex Kang**, Rice University

    Utilizing *in vitro* pathosystems to identify novel antivirulence therapeutics against *Pseudomonas aeruginosa*

11:55 - 12:10 pm    **Lauren Lynch**, Baylor College of Medicine

    Neonatal cholestasis hinders microbiome maturation and bile salt deconjugation in preterm infants


**General Microbiology (Event Space)**

    <u>O.B. Williams Award - Oral graduate student presentation award in General Microbiology</u>

Chair: **Cathy Wakeman**, Texas Tech University

10:40 - 10:55 am    **Alexis Carey**, TAMU

    Utilization of growth rates and swimming motility to evaluate fitness of *S. Typhimurium* after phase I or II flagellin loss

10:55 - 11:10 am    **Kristen Curry**, Rice University

    Emu: species-level microbial community profiling of full-length 16S rRNA Oxford Nanopore sequencing data

11:10 - 11:25 am    **Kyra Elise Groover**, University of Texas at Austin

    Development of a synthetic serum active peptide

11:25 - 11:40 am    **Allison Judge**, Baylor College of Medicine

    Mapping the determinants of catalysis and substrate specificity of the antibiotic resistance enzyme CTX-M β-lactamase

11:40 - 11:55 am    **Ashvini Ray**, The University of Texas at Dallas

    Roles of *dksA*-like genes in *Paracoccus denitrificans*

11:55 - 12:10 pm    **Xinyi Zhang**, Baylor University

Nonsense-mediated mRNA decay of metal-binding activator MAC1 is dependent on copper levels and 3'-UTR length in *Saccharomyces cerevisiae*

**Undergraduate Presentations (BRC 106)**

Sarah A. McIntire Award - Oral undergraduate student presentation award

Chair: **Blake Hanson**, UT Health

10:40 - 10:55 am    **Camille Condron**, University of Texas at Arlington
Inoculant carrier formulation on survivability of a drought-tolerant *Bradyrhizobium i*solate under desiccation stress

10:55 - 11:10 am    **Saoirse Disney-McKeethen**, Rice University
Evolving *Pseudomonas aeruginosa* to colistin in microfluidic emulsions recapitulates clinically relevant mutations that are depleted in bulk bulture

11:10 - 11:25 am    **Irene Hau**, University of Texas at Dallas
Gain of function cytolysin variant expressed by clinically isolated *Enterococcus faecalis*

11:25 -11:40 am    **Aeron Pennington**, Tarleton State University
Modulation of bacterial host phenotypes by mycobacteriophage pixie gene products

11:40 - 11:55 am    **Star Okolie**, University of Texas at Dallas
Defining the spatial and temporal dynamics of the urogenital microbiome in postmenopausal women

11:55 - 12:10 pm    **Filemon C. Tan**, Rice University
Pyocins contribute to ST111 strain dominance in *P. aeruginosa* inter-strain competition

12:10 – 12:30 pm    **Break**

12:30 – 1:00 pm    **Lunch** (Event Space)

1:00 – 2:00 pm    **Poster session A** (Event Space)
2:00 – 3:00 pm    **Poster session B** (Event Space)

3:00 – 3:30 pm    **Break**

3:30 – 4:40 pm    **Parallel sessions**

**Careers in Transition (Main Auditorium)**

Thomas S. Matney Postdoctoral Fellow Oral Presentation Award

Chair: **James Chappell**, Rice University

3:30 - 3:55 pm    **Chelsea Hu**, TAMU (Faculty)
System dynamics and feedback control in synthetic biology

3:55 - 4:10 pm    **Giuseppe Buda De Cesare**, Univ. of Texas McGovern Medical School
Characterization of activity and mechanism of action of the *Enterococcus faecalis* bacteriocin EntV on *Candida albicans*

4:10 - 4:25 pm    **Anwar Kalalah**, University of Texas at San Antonio
Pathogenomes and phylogenomic comparison Of 'Big Six' Non-O157 Shiga toxin-producing *Escherichia coli*

4:25 - 4:40 pm    **Saugata Mahapatra**, Texas A&M University

*Coxiella burnetii* requires type IVB secretion system to suppress host  TLR3/TRIF-dependent NF-kB-activation

**Environmental Biology/Ecology (BRC 106)**
   O.B. Williams Award - Oral graduate student presentation award in General Microbiology
Chair: **Lory Santiago-Vázquez**, U of H Clear Lake
3:30 - 3:55 pm     **Michael LaMontagne**, U of H Clear Lake (Faculty)
   Application of MALDI-ToF mass spectrometry systems to environmental microbiology
3:55 - 4:10 pm     **Chahat Upreti**, The University of Texas at Dallas
   The clinic vs the farm: exploring prevalence and function of CRISPR-Cas in agriculturally relevant niches
4:10 - 4:25 pm     **Meaghan Rose**, University of Texas at Arlington
   Induction of root nodulation independent of nitrogen fixation by *Leifsonia shinshuensis* in *Aeschynomene indica* plants
4:25 - 4:40 pm     **Stephan Joseph**, University of Texas at Tyler
   Mercury contamination characterized by microbial Hg methylation genes in Martin Lake, East Texas

4:40 - 6:00 pm     **Break**

5:00 - 6:00 pm     **Closing Keynote (Main Auditorium)**
                     **ASM Distinguished Lecturer Miriam Braunstein**, UNC, School of Medicine
   The bacterial protein export zoo

6:00 - 6:30     **Walk to new location: Glasscock School of Continuing Studies, Commons area**

6:30 - 7:45 pm     **Dinner**
7:45 pm – 8:10 pm   **Student Awards / Other Awards and Distinctions**
8:10 pm     **Closing remarks**

**Day 3, Nov 12:**
8:00 – 8:50 am     **Breakfast**
8:00 – 8:50 am     **Mentoring event (BRC 106): Improving DEI in Research Environments**
                     Panelists: **Cecilia Fernandez** (Assistant Director of Diversity, Equity, Inclusion and Outreach) and **Jorge Loyo Rosales** (Associate Director of Education)

9:00 - 12:30 pm     **Parallel sessions**
**Education and Pedagogy Session (BRC 106)**
   Co-chairs: **Todd Primm**, Sam Houston State University & **Gregory Frederick**, American University of the Caribbean School of Medicine
9:00 - 9:45 am     **Todd Primm**, SHSU: Teaching metacognition to students: I never metacognition I didn't like
9:45 - 10:30 am     **Jacqueline Horn**, Houston Baptist University

Learning to think critically: performing CURE research to Hone students' thinking skills

10:30 - 10:45 am     **Break**

10:45 - 11:30 am     **Greg Frederick**, AUC School of Medicine
Have medical schools flipped out? Or is it a case for team-based Learning

11:30 - 12:15 pm     **Panel Discussion**

**Workshops (Event Space)**

9:00 - 9:55 am     **Michael LaMontagne**, U of H Clear Lake
Strain-level bacterial identification with MALDI-TOF MS: A hands-on workshop from isolate to data analysis

10:00 - 10:45 am     **Jennifer Spinler & Ruth Ann Luna**, Baylor College of Medicine
Pathogen epidemiology using whole genome sequencing

10:50 - 11:35 am     **Todd Treangen**, Rice University
Methods for strain-level characterization of metagenomic sequencing data

11:40 - 12:25 pm     **Yahan Wei**, UT Dallas
RNA-sequencing analysis for bacterial gene expression

12:25 pm     **Adjourn**

# Posters

| | | | | GENERAL MICROBIOLOGY - graduate students<br>Samuel Kaplan Award Poster graduate student presentation |
|---|---|---|---|---|
| A | GS P1 | Jacqueline Carroll | Baylor University | Differential gene regulation of the iron transcriptome by nonsense-mediated mRNA decay in Saccharomyces cerevisiae |
| B | GS P2 | Andrea Garza Elizondo | Rice University | Targeted, high-throughput transcriptional activation via a CRISPR-associated transposon System |
| A | GS P3 | Sun-Young Kim | The University of Texas at Austin | Secretion of heterologous peptides from Gram-negative bacteria |
| B | GS P4 | Sinjini Nandy | The University of Texas at Arlington | Molecular interactions between peptidoglycan integrity maintenance and outer membrane lipid asymmetry in *Acinetobacter baumannii* |
| A | GS P5 | Trusha Parekh | University of Texas at Dallas | New insights into the regulation of methylotrophic growth in *Paracoccus denitrificans* |
| B | GS P6 | Xinhao Song | Rice University | Methyl halide transferase-based gas reporters for quantification of filamentous bacteria in microdroplet emulsions |
| A | GS P7 | Mady Telford | The University of Texas at Austin | Bacterial secretion of affibodies and other biologics. |
| B | GS P8 | Suman Tiwari | University of Texas at Dallas | Development of a high-throughput minimum inhibitory concentration (HT-MIC) testing workflow |
| A | GS P9 | Aparna Uppuluri | University Of Texas At Dallas | Assessing lysine-lipid asymmetry in the Group B streptococcal membrane by lipid labeling |
| B | GS P10 | Fabiha Zaheen Khan | University of Texas at Dallas | Elucidating the function of an unusual hydrophobic peptide in *Pseudomonas aeruginosa* |
| A | GS P11 | Brenda Zarazua-Osorio | University of Houston | Characterizing the autoregulation of Spo0A, the master regulator of biofilm and sporulation in *Bacillus subtilis* |

| | | | | PATHOGENIC MICROBIOLOGY - graduate students<br>Samuel Kaplan Award Poster graduate student presentation |
|---|---|---|---|---|
| B | GS P12 | Priya Christensen | University of Texas at Dallas | Expression of diverse streptococcal multiple peptide resistance factors and lipid hydrolase in Streptococcus mitis |
| A | GS P13 | Shane Cristy | University of Texas Health Sciences Center Houston | Candida albicans biofilm development in urinary catheters |
| B | GS P14 | Jacob Hogins | The University of Texas at Dallas | The distinct transcriptome of virulence-associated phylogenetic group B2 *Escherichia coli* |
| A | GS P15 | Bhuvana Lakkasetter Chandrashekar | The University of Texas at Dallas | Development of a co-culture model for assessing competing mammalian host cell and bacterial attachment on dental biomaterials |

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|---|---|---|---|---|
| B | GS P16 | Melissa Martinez | UT Health Houston | A tractable nematode model for the emerging fungal pathogen, *Candida auris* |
| A | GS P17 | Stephany Navarro | Texas Tech University Health Sciences Center | *Gardnerella vaginalis* growth is eliminated by a novel narrow-spectrum factor secreted by *Lactobacillus jensenii* |
| B | GS P18 | Jessica O'Berry | University of Texas at San Antonio | Role of Borrelia unfed tick induced protein (BtiP) in the colonization of the Lyme disease agent within tick and mammalian hosts |
| A | GS P19 | Irvin Rivera | The University of Texas at San Antonio | Removal of phosphate from lysate protein by a recombinant phosphase from *Acinectobacter baumannii* |
| B | GS P20 | Qi Xu | Rice University | A novel type of cytotoxic membrane vesicles produced by *Pseudomonas aeruginosa* |

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|---|---|---|---|---|
| **AMR & MICROBIAL ECOLOGY - graduate students** <br> **Samuel Kaplan Award Poster graduate student presentation** | | | | |
| A | GS P21 | Francesca Agobe | Texas A&M University School of Medicine | Novel drug combinations to treat *Rhodococcus equi* Infection |
| B | GS P22 | Samuel Cornelius | University of Texas at Dallas | Antigen stabilized vaccines against recurrent urinary tract infection |
| A | GS P23 | Jiayi Fan | Baylor College of Medicine | Discovery of novel broad-spectrum antibiotics and inhibitors for Œ≤-lactamases using combinatorial chemistry approaches |
| B | GS P24 | Jindanuch Maneekul | University of North Texas | Novel *Streptomyces* bacteriophage endolysins: isolation, purification, and functional domain testing. |
| A | GS P25 | Angela O'Donnell | The University of Texas at Austin | Nanobodies: overcoming the outer membrane barrier with small, charged proteins |
| B | GS P26 | Angelica Ponce | University of Texas-Arlington | Effects of a drought-tolerant *Bradyrhizobium i*solate on soybean growth in Arkansas |
| A | GS P27 | Ariel Robles | Texas A&M University - San Antonio | Microbial Source Tracking in Ambient Waters |
| B | GS P28 | Gloria Rodriguez | Texas A&M University-San Antonio | Developing a faster, inexpensive, accessible, microbial detection method for wastewater surveillance |
| A | GS P29 | Malyn Selinidis | Rice University | A ribozyme for non-destructive reporting of gene transfer within a soil consortium |
| B | GS P30 | Lyndsy Stacy | Texas A&M University San Antonio | Agent-based modeling to establish a protocol for sampling DNA from the air |
| A | GS 31 | Adeline Supandy | Rice University | Activated charcoal as a sink for diffusing AHL molecules in the microdroplets system |
| B | GS P32 | Arshya Tehrani | University of Texas at Arlington | Characterizing putative DD-carboxypeptidases that promote outer membrane integrity in *Acinetobacter baumannii* |
| A | GS P33 | Jacob Zulk | Baylor College of Medicine | Bacteriophage resistance associated with reduced bacterial fitness in the urinary environment |

| | | MICROBIOME & COMPUTATIONAL BIOLOGY - graduate students | | |
|---|---|---|---|---|
| | | **Samuel Kaplan Award Poster graduate student presentation** | | |
| B | GS P34 | A H M Zuberi Ashraf | The University of Texas at Austin | Stability of honey bee gut symbiont *S. alvi* traits during laboratory propagation |
| A | GS P35 | Tallon Coxe | University of North Texas | DL-ARG: leveraging deep learning to predict and classify antimicrobial resistance from long and short-sequence reads |
| B | GS P36 | Sarobi Das | University of Texas at Arlington | The effect of inoculation of beneficial bacteria on microbial diversity in soil infected with a pathogenic fungus. |
| A | GS P37 | Ronika De | University of North Texas | CAFÉ_GI: A tool for identification of genomic islands in bacterial genomes |
| B | GS P38 | Ken Dickinson | University of Houston - Clear Lake | Assembly of quality genomes from metagenomic reads generated from the rhizoplane of wheat |
| A | GS P39 | Kaelyn Dobson | Texas State University | Multi-species housing impacts: overlapping microbiomes - Preliminary Data |
| B | GS P40 | Marlyd Mejia | Baylor College of Medicine | The second mouse gets the cheese: how the field of reproductive tract microbiology benefits off the generation of a humanized gut-microbiota mouse model |
| A | GS P41 | Christian Peterson | University of Texas - Arlington | Effects of a drought-tolerant *Bradyrhizobium* isolate on soybean yield and the soybean rhizosphere microbiome |
| B | GS P42 | Vaidehi Pusadkar | University of North Texas | Benchmarking metagenomic classifiers on simulated ancient and modern metagenomic data |
| A | GS P43 | Shrestha Sujan | University of North Texas | Modelling the transmission of COVID-19 during the first wave in India using a data driven SEIRD model |
| B | GS P44 | Muneer Yaqub | University of Texas at Dallas | Defining the evolutionary framework of colistin resistance in *Acinetobacter baumannii* |

| | | GENERAL MICROBIOLOGY - undergraduate students | | |
|---|---|---|---|---|
| | | **Joan Abramowitz Award - Poster undergraduate student presentation** | | |
| A | UP 1 | Stephanie Marie Davidson | Texas A&M University-San Antonio | Use of *S. Aureus* to study airflow and filtration in a collegiate environment |
| B | UP 2 | Taylor Holly | Sam Houston State University | Models for cellular aging in yeast |
| A | UP 3 | Pranav Kumar | University of Texas at Dallas | Examining the effect of antibiotics on CRISPR-Cas defense efficacy against conjugative plasmids |
| B | UP 4 | Jenny Le | University of Texas at Arlington | Nodule formation inhibited by *Paenibacillus sp.* isolated from Texas native *Aeschynomene indica* plants |
| A | UP 5 | Cassandra Maldonado | Texas A&M San Antonio | Characterization of antibiotic production and microbial diversity in the soils of San Antonio |
| B | UP 6 | Kyren Miller | The University of Texas at Tyler | Mercury reduction gene merA detection in Martin lake |

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|---|---|---|---|---|
| A | UP 7 | Julie Nguyen | The University of Texas at Dallas | Antimicrobial effects of human metabolite lysophosphatidylcholine |
| B | UP 8 | Catherine Nickel | St. Edward's University | Standardization of Saccharomyces cerevisiae microplate reader covering parameters |
| A | UP 9 | Heather Nolte | University of Houston - Clear Lake | Prevalence of antibiotic-resistant Vibrio strains in oysters harvested from Galveston Bay |
| B | UP 10 | Madison Wolfrom | Sam Houston State University | Developing a cellular aging model in yeast |
| A | UP 11 | Allison Wyrick | University of Houston - Clear Lake | Prevalence of antibiotic resistant bacteria on microplastics in Galveston Bay |

| PATHOGENIC MICROBIOLOGY - undergraduate students<br>Joan Abramowitz Award - Poster undergraduate student presentation | | | | |
|---|---|---|---|---|
| B | UP 12 | Withdrawn | | |
| A | UP 13 | Alex Caron | Texas Christian University | Characterization of antibacterial mechanisms of zinc oxide in *Staphylococcus aureus* |
| B | UP 14 | Guan Chen | University of Texas at Dallas | The Role of glycolipids in *Streptococcus sp.* 1643 |
| A | UP 15 | Luke Hamilton | Texas Christian University | Identifying novel mutants with increased susceptibility to hydrogen peroxide and reduced virulence in *Bacillus anthracis* Sterne |
| B | UP 16 | Alexis Ho | University of Texas at San Antonio | Role of *Borrelia* sugar phosphorylation protein (BsuP) in the patho-physiology of Lyme disease agent. |
| A | UP 17 | Jerril Jacob | McGovern Medical School, University of Texas Health Science Center at Houston, TX, | Bacteriophage-containing biodegradable microsphere technology to treat osteomyelitis |
| B | UP 18 | Rebecca McGehee | Texas A&M University-San Antonio | Establishing an invertebrate infection model for *Staphylococcus hemolyticus* |
| A | UP 19 | Christina Nguyen | University of Texas at Arlington (UTA) | Transcriptional regulation of lipoproteins Lpp1 and Lpp2 in the nosocomial pathogen, *Acinetobacter baumannii* |
| B | UP 20 | Nikita Singh | Rice University | Exploring host-pathogen interactions in the liquid killing assay |
| A | UP 21 | Justin Wright | University of Texas at Tyler | Genotypic and phenotypic association of antibiotic resistance in *Pseudomonas aeruginosa* |

| | | | | **POSTDOCTORAL FELLOW POSTERS**<br>**Samuel Kaplan Award - Poster graduate student presentation** |
|---|---|---|---|---|
| A | PDP 1 | Ayan Chatterjee | University of Texas | The role and dynamics of ethanolamine-utilizing bacterial microcompartments |
| A | PDP 2 | Carolaing Gabaldon | The University of Texas Health Science Center at Houston | CDC-48 influences SKN-1 activity in response to pathogen infection |
| A | PDP 3 | Shantanu Guha | University of Texas Health Sciences Center in Houston | Development of novel antifungals against candida based on an antifungal peptide produced by *E. faecalis* |
| A | PDP 4 | Venkatesh Kumaresan | UTSA | Cellular and transcriptional signatures of innate immune response following *Borrelia burgdorferi* infection of murine splenocytes unveiled by single cell RNA-Seq (scRNA-Seq) |
| A | PDP 5 | Joana Rocha | Texas A&M University | Toxic mechanisms of STM3845 in *Salmonella Typhimurium* |

| | | | | **STAFF POSTERS** |
|---|---|---|---|---|
| B | SP 1 | Muqaddas Amer | The University of Texas at Dallas | Uncovering the mechanism behind metronidazole inactivation in *Enterococcus faecalis* and its role in protecting metronidazole-susceptible bacteria |
| B | SP 2 | Sydney Hall | The University of Texas at Dallas | Putrescine as a requirement for pili-mediated surface motility in *Escherichia Coli* |
| B | SP 3 | Deborah Omoregie | University of Texas at Arlington | LD-Transpeptidase regulatory elements promote the viability of lipooligosaccharide deficient *Acinetobacter baumannii* |

| | | | | **FACULTY POSTERS** |
|---|---|---|---|---|
| B | FP 1 | Yajuan Lin | Texas A&M University - Corpus Christi | Linking community structure to ecosystem functioning - specific plankton and interactions are good predictors of carbon export at the Western Antarctic Peninsula |
| B | FP 2 | Alex Wong | Texas A&M | The fitness effects of antimicrobial resistance mutations in *E. coli* are modulated by strong genotype by environment interactions |

# ORAL PRESENTATION ABSTRACTS

## Opening Keynote

**1.**
**Infectious diseases discovery using the microbiovore *Caenorhabditis elegans***

**Danielle A. Garsin[1],** Melissa R. Cruz[1], Shane Cristy[1], Shantanu Guha[1], Giuseppe Buda De Cesare[1], Hiram Sanchez[2], Pedro Miramón[1], Junko Yano[3], Paul L. Fidel, Jr[3], Alexei Savchenko[4], David R. Andes[2], Peter J. Stogios[5], and Michael C. Lorenz[1]

[1]University of Texas Health Science Center Houston, [2]University of Wisconsin Madison, [3]Louisiana State University, [4]University of Calgary, 5University of Toronto

Fungal pathogens are a continuing challenge due to a lack of effective antifungals and a rise in both acquired and intrinsic resistance. We previously described the inhibition of *Candida albicans* virulence following exposure to the bacteriocin EntV, secreted by *Enterococcus faecalis,* in a variety of in vitro and in vivo models. To optimize EntV as a potential therapeutic and better understand its antifungal features, an X-ray structure was obtained that revealed "clasping palms" of three alpha helices each enclosing a seventh (a7) of 16 amino acids. Using the in vitro adhesion and nematode infection assays we demonstrated that the antifungal activity resides entirely in a7, which could be reduced to 12 amino acids and retain full activity. Shorter peptides, down to 10 amino acids, had reduced activity. Using *C. elegans* we demonstrated that the 12 amino acid peptide was also effective against the emerging multidrug resistant pathogen *Candida auris* and *Cryptococcus neoformans*, an agent of fungal meningitis that is very distantly related to *Candida*. Moving to rodents, we found excellent protection in the OPC model, a catheter infection model, and in disseminated disease. The mechanism of action is not yet understood, but these peptides are not toxic to *C. albicans* and appear to bind the cell envelope. To further develop the potential of these peptides, we generated and tested a library of peptide derivatives using high throughput worm screen, identifying several candidates with enhanced activity. Together, these results showcase EntV-derived peptides as promising candidates for antifungal therapeutic development.

## Medical Microbiology, Faculty Presentations

**MMF 2.**
**Characterizing the role of *Candida* species during gastrointestinal infection by *Clostridioides difficile***

**Jesús A. Romo[1]** and Carol A. Kumamoto[2]
[1]The University of Texas at San Antonio, [2]Tufts University School of Medicine

*Candida* species are opportunistic fungal pathogens and common colonizers of the human gastrointestinal tract. They have been shown to affect the host immune system and interact with the gut microbiome. Therefore, *Candida* species could be expected to play important ecological roles in the host gastrointestinal tract. Previously, our group demonstrated that pre-colonization of mice with *C. albicans* protected them against lethal *Clostridioides difficile* infection (CDI). In contrast, the findings presented here show that mice pre-colonized with *C. glabrata* succumbed to CDI more rapidly than mice that were not pre-colonized. Moreover, mice pre-colonized with *C. glabrata* showed increased bacterial burden and toxin titers earlier in the infection, suggesting enhancement of *C. difficile* pathogenesis. We also show that when *C. difficile* is added to *C. glabrata* biofilms, an increase in matrix and overall biomass is observed. These findings suggest a model in which *C. albicans* and *C. difficile* have an antagonistic relationship which reduces the pathogenicity of *C. difficile,* while *C. glabrata* and *C. difficile* have a relationship which enhances the pathogenicity of *C. difficile.* Thus, the ecological roles of *C. albicans* and *C. glabrata* in the

gastrointestinal tract ecosystem are not identical. Interestingly, the presence of *C. difficile* increases *C. glabrata* biofilm susceptibility to Caspofungin and induces the secretion of cell wall-like particles into the environment, indicating that co-culture with *C. difficile* leads to fungal cell wall changes. Defining this intricate relationship will enhance our understanding of the role of *Candida* species in the context of intestinal disease such as CDI.

## MMF 3.
### COVID-19-associated mucormycosis
**Dimitrios P. Kontoyiannis**
Baylor College of Medicine, UT School of Public Health, University of Houston

In May 2021, a massive number of mucormycosis cases in patients with COVID19 was encountered throughout India, a country with a very high prevalence of mucormycosis to begin with, and overwhelmed in already fragile medical system. COVID19-associated mucormycosis (CAM) was seen typically in patients with new onset or poorly controlled diabetes and manifested as a rhino-orbital or rhino-cerebral disease or sinusitis in the vast majority of cases. Most affected patients had received systemic corticosteroids, often without a firm indication for use based on their COVID19 condition. Outcomes including loss of life and severe debility such as vision loss were severe. In this lecture, I will provide an overview the fascinating epidemiology, putative pathogenesis, clinical manifestations and management of CAM. The CAM syndemic exposed the unpreparedness to face the overwhelming burden of cases of a serious mycosis and emphasized the inequities of care delivery and infrastructure in high risk countries. Importantly, it reminded us on the need to be more prepared for the next big fungal outbreak.

## MMF 4.
### Investigating the recalcitrance of *S. aureus* clinical isolates to prophylactic antibiotic treatment
Jesus Duran Ramirez[ab], Jana Gomez[a], Terence Myckatyn[c], **Jennifer N Walker[ab]**
[a]Department of Microbiology and Molecular Genetics, McGovern Medical School, UTHealth
[b]Department of Epidemiology, Human Genetics, and Environmental Sciences, School of Public Health, UTHealth
[c]Division of Plastic and Reconstructive Surgery, Washington University School of Medicine

Breast implant-associated infections (BIAIs) are a common complication following breast prostheses placement and account for ~100,000 infections annually. The frequency, high cost of treatment, and morbidity make BIAIs a significant health burden for women. Thus, effective BIAI prevention strategies are urgently needed. We set out to examine the efficacy of one infection prevention strategy: the use of a prophylactic triple antibiotic pocket irrigant (TAPI) against *Staphylococcus aureus*, the most common cause of BIAIs. TAPI consists of 50,000 U bacitracin, 1 g cefazolin, and 80 mg gentamicin diluted in 500 mL of saline and is used to irrigate the breast implant pocket during surgery. We demonstrate that while planktonically grown *S. aureus* BIAI isolates are susceptible to TAPI, biofilm formation was enhanced following exposure to TAPI. Furthermore, genome sequencing indicated these strains did not carry many acquired antimicrobial resistance genes. Lastly, using a mouse model of BIAI, we compared TAPI treatment of a *S. aureus* reference strain (JE2) to a BIAI isolate (117). TAPI significantly reduced infection of JE2 at 1- and 7-days post infection (dpi). In contrast, BIAI strain 117 displayed high bacterial burdens in tissues and implants, which persisted out to 14-dpi despite TAPI treatment. Lastly, we demonstrated that this recalcitrance was unique to *S. aureus* species, as TAPI was effective against *P. aeruginosa* BIAI strains *in vitro* and *in vivo.* Together, these data suggest *S. aureus* BIAI strains employ unique mechanisms to resist prophylactic antibiotic treatment and promote chronic infection.

**MMF 5.**
**Know your enemy: characterization of acute virulence factors from *P. aeruginosa*.**

**Natalia V. Kirienko[1]**, Donghoon Kang[1], Qi Xu[1], Alexey V. Revtovich[1], Carolyn L. Cannon[2]
[1]Department of BioSciences, Rice University Houston, Texas, United States
[2]Department of Microbial Pathogenesis and Immunology, Texas A&M University Health Science Center, College Station, Texas, United States

The increasing display of multidrug resistance by the bacterial pathogen *Pseudomonas aeruginosa* is an imminent threat to world-wide health. This opportunistic, re-emerging pathogen is responsible for a large number of nosocomial infections, which have shown marked increases in morbidity and mortality over the last decade as it rapidly acquires resistance to many frontline antimicrobials and as pan-drug resistant strains have begun to emerge. Patients who require frequent or maintenance treatment (e.g., cystic fibrosis (CF) patients, cancer patients, etc.) face a looming need for new treatment.

One increasingly popular option is to target virulence instead of growth. Toward this approach, my lab has focused on pyoverdine, a major siderophore produced by *P. aeruginosa.* Pyoverdine is cytotoxic to *Caenorhabditis elegans* and murine macrophages, where it damages mitochondria. Pyoverdine is required for full virulence for *P. aeruginosa* in acute murine infection models. We developed synergistic drug combinations targeting pyoverdine, including 5-fluorocytosine (a pyoverdine biosynthetic inhibitor) and gallium (a direct inhibitor of pyoverdine). Mutants, which arose rarely, exhibited resistance only to gallium, but not to 5-fluorocytosine.

We also discovered a novel type of cytotoxic membrane vesicle produced by *P. aeruginosa*. Vesicles cause severe membrane stress in host cells, killing macrophages within minutes and epithelial cells within 3-4 hours. Cytotoxicity is markedly higher than previously-described lysozyme- or quorum sensing-dependent membrane vesicles produced by *P. aeruginosa.*

# General Microbiology, Faculty Presentations

**GMF 6.**
**Phospholipid transporters—why have three?**

**Angela M. Mitchell**
Department of Biology, Texas A&M University

The gram-negative outer membrane acts as a barrier protecting the cell from chemical and physical stress. The long-standing mystery of phospholipid transport to the outer membrane has been recent relieved by the discovery of three phospholipid transporters in *E. coli* K-12, YhdP, TamB, and YdbH. Evidence suggests these proteins provide hydrophobic channels for the diffusion of phospholipids between the membranes with YhdP and TamB playing major roles. However, YhdP and TamB have several quite different phenotypes suggesting distinct functions in phospholipid transport. This seminar will discuss a unique cold sensitivity phenotype of a double mutant of *yhdP* and a major regulator of fatty acid synthesis and degradation, and the genetic investigations that have lead us to be able to differentiate the roles of YhdP and TamB. Our data clarifies the roles of the individual transporters, suggesting a rationale for the redundancy in this system.

**GMF 7.**

**Cell envelope protein homeostasis underpins the evolution of antibiotic resistance**

**Nikol Kaderabkova**[1], Ayesha Mahmood[1], Daniel E. Deatherage[1],
Jeffrey E. Barrick[1], Despoina A.I. Mavridou[1,2]
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[2]John Ring LaMontagne Center for Infectious Diseases, University of Texas at Austin, Austin, 78712, Texas, USA

The continuous emergence of new variants of β-lactamases, enzymes that hydrolyze compounds like penicillins, poses a significant threat to the most used class of antibiotics worldwide. While successful clinical β-lactamase inhibitors exist, resistance towards these compounds also frequently arises. Evolution of β-lactamases mostly occurs through mutations that alter the active site of the protein, often imposing a burden to its stability. Our previous work has shown that disulfide bonds are essential for the folding, stability and activity of clinically important enzymes with broad hydrolytic activities but are less important for the function of narrow-spectrum β-lactamases, like the prototypical TEM and SHV enzymes. Here, we investigate the importance of oxidative protein folding for the transformation of narrow-spectrum enzymes to variants with activity towards invaluable more complex β-lactam antibiotics, such as cephalosporins and carbapenems. By exposing cells expressing TEM-1 and SHV-1 to increasing concentrations of the 4th generation cephalosporin ceftazidime, we obtain clinically relevant mutants with expanded hydrolytic spectra. However, loss of disulfide bond formation, either through removal of cysteine residues on the enzyme or deletion of the gene encoding for the major cell envelope oxidase DsbA, results in a smaller mutational landscape that contains evolved enzymes with limited hydrolytic capabilities. We find that this constrained pool of evolved enzymes is generated because most variants with expanded hydrolytic spectra fail to fold in the absence of their disulfides. Our findings open unique avenues towards hampering the evolution of antibiotic resistance through targeting pathways that safeguard protein homeostasis in the cell envelope.

**GMF 8.**

**Outbreak strains of fluoroquinolone resistant *Clostridioides difficile* exhibit a cryptic mechanism of metronidazole resistance**

Abiola O. Olaitan[1*], Chetna Dureja[1*], Madison A. Youngblom[2], Madeline A. Topf[2], Wan-Jou[3] Shen1, Anne J. Gonzales-Luna3, Aditi Deshpande[1], Kirk E. Hevener[4], Jane Freeman[5,6], Mark H. Wilcox[5,6], Kelli L. Palmer[7], Kevin W. Garey[3], Caitlin S. Pepperell[8,9#], **Julian G. Hurdle**[1#]

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[8]Department of Medical Microbiology and Immunology, School of Medicine and Public Health, University of Wisconsin-Madison, Madison, Wisconsin, USA

[9]Department of Medicine, Division of Infectious Diseases, University of Wisconsin-Madison, Madison, Wisconsin, USA

The global spread of fluoroquinolone resistant *Clostridioides difficile* has been linked with major outbreaks of *C. difficile* infection and deaths over the past two decades. During the same time, the clinical efficacy of the CDI antibiotic treatment metronidazole has decreased. Most *C. difficile* strains that are resistant to metronidazole have a peculiar resistance profile that can only be detected using molecularly intact heme in susceptibility testing agars. This presentation will detail the mechanism behind this characteristic, which we discovered using molecular genetics, phylogenetics, and population analysis. Our research also discovered that the fluoroquinolone-resistant strains are also resistant to metronidazole. Hence, resistance to these two antibiotics co-mediated the recent pandemic of healthcare-associated *C. difficile*, which is associated with suboptimal treatment outcomes in CDI patients treated with metronidazole.

## GMF 9.
**More than one way to skin a bacterium: the bacteriophage-encoded antimicrobial peptide**
**Jolene Ramsey[1]**
[1]Department of Biology & Center for Phage Technology, Texas A&M University

Bacterial viruses, or phages, are swift executioners of their infected host cells. After exploiting bacterial resources to assemble progeny, phages trigger host cell lysis, an explosive event releasing newly made virions into the environment. In Gram-negative hosts, tailed phage encode a class of proteins to actively target each physical layer of the bacterial cell: the inner membrane (holins), peptidoglycan cell wall (endolysins), and outer membrane (usually spanins). We previously demonstrated that the T7-like coliphage phiKT causes the explosive cell lysis associated with spanin activity using a small 56-amino acid cationic membrane protein called gp*28*. In predicted properties and classic assays that measure bactericidal and inhibitory effects gp*28* is similar to the human cathelicidin antimicrobial peptide LL-37. We designated gp*28* the founding member of a new class of phage lysis proteins, the disruptins, which are phage-encoded cationic antimicrobial peptide that disrupt the bacterial outer membrane during host lysis. Bioinformatic searches identified a second coliphage disruptin candidate that complements outer membrane disruption in *E. coli*. Surprisingly, the second disruptin is found in a phage that also encodes spanins. Lack of sequence similarity among antimicrobial peptides and the disruptins necessitates the use of functional assays for their discovery. Although many mechanisms are proposed for antimicrobial peptide function, lack of an established genetic system has hampered clear consensus on the molecular basis of membrane disruption. Therefore, the study of these genetically tractable phage disruptins with antimicrobial peptide properties may facilitate mechanistic analyses to elucidate their interactions with specific components of the cell membrane.

# Medical Microbiology, Trainee Presentations

## MMT 10.
**Mechanisms of altered antibiotic susceptibilities in a polymicrobial community**
**Caroline Black**, Viviann Robles, Catherine Wakeman
Department of Biological Sciences, Texas Tech University

Recent advances in sequencing technologies have demonstrated that many chronic wounds are polymicrobial in nature. Species interactions within a polymicrobial community can lead to decreases in antibiotic efficacy through polymicrobial cooperation or increases through polymicrobial competition.

Despite the knowledge that polymicrobial communities are common occurrences in persistent infections, current antimicrobial susceptibility testing (AST) is performed on monomicrobial suspensions. This research demonstrates the role of a polymicrobial community in shifting antimicrobial susceptibilities. Four relevant chronic wound pathogens (*Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Acinetobacter baumannii*, and *Enterococcus faecalis*) were grown in both monomicrobial and polymicrobial conditions. When *E. faecalis* was grown in a polymicrobial community, it demonstrated increased susceptibility to gentamicin. It was determined this phenomenon is due to *E. faecalis* acquiring heme from the community, which activates cellular respiration, and also allows more gentamicin to enter the cell via proton motive force. When *E. faecalis* was grown in community with *A. baumannii*, it exhibited decreased susceptibility to cephalexin. Further investigation determined that *A. baumannii* likely produces a beta-lactamase in the presence of cephalexin, allowing for the neutralization of the antibiotic and protection for susceptible *E. faecalis*. These mechanisms demonstrate that the community plays a role in determining an individual bacterium's antibiotic susceptibility, meaning that current AST testing, which focuses on the monomicrobial agent of disease, may not be truly reflective of the infection environment. However, by acknowledging the role of community interactions within infections in determining antibiotic susceptibilities, we can more effectively treat persistent infections, leading to improved patient outcomes.

**MMT 11.**
**Identification and characterization of chondroitin sulfate degradation and metabolism by uropathogenic *Proteus mirabilis***

**Braden Shipman[1]**, Serena Zhou[1], Benjamin Hunt[2], Belle Sharon[1], Chelsie Armbruster[2], Phillipe Zimmern[3], Nicole De Nisco[1,3]
[1]Department of Biological Sciences, University of Texas at Dallas, Richardson, TX [2]Department of Microbiology and Immunology, Jacobs School of Medicine and Biomedical Sciences, State University of New York at Buffalo, Buffalo, NY [3]Department of Urology, University of Texas Southwestern Medical Center, Dallas, TX

Urinary tract infection (UTI) affects over 150 million individuals worldwide annually with between 50 – 60% of adult women affected by UTI in their lifetime. To establish infection, uropathogens must overcome several host defenses including the glycosaminoglycan (GAG) layer which coats the apical surface of the urothelium. GAGs are thought to protect against UTI by serving as scaffolding sites for commensal bacteria and by preventing adherence of uropathogenic bacteria to the urothelium. Previous studies have established that chondroitin sulfate (CS) is the most prevalent GAG in urine. However, the ability of urinary bacteria to degrade and utilize GAGs and the contribution of these activities toward UTI progression is largely unknown. While screening multiple urinary bacterial isolates for GAG degradation and utilization, we discovered that the uropathogen *Proteus mirabilis* degrades CS and potentially utilizes it as a carbon source. We used a GAG degradation and utilization assay developed within our lab to characterize the degradation phenotype of seven *P. mirabilis* urinary isolates in both basal and artificial urine media. We found a wide range of variation in the kinetics of CS degradation between strains and media types. Using closed, hybrid genome assemblies, we identified conserved chondroitinase ABC endo- and exolyase genes and successfully generated knockout mutants for these genes. Using the GAG assay, we found that loss of both genes is required to abolish CS degradation activity. We hypothesize that the ability to degrade CS may be an integral virulence mechanism allowing for successful colonization of the urinary tract by *P. mirabilis*.

**MMT 12.**
***Borrelia burgdorferi* BosR binds small non-coding RNAs (sRNAs): Implications for borrelial post-transcriptional gene regulation and pathogenesis**

**Brittany Shapiro[1]**, Prashant Jaiswal[1], Taylor Van Gundy[2], Sourav Roy[3], Brandon L. Garcia[3],

Meghan Lybecker[2], Jenny A. Hyde[1], and Jon T. Skare[1]
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Post-transcriptional regulation is a mechanism that modulates protein levels in living systems in a dynamic manner. In the case of *Borrelia burgdorferi*, the etiologic agent of Lyme disease, the most common vector-borne disease in the U.S., dramatic gene expression changes are a hallmark of the bacterium moving between the arthropod vector and infected hosts. However, the details of how *B. burgdorferi* adapts to host-specific signals are still being determined. Previously, BosR was identified as a global regulator that affects *rpoS*, a master switch for infectivity-associated mammalian-specific gene expression. These studies demonstrated that a *bosR* mutant no longer produced RpoS, thereby abrogating global RpoSregulation, including the virulence-associated *ospC* locus, which is essential for mammalian infection. Recent data indicates that BosR also serves as a chaperone for small non-coding RNAs (sRNAs). We hypothesize that BosR-bound sRNAs are used as an additional layer of regulation to modulate responses needed to adapt to their environment appropriately. Specifically, BosRbound sRNAs are predicted to target mRNA transcripts, resulting in either their degradation or enhanced translation. This adds new BosR-mediated post-transcriptional regulation to borrelial pathogenesis. Herein, we demonstrate that BosR binds to several sRNAs, including SR0735. We genetically inactivated SR0735 and found that cells lacking this sRNA alter virulence-associated gene expression and protein production. This suggests that SR0735 targets transcripts important for virulence-associated function(s). Characterizing the mechanisms of BosR::sRNA::mRNA interactions provides a new perspective regarding the exploitation of a post-transcriptional regulatory scheme in *B. burgdorferi* that alters transcript stability and/or protein production.

**MMT 13.**

**Peptidoglycan recycling promotes outer membrane integrity and carbapenem tolerance in**
***Acinetobacter baumannii***

<u>Nowrosh Islam</u>[1], Misha I. Kazi[*1], Katie N. Kang[1], Jacob Biboy[2], Joe Gray[3], Feroz Ahmed[1], Richard D. Schargel[1], Cara C. Boutte[1], Tobias Dörr[4], Waldemar Vollmer[2], Joseph M. Boll[1]
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β-Lactam antibiotics exploit the essentiality of the bacterial cell envelope by perturbing the peptidoglycan layer, typically resulting in rapid lysis and death. Many Gram-negative bacteria do not lyse but instead exhibit "tolerance," the ability to sustain viability in the presence of bactericidal antibiotics for extended periods. Antibiotic tolerance has been implicated in treatment failure and is a stepping-stone in the acquisition of true resistance. However, the molecular factors that promote intrinsic tolerance are not well understood. *Acinetobacter baumannii* is a critical-threat nosocomial pathogen notorious for its ability to rapidly develop multidrug resistance. Carbapenem β-lactam antibiotics (i.e., meropenem) are first-line prescriptions to treat *Ab* infections, but treatment failure is increasingly prevalent. Meropenem tolerance in Gram-negative pathogens is characterized by morphologically distinct populations of spheroplasts, but the impact of spheroplast formation is not fully understood. Here, we found that susceptible *A. baumannii* clinical isolates demonstrate high level of meropenem tolerance, form spheroplasts and revert to normal growth after antibiotic removal. Using transcriptomics and genetic screens, we characterized several genes associated with outer membrane integrity maintenance and efflux promote tolerance, likely

by limiting drug entry into the periplasm. We also defined the enzymatic activity of the tolerance determinants penicillin-binding protein 7 (PBP7) and ElsL (a cytoplasmic LD-carboxypeptidase). Together these data show that outer membrane integrity and peptidoglycan recycling are tightly linked and contribute to meropenem tolerance in *A. baumannii.*

## MMT 14.
### Utilizing *in vitro* pathosystems to identify novel antivirulence therapeutics against *Pseudomonas aeruginosa*

**Donghoon (Alex) Kang**[1], Alexey V. Revtovich[1], Xu Wang[1], Carolyn L. Cannon[2],
and Natalia V. Kirienko[1]
[1]Department of BioSciences, Rice University Houston, Texas, United States
[2]Department of Microbial Pathogenesis and Immunology, Texas A&M University Health Science Center, College Station, Texas, United States

*Pseudomonas aeruginosa* is a multidrug-resistant pathogen that causes life-threatening infections in immunocompromised patients. One key virulence factor is the siderophore pyoverdine, which not only provides the bacterium with iron, but also regulates the production of secreted toxins. Consequently, pyoverdine is necessary for virulence during murine lung infection. We have further developed various *in vivo* and *in vitro* pathosystems using *Caenorhabditis elegans*, alveolar macrophages, and bronchial epithelial cells to model pyoverdine-dependent virulence. A survey of panels of clinical isolates in these pathosystems demonstrated that pyoverdine production correlates to virulence.

Importantly, these models allowed us to identify several novel antivirulents. A biochemical screen of ~45,000 compounds, yielded molecules that directly interact with the siderophore (validated by NMR) and attenuate the production of pyoverdine-regulated toxins and rescue *C. elegans* during pathogen exposure.

In a whole-organism, host-pathogen drug screen using *C. elegans*, we identified fluoropyrimidines, most notably 5-fluorocytosine (5-FC), that curtail pyoverdine production without overtly affecting bacterial titer. 5-FC was sufficient in rescuing all aforementioned hosts during pathogenesis, including murine lung infection. Furthermore, we demonstrated that 5-FC synergizes with the antimicrobial gallium nitrate to inhibit bacterial growth. This is likely due to pyoverdine's ability to sequester the metal. Interestingly, even in the presence of gallium, 5-FC functioned as an antivirulent. Spontaneous mutants emerged in the presence of both drugs were resistant to gallium but remained sensitive to 5-FC. We expect these populations to remain less virulent during dual-drug treatment due to pyoverdine inhibition. Overall, these results demonstrate the promise of antivirulents against multidrug-resistant pathogens.

## MMT 15.
### Neonatal cholestasis hinders microbiome maturation and bile salt deconjugation in preterm infants

**Lauren E. Lynch**[1], Amy B. Hair[2], Krishnakant G. Soni[1], Heeju Yang[2], Laura A. Gollins[2], Monica Narvaez-Rivas[3], Kenneth D. R. Setchell[3,4], and Geoffrey A. Preidis[1]
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Cholestasis (impaired bile flow from the liver to the intestine) affects 10-20% of preterm births, impairs growth, and can cause liver failure and death. Bile salt hydrolase (BSH) enzymes mediate liver-gut-microbiome crosstalk by catalyzing the deconjugation of primary bile acids into secondary bile acids. The

secondary bile acid ursodeoxycholic acid (UDCA) is prescribed to treat cholestatic liver diseases; however, its effects are poorly understood in preterm neonates and treatment is not always effective. We sought to determine how the liver-gut-microbiome axis develops over time in extremely preterm neonates and whether cholestasis alters this pattern of development. We performed a nested case-control study of 24 preterm infants (mean 27.2 weeks gestation), half of whom developed cholestasis. Longitudinally collected stool samples (n=124) were analyzed by metagenomic sequencing, an *in vitro* BSH enzyme activity assay optimized for low biomass fecal samples, and quantitative mass spectrometry to measure the bile acid metabolome. The most distinctive features of preterm gut microbiome development were increasing abundance of the BSH carrier *Clostridium perfringens* and increasing genomic capacity for secondary bile salt biosynthesis over time. Cholestatic neonates did not acquire *C. perfringens*, exhibited impaired BSH enzyme activity, and had decreased abundance of unconjugated bile acids. Administration of UDCA dramatically altered fecal bile acid profiles by increasing the amount of UDCA 522-fold. Atypical isomeric bile acids dominated the fecal bile acid profile in early preterm development and directly correlated with neonatal growth. These data demonstrate that BSH activity, which is impaired in preterm neonates with cholestasis, predicts somatic growth.

# General Microbiology, Trainee Presentations

### GMT 16.
### Utilization of growth rates and swimming motility to evaluate fitness of *S.* Typhimurium after phase I or II flagellin loss

**Alexis Carey[1]**, Sara Lawhon[2], Helene Andrews-Polymenis[2,3], Sargurunathan Subashchandrabose[2], Keri Norman[1]

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Salmonella *enterica* subspecies *enterica* serovars I 4,[5],12:i:- and Typhimurium are among the top five most prevalent in culture-confirmed human non-typhoidal *Salmonella* cases. Evidence suggests that serovar I 4,[5],12:i:- has repeatedly evolved from serovar Typhimurium through acquisitions of chromosomal antibiotic resistance (AMR) or heavy metal resistance (HMR) genes within the phase II flagellin operon (*fljB*). Most *S.* I 4,[5],12:i:- studies focus on genetic elements associated with AMR or HMR acquisition but fail to evaluate the impact of flagellin loss. While *S.* I 4,[5],12:i:- still confers the benefits of phase I flagellin, the current mode of inquiry fails to address potential ecological advantages of *S.* I 4,[5],12:i:- contributing to increasing prevalence in swine and human populations. We hypothesize that environmental conditions favorable for the loss of *fljB* in *S.* Typhimurium will also be advantageous for the initial survival and proliferation of *S.* I 4,[5],12:i:-. Therefore, we aim to determine conditions favorable for the ecological success of *S.* Typhimurium following flagellin loss by examining the impact of growth under two nutrient conditions and the motility of *S.* Typhimurium strains lacking *fljB* or *fliC* flagellin genes. Swimming motility in all strains without *fljB* was less than wildtype strains at 6- and 9-hours growth, while strains without *fliC* were comparable to wildtype. In contrast, generation times in plain Luria broth showed similar results for strains lacking *fljB* or *fliC* genes. Results indicate that *fljB* loss is deleterious for swimming motility, suggesting motility may not be critical for the initial survival of *S.*

# GMT 17.

## Emu: species-level microbial community profiling of full-length 16S rRNA Oxford Nanopore sequencing data

**Kristen D. Curry[1]**, Qi Wang[2], Michael G. Nute[1], Alona Tyshaieva[3], Elizabeth Reeves[1], Sirena Soriano[4], Qinglong Wu[5,6], Enid Graeber[2], Patrick Finzer[2], Werner Mendling[7], Tor Savidge[5,6], Sonia Villapol[4], Alexander Dilthey[3], and Todd J. Treangen[1]

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16S rRNA based analysis is the established standard for elucidating microbial community composition. However, with short-read data delivering only a portion of the 16S gene, this analysis is limited to genus-level results at best. Obtaining species-level accuracy is imperative since two bacterial species within the same genus have proven to express drastically different behaviors on their community and human health. Full-length 16S sequences have the potential to provide species-level resolution. Yet, taxonomic identification algorithms designed for previous generation sequencers are not optimized for the increased read length and error rate of Oxford Nanopore Technologies (ONT). Here, we present Emu, a novel approach that employs an Expectation-Maximization (EM) algorithm, to generate a taxonomic abundance profile from full length 16S rRNA reads. We demonstrate accurate sample composition estimates by our new software through analysis on two mock communities and two simulated data sets. We also show Emu to elicit fewer false positives and false negatives than previous methods on both short and long read data. In summary, full-length 16S ONT sequences, paired with Emu, opens a new realm of microbiome research possibilities. Emu proves, with the appropriate method, increased accuracy can be obtained with nanopore long reads despite the increased error rate. Our novel software tool Emu allows researchers to further leverage portable, real-time sequencing provided by ONT for accurate, efficient, and low-cost characterization of microbial communities.

# GMT 18.

## Development of a synthetic serum active peptide

**Kyra E. Groover[1]**, Bryan W. Davies[1]
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The need for new antibiotics with mechanisms of action that circumvent existing resistance mechanisms is desperate, as deaths caused by antimicrobial resistant infections are on the rise. Peptides both synthetic and natural are being re-evaluated for their use in treating antibiotic resistant pathogens as their mechanism of actions differ when compared to traditional antibiotics. Peptides, however, have significant hurdles to overcome when moving into the therapeutic space. These hurdles include proteolytic susceptibility, cytotoxicity, and renal clearance. While proteolysis and renal clearance can be mitigated by chemical modifications it is difficult decrease the cytotoxicity without altering activity of a peptide. Making it desirable to start with a non-cytotoxic peptide. From a large screen we identified a peptide of interest that had antimicrobial activity. Additionally, the peptide had a small number of hydrophobic residues. It has been seen previously that an increased number of large aromatic residues correlates with greater mammalian cell toxicity. Therefore, we hypothesized that the peptide would be a promising lead for further development. The peptide was then modified via truncation and the incorporation of unnatural amino acids with the goal to reduce proteolytic susceptibility. This modified peptide was found to have

broad spectrum antimicrobial activity as well as low mammalian cell cytotoxicity. Most notably, the modified peptide retains most of its activity in 100% human serum with only a two-fold decrease in its minimum bactericidal concentration. These qualities provide a strong foundation for further therapeutic studies.

## GMT 19.
## Mapping the determinants of catalysis and substrate specificity of the antibiotic resistance enzyme CTX-M β-lactamase

**Allison Judge[1]**, Liya Hu[1], Banumathi Sankaran[2], Justin Van Riper[1], B.V. Venkataram Prasad[1], and Timothy Palzkill[1]
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CTX-M β-lactamases are prevalent antibiotic resistance enzymes in Gram-negative pathogens and are notable for their ability to rapidly hydrolyze the extended-spectrum cephalosporin, cefotaxime. We used codon randomization, antibiotic selection, and deep sequencing to systematically determine the CTX-M active-site residues required for hydrolysis of cefotaxime and a representative penicillin, ampicillin. These experiments revealed active-site positions required for hydrolysis of all β-lactams, as well as residues controlling substrate specificity. Further, CTX-M enzymes poorly hydrolyze the extended-spectrum cephalosporin, ceftazidime. We used our codon-randomized libraries to reveal that the sequence requirements for ceftazidime hydrolysis largely follow those of cefotaxime, with the exception that key active-site omega loop residues are not required and may, in fact, be detrimental for ceftazidime hydrolysis. These results provide insights into the unique ability of CTX-M enzymes to hydrolyze extended-spectrum cephalosporins and demonstrate that changes to the active-site omega loop are likely required for the evolution of CTX-M hydrolysis of ceftazidime to provide resistance.

## GMT 20.
## Roles of *dksA*-like genes in *Paracoccus denitrificans*

**Ashvini Ray[1]**, Stephen Spiro[1]
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*Paracoccus denitrificans* reduces nitrate to dinitrogen during anaerobic respiration (the pathway called denitrification). Nitric oxide (NO), a cytotoxic signaling molecule and nitrous oxide ($N_2O$), a greenhouse gas, are produced as intermediates. Identifying factors which regulate denitrification is important for understanding the control of the production and consumption of these nitrogen oxides. DksA, a transcription factor that binds RNA polymerase, has a role in NO sensing and detoxification, redox balance, and the stringent response in ⍺-proteobacteria. Analysis of the *P. denitrificans* genome revealed two *dksA*-like genes. We show that ⍺*dksA1* strains exhibit an aerobic growth defect on reduced carbon substrates such as butyrate. ⍺*dksA1* strains fail to upregulate expression and activity of the periplasmic nitrate reductase NAP, which disposes of excess reducing equivalents during growth on reduced substrates. Thus, one DksA-like protein is implicated as a regulator of expression of the *nap* operon. NO stimulates biofilm production in *P. denitrificans*, either directly or indirectly. We observed a 4-fold reduction in biofilm production in the ⍺*dksA2* strain. Reporter fusion assays show that *dksA2* is a negative regulator of *hmp*, which encodes an NO-scavenging flavohemoprotein. We suggest that increased *hmp* expression in ⍺*dksA2* strains reduces NO accumulation in static cultures leading to reduced biofilm formation. DksA sometimes acts as a complex with the nucleotide ppGpp, which is synthesized by the product of the *relA* gene.  We show that *relA* modulates the stringent response in *P. denitrificans* and that loss of both *dksA1* and *relA* leads to non-viability on reduced carbon substrates.

**GMT 21.**
**Nonsense-mediated mRNA decay of metal-binding activator *MAC1* is dependent on copper levels and 3'-UTR length in *Saccharomyces cerevisiae***
Xinyi Zhang[1] and Bessie W. Kebaara[1]
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The nonsense-mediated mRNA decay (NMD) pathway was primarily identified as a surveillance system that degrades mRNAs with premature termination codons (PTC). Now NMD is also recognized as a post-transcriptional gene regulatory pathway, which regulates expression of functional natural mRNAs. *Saccharomyces cerevisiae* has been extensively used as a model to study NMD. Earlier studies demonstrated that regulation of functionally related mRNAs by NMD can be differential and conditional in *S. cerevisiae*. Here, we elucidate the regulation of *MAC1* mRNAs by NMD in response to copper, and the role *MAC1* 3'-UTR plays in this regulation. *MAC1* is a copper-sensing transcription factor that regulates high affinity copper transport and is activated under low copper conditions in *S. cerevisiae*. We found that *MAC1* mRNAs were regulated by NMD under normal growth conditions but escape NMD under low copper and high copper conditions. We also found that the *MAC1* 3'-UTR contributes to the degradation of the mRNAs by NMD and that *MAC1* mRNAs lacking the 3'-UTR were stabilized in response to copper deprivation. Taken together, our results demonstrate that condition-specific gene expression modulates an interaction between the NMD machinery and environmental cues. This study supports a model of a novel mechanism of regulating a metal sensing transcription factor, where *MAC1* mRNA levels are regulated by NMD and copper, while the activity of Mac1p is controlled by copper levels.

# Undergraduate Oral Presentations

**UOP 22.**
**Inoculant carrier formulation on survivability of a drought-tolerant *Bradyrhizobium* isolate under desiccation stress**
Camille Condron, Christian Peterson, Spencer Reiling, and Woo-Suk Chang
University of Texas at Arlington

The soybean symbiont *Bradyrhizobium japonicum* fixes atmospheric nitrogen ($N_2$) into ammonia ($NH_3$) through root nodules. *B. japonicum* cultures have been applied as inoculants (i.e., biofertilizers) to soybean fields and its application has been shown to be beneficial to rhizosphere microbiomes, plant growth, and final yield. However, drought is detrimental to the ability to fix nitrogen due to the inability of the symbiont to survive in desiccated conditions. Previously, Chang Lab isolated a Texas-native drought-tolerant strain, *B. japonicum* sp. TXVA. In addition to field trials against other commercially available inoculants, it's been realized that inoculant carrier formulation testing is crucial to success as a commercial product. Using filter disk desiccation assays and CFU plate counting, we have established baseline survivability and tested the first media alteration of the TXVA inoculant alongside *B. japonicum* USDA 110. Cultures were grown up to the late exponential phase and vacuum filtered onto polycarbonate membranes to be stored under fully hydrated and desiccated conditions (i.e., 100% vs. 27% relative humidity) for 24 and 72 h before being resuspended for plate counts. Results from the first medium alteration show that widened carbon source utilization, by the addition of sucrose and trehalose, leads to increased survivability of both strains at both timepoints. The use of compatible solutes and polymeric additives shows potential for increasing the survivability of inoculants in the field.

**Evolving *Pseudomonas aeruginosa* to colistin in microfluidic emulsions recapitulates clinically relevant mutations that are depleted in bulk culture**

__Saoirse Disney-McKeethen__[1], Seokju Seo[1], Heer Mehta, Yousif Shamoo[1]
[1]Rice Universiy

Antibiotic resistance is a global health crisis. Colistin is often the last resort antibiotic used to treat infection by the opportunistic pathogen Pseudomonas aeruginosa, but resistance has already been observed in clinical settings. Understanding mechanisms of resistance through experimental evolution allows for the design of novel therapies to combat resistance. However, in vitro evolution is typically done in a nutrient rich bulk culture environment that 1) does not mimic the nutritional cues of a host environment, 2) lacks the fine spatial structure often present at infection sites such as the lung, and 3) are highly competitive which leads to loss of less fit, slower growing strains. Microfluidic emulsions can be used to create spatially structured, low competition environments that use complex media such as synthetic cystic fibrosis sputum media, which may potentiate clinically relevant evolutionary trajectories that would not be seen in a traditional flask evolution experiment.

Our project aimed to adapt P. aeruginosa to colistin in a microfluidic environment with synthetic sputum media, and compare the evolutionary trajectories observed in flask, large droplet, and small droplet environments through longitudinal whole genome analysis of individual populations. We observed mutations in our small droplet populations that are linked to hallmark phenotypic adaptations to a cystic fibrosis lung environment, such as loss of motility, increased exopolysaccharide production, and loss of O antigen biosynthesis. Furthermore, these mutations were quickly lost when droplet populations were reintroduced to a bulk culture, indicating that these trajectories can only be recapitulated in a microdroplet environment.

**Gain of function cytolysin variant expressed by clinically isolated *Enterococcus faecalis***

__Irene Hau__[1], Neha Hulyalkar[1], Belle Sharon[1], Vivian Nguyen[3], Philippe Zimmern[2], Nicole De Nisco[1,2]
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*Enterococcus faecalis* can cause serious nosocomial infections, most commonly urinary tract infections. While prescribed antibiotic therapies are available, UTIs are often refractory to these treatments, resulting in diminished quality of life. Cytolysin, an enterococcal exotoxin & bacteriocin encoded by the *cyl* operon, can enhance virulence in endocarditis models. Cytolysin targets various mammalian cells, including erythrocytes, macrophages, neutrophils, and even bacteria. Cytolysin is active against human, rabbit, and horse erythrocytes due to their high phosphatidyl choline (PC) content, but sheep erythrocytes (SE) with low PC content are resistant.

We identified a urinary *E. faecalis* isolate, C33, that demonstrated enhanced hemolysis against SEs. Studies using markerless gene exchange approach confirmed C33 SE hemolysis was dependent on the *cyl* operon, but sufficiency of *cyl* operon is yet to be confirmed. Since cytolysin has been previously reported to target bacterial cells, we hypothesized that C33 gain-of-function cytolysin is important for competition with the urinary microbiota. We observed that *E. faecalis* C33 inhibited the growth of *Enterococcus raffinosus* and *Streptococcus parasanguinis* isolated from the same urine. Additionally, under anaerobic conditions, C33 showed increased survival against *Staphylococcus capitis*, which was highly inhibitory in aerobic conditions. Interestingly, C33 Δ*cyl* mutant inhibited the growth of *E. faecalis* reference strains OG1RF (*cyl-*) & DS16 (*cyl+*), hinting at the presence of an additional bacteriocin in this strain. We identified

a candidate bacteriocin operon on a 72 kilobase plasmid in C33 and are currently evaluating the contribution of this operon to enhanced SE hemolysis and competition against the urinary microbiota.

## UOP 25.

**Modulation of bacterial host phenotypes by mycobacteriophage pixie gene products**

Aeron Pennington, SEA-GENES, Selina Alvarado, Harold Rathburn, and Dustin Edwards
Tarleton State University, SEA-GENES, Howard Hughes Medical Institute

Bacteriophage genes are being studied for their potential clinical use in phage therapy for antibiotic-resistant infections. With the support of the Howard Hughes Medical Institute, as part of the SEA-GENES network, we conducted a cytotoxicity study with all 100 genes from Mycobacteriophage Pixie. Genes were amplified from Pixie high titer lysate by PCR amplification with gene-specific primers, and the products were purified and ligated into pExTra plasmids by isothermal assembly. Plasmids were transformed into 5-alpha F'Iq Escherichia coli, and the extracted plasmid DNA was electroporated into Mycobacterium smegmatis mc$^2$155. Phenotypic assays were conducted by plating transformed M. smegmatis on agar containing anhydrotetracycline to induce the production of gene inserts. Cytotoxicity was determined by spotting serially diluted transformed M. smegmatis growth versus controls. Our study revealed 34 potentially cytotoxic genes: a putative major capsid protein, a holin protein downstream of the lysin A/B proteins, portal protein, putative tyrosine integrase, and a series of uncharacterized cytotoxic genes adjacent to the immunity repressor. We are currently assessing bacteriophage-bacteria protein-protein interactions of cytotoxic genes using bacterial 2-hybrid assays. This information broadens our understanding of bacteriophage-host interactions and the potential clinical use of cytotoxic genes to treat bacterial infections.

## UOP 26.

**Defining the spatial and temporal dynamics of the urogenital microbiome in postmenopausal women**

Star Okolie[1,2], Amanda Arute[1], Dr. Michael Neugent[1], Namrata Sawant[1], Neha Hulyalkar[1], Dr. Philippe Zimmern[2], Dr. Nicole De Nisco[1,2]
[1]University of Texas at Dallas, [2]UT Southwestern Medical Center

Urinary tract infection (UTI) is among the most common infections in the United States and primarily affects women.[1] The prevalence of UTI generally increases with age, and recurrent UTI (rUTI), defined as 3 UTIs in the past year, doubles in women over 65.[1] The differential incidence of rUTIs in pre- and post-menopausal women postulates that hormone status and the urogenital microbiome may be key to rUTI susceptibility.[2] Because little is known about the spatial and temporal dynamics of the female urogenital microbiome in healthy post-menopausal (PM) women, this study seeks to define temporal changes in the urinary, vaginal, and peri-anal microbiomes associated with estrogen hormone therapy (EHT) in PM women with no UTI history.[2] Patients selected for the study donated urine and swabs at 4 time points. At each visit, a wet and dry swab were collected from the vagina and the peri-anal region, followed by clean-catch urine. Genomic DNA was extracted from dry swabs and microbiome composition was determined via 16S rRNA amplicon sequencing of the entire 16S gene using PacBio sequencing for species-level resolution. The wet swabs were cultivated by a recently published Advanced Culture method with PCR and Sanger sequencing of the 16S rRNA gene for species identification.[2] To date, full taxonomic profiles have been generated for 6 patients and over 200 isolates have been cultivated and identified.

**UOP 27.**
**Pyocins contribute to ST111 strain dominance in *P. aeruginosa* inter-strain competition**
**Filemon C. Tan**, Liyang Zhang, Natasha Kirienko
Rice University, BioSciences

Previous research identified that multi-locus sequence type (ST) 111 dominated over other *P. aeruginosa* strains in patients with hematologic malignancy or those receiving hematopoietic cell transplants. In part, this was due the production of >100 kDa protease-sensitive factor(s) secreted by ST111 isolates that inhibited a panel of other ST's identified in these patients. Importantly, lab-adapted strain PA14 also produced factor(s) with the same characteristics. Both ST111 and PA14 cell-free spend media (filtrate) inhibited the growth of the same panel of non-ST111 isolates. Considering the matching characteristics of PA14 and ST111 effector(s), studying PA14 could reveal a mechanism of ST111 dominance. To identify genes necessary for PA14 to inhibit non-ST111 isolates, a high-throughput, PA14 transposon-insertion mutant library screen was developed by 1) transforming non-ST111 M0104 sensor strain with dsRed and 2) exploiting strains' differing antibiotic susceptibility. Of 5,810 mutants, 46 hits were identified. Hits included adjacent R-pyocins genes, a class of bactericidal phage-like particles. R-pyocin mutations abolished PA14's ability to inhibit M0104 and other non-ST111 strains. Furthermore, PA14 filtrate exhibited killing activity against M0104, consistent with a phage-like particle. Production of R-pyocin by ST111 strains was confirmed via specialized R-pyocin indicator strain. In summary, we observed that contact-independent effector(s) mediate both PA14 and ST111 ability to inhibit non-ST111 hospital isolates. A PA14 library screen identified R-pyocins, phage-like particles produced by both PA14 and ST111 strains, as a key effector.

# Careers in Transition

**CT 28.**
**System dynamics and layered feedback control in synthetic biology**
**Chelsea Y. Hu**[1,2] and Richard M. Murray[1]
[1]California Institute of Technology, Division of Biology and Bioengineering
[2]Texas A&M University, Department of Chemical Engineering

Layered feedback is an optimization strategy in feedback control designs widely used in engineering. Control theory suggests that layering multiple feedbacks could overcome the robustness-speed performance trade-off limit. In natural biological networks, genes are often regulated in layers to adapt to environmental perturbations. It is hypothesized layering architecture could also overcome the robustness-speed performance trade-off in genetic networks. In this work, we validate this hypothesis with a synthetic biomolecular network in living E. coli cells. We start with system dynamics analysis using models of various complexities to guide the design of a layered control architecture in living cells. Experimentally, we interrogate system dynamics under three groups of perturbations. We consistently observe that the layered control improves system performance in the robustness-speed domain. This work confirms that layered control could be adopted in synthetic biomolecular networks for performance optimization. It also provides insights into understanding genetic feedback control architectures in nature.

**CT 29.**
**Characterization of activity and mechanism of action of the *Enterococcus faecalis* bacteriocin EntV on *Candida albicans***

**G. Buda De Cesare**[1], S. Guha[1], MR. Cruz[1], SA. Cristy[1], A. Zito[2], E. Evdokimova[3], H. Sanchez[4], D. Borek[5], P. Miramón[1], J. Yano[6], PL. Fidel Jr[6], A. Savchenko[3], DR. Andes[4], PJ. Stogios[3], MC. Lorenz[1], and DA. Garsin[1].
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*Candida albicans*, an opportunistic fungal pathogen, causes systemic and superficial infections, especially in immunocompromised patients. Treatment of fungal infections is complicated by limited antifungal options and the development of drug resistance. Previous work from our group demonstrated that the bacterium *Enterococcus faecalis*, a normal constituent of the oral and gut microbiome that is often co-isolated with *C. albicans*, antagonizes hyphal morphogenesis, biofilm formation, and virulence in *C. albicans*. These effects are mediated by EntV, a 68aa bacteriocin produced by *E. faecalis*. Based on structural data, we identified a 12aa fragment of EntV that was fully active in both *in vitro* and *in vivo* experiments, including mouse oropharyngeal candidiasis and disseminated infection models. The 12mer localizes to the cell surface, with a greater binding of the peptide to hyphae compared to yeast cells, indicative of a higher amount of the peptide target in hyphae. The binding of the peptide to extracellular vesicles in *C. albicans* and *Cryptococcus neoformans*, another opportunistic fungal pathogen, suggests a connection with the antivirulence activity of EntV. In fact, the EVs are involved in virulence and biofilm formation in multiple fungal species and the similar localization of EntV in both species highlights the potential for this peptide as a broad-spectrum antifungal. Lastly the 12mer induces transcriptome changes indicating possible metabolic and cell wall remodelling mechanisms associated with peptide exposure. Together these approaches are working to identify the molecular mechanism of EntV and enhancing its activity, both important step in its further development as a potential therapeutic.

**CT 30.**
**Pathogenomes and phylogenomic comparison Of 'Big Six' Non-O157 shiga toxin-producing *Escherichia coli***

**Anwar A. Kalalah**[1,2], Sara S.K. Konig[1,2], James L. Bono[3], Joseph M. Bosilevac[3], Mackenzie M. Nolen[1], Armando Rodriguez[4], Mark Eppinger[1,2]
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**Background:** Shiga toxin (Stx)-producing *Escherichia coli* (STEC) of serotype O157:H7 are a global public health threat responsible for widespread human disease. The incidence of nonO157 STEC infections, however, is steadily increasing. The predominant non-O157 serotypes; namely O26, O45, O103, O111, O121, and O145 are colloquially referred to as the 'big six', and account for the majority of clinical non-O157 STEC infections. In this study, we established phylogenetic hypotheses for all available 185 closed 'big six' genomes including prevalent Hserotypes and recorded virulence profile. **Method:** We closed

genomes of representative 'big six' strains obtained from the American Type Culture Collection (ATCC MP-9) to serve as references for our comprehensive analyses. All genomes were profiled *in silico* for virulence gene inventories dissecting the core and mobilome. We determined the Sequence Type (ST) by applying the 7-gene Warwick *E. coli* multilocus sequence typing (MLST) schema, and developed a core genome (cg) MLST using Ridom SeqSphere+. Computations of the pangenome allowed us to identify unique, shared and common gene content. **Results and conclusions**: Comprehensive analyses revealed the genome plasticity and individual relationships of sampled 'big six' STEC isolates at a high-level of phylogenetic accuracy and resolution. This is reflected in the individual virulence complements and genome sizes, ranging from 4.7 to almost 6 Mbp. The latter is driven largely by the mobilome acquisition of prophages and genomic islands. Our data provide a robust foundation to further elucidate the virulence potential and niche adaptations in the 'big six', which are major culprits of food-borne disease.

### CT 31.
### *Coxiella burnetii* requires type IVB secretion system to suppress host TLR3/TRIF-dependent NF-kB-activation

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*Coxiella burnetii (Cb)*, the causative agent of Q fever, replicates inside a Coxiella-containing vacuole within macrophages modulating NF-κB-dependent host innate immune processes. A *Cb* growth defect in C57Bl/6 bone marrow-derived macrophages (BMDM) is restored in BMDMs deficient in TLR2, MyD88 or TNFαR, supporting a model for NF-kB-regulation-dependent restrictions during infection. *Cb* requires a functional type IVB secretion system (T4SS) to modulate host NF-kB signaling, and yet, characterization of the range of *Cb* T4SS effectors which directly or indirectly modulate NF-kB signaling during infection remains incomplete. To determine at which steps of NF-kB signaling the *Cb* T4SS effectors modulate the host, we tested molecular components both up and down-stream in the NF-kB pathway. We used multiple Tolllike receptor (TLR) ligand-inducing NF-kB activation screens to identify how *Cb* regulates NFkB activation. TLR ligands in THP1 NF-kB reporter cells quantitatively demonstrated that *Cb* utilized T4SS to selectively inhibit host TLR3/TRIF-induced NF-kB activation. Furthermore, RTqPCR confirmed that *Cb* used T4SS to suppress the transcription of NF-kB-mediated host TNFalpha, IL8, and BCL-3 in THP-1 cells induced with Poly IC (a TLR3 agonist). Comparison between *Cb* NMII and DotA (T4SS deletion mutant) in TRIF$^{-/-}$-THP-1 NF-kB reporter cells demonstrated a loss of TLR3-induced NF-kB activation in cells infected with DotA. Moreover, laser scanning confocal microscopy showed that both TLR3 and TRIF co-localize with *Cb* NMII vacuoles. Collectively, these data demonstrate that during infection, *Cb* blocks TLR3/TRIFdependent NF-kb signaling in a T4SS-dependent manner and provides an opportunity to identify specific T4SS effectors which mediate this process.

# Environmental Microbiology & Ecology

### EME 32.
### Application of MALDI-ToF mass spectrometry systems to environmental microbiology

**Michael G. LaMontagne**

University of Houston – Clear Lake

Matrix-assisted laser desorption – time of flight mass spectrometry (MALDI-ToF MS) systems provide strain-level identification of microbes by pattern matching between protein and reference spectra.

Reagents cost pennies per spectra and the systems have a throughput of thousands per day. These systems have transformed clinical microbiology by providing rapid and accurate identification of pathogens. In environmental microbiology, MALDI-ToF systems allow
investigators the opportunity to characterize libraries of isolates with the depth that approaches metagenomic sequence libraries. This proteomics technique has reinvigorated applications in microbiology that largely depend on culturing microbes, including microbial discovery, and shows promise for culture-dependent microbial source tracking (MST), which is essential to identifying the source of the fecal indicator bacteria (FIB) that result in regulatory action. In this talk, I will present work from my laboratory where we apply MALDI-ToF to discovery of plant growth promoting rhizobacteria (PGPR) and culture-dependent microbial source tracking (MST). For PGPR, the resolution of MALDI-ToF was superior to the widely used method of 16S rRNA sequencing. Indeed, preliminary results suggest that MALDI-ToF has a resolution comparable to the gold standard of whole genome sequencing. For MST. MALDI-ToF consistently differentiated FIB (*E. coli* and *Enterococci* sp) isolated from human and animal sources. These results confirm that MALDI-ToF systems have wide applications in environmental microbiology.

**EME 33.**
**The clinic vs the farm: exploring prevalence and function of CRISPR-Cas in agriculturally relevant niches**

<u>Chahat Upreti</u>[1], Pranav Kumar[1], Savannah Pas[2], Lisa M. Durso[3], Kelli L. Palmer[1]
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Global efforts to combat the spread of antibiotic resistance have highlighted the importance of a OneHealth approach that combines human, animal and environmental perspectives. A key driver of antibiotic resistance is horizontal gene transfer (HGT). As a native defense system against HGT in bacteria, CRISPR-Cas is a promising tool to address this crisis. Due to historical study biases favoring clinically-derived strains, we have a poor understanding of CRISPR-Cas prevalence and efficacy in agriculturally relevant strains. Here, we analyzed 1,984 genomes of the zoonotic pathogen, *Enterococcus faecalis,* from human and animal sources. We found that strains from humans had larger genomes and greater sequence diversity, whereas those from animals carried a higher proportion of multidrug resistant genomes and had more diverse sequence targets in their CRISPR systems. To further explore animal-origin strains, we obtained 130 agricultural *E. faecalis* isolated from farms across the USA. We found that apart from geography, the presence of a CRISPR-Cas system and host animal identity drove genetic similarity. Finally, to evaluate its therapeutic potential against the spread of resistance in agricultural environments, we tested the efficacy of CRISPR-Cas in this niche. For this, we determined the ability of CRISPR-Cas to block plasmid transfer in manure. We found that not only is CRISPR-Cas effective in manure, it maintains its efficacy longer than in solid agar or liquid BHI media. Together, our work shows for the first time a comprehensive picture of CRISPR-Cas prevalence and efficacy in agriculturally relevant niches for *E. faecalis*.

**EME 34.**
**Induction of root nodulation independent of nitrogen fixation by *Leifsonia shinshuensis* in *Aeschynomene indica* plants**

<u>Meaghan Rose</u>, Jenny Le, and Woo-Suk Chang
University of Texas at Arlington

The formation of root nodules has been understood as one of the most agriculturally important systems for biological nitrogen fixation. Nitrogen-fixing bacteria form symbiotic relationships with leguminous host plants providing usable forms of nitrogen to the host in exchange for environmental stability and access to energy packed carbon sources. Host plants produce nodules and leghemoglobin, a pigmented molecule that facilitates nitrogen fixation by sequestering oxygen, in response to the invasion of bacteria into their root cortical tissues. It has been long assumed that the number of pigmented nodules (from the leghemoglobin) is directly proportional to the amount of nitrogen fixed within the nodules. To test this, *Aeschynomene indica* plants were inoculated with a non-nitrogen fixing endophyte, *Leifsonia shinshuensis,* and the resulting nodule formation was observed. Nitrogen fixation rates were determined indirectly by measuring the activity of nitrogenase via an acetylene reduction assay. *A. indica* plants that were inoculated with *L. shinshuensis*, were found to have increased pigmented nodule formation and a deficit in nitrogenase activity. These results suggest that our assumption on the role of pigmented nodules needs to be reevaluated to be cognizant of the idea that the presence of pigmented nodules does not necessarily indicate nitrogen fixation is occurring in root nodules.

**EME 35.**
**Mercury contamination characterized by microbial Hg methylation genes in martin lake, East Texas**

<u>Stephan Joseph</u>[1], Sharon Schmidt[1], Javid F. McLawrence[2], Anil Somenahally[2], Ri-Qing Yu[1]

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As the US largest airborne mercury (Hg) emitter of the four coal-fired power plants in East Texas, Luminant's Martin Lake Steam Station on Martin Creek Lake in Tatum, Texas emits atmospheric Hg while burning coal to generate electricity, and releases cooling water into the lake. Inorganic Hg in the sediment could be methylated by anaerobic microbes into neurotoxicant methylmercury (MeHg). MeHg can easily bioaccumulate along the aquatic food web and cause detrimental health risks to local populations and wildlife. The fate and microbial synthesis of Hg in the lake ecosystem and the dominant groups of Hg methylating microbes, however, are unknown in the lake.

We investigated sediment, lake water, and pore water samples in Martin Lake in October 2019 and January 2020. Sediment samples were taken using Ekman Sediment Grab Samplers, while lake water was collected using half-liter bottles. Biogeochemical analyses of sulfate, iron (III), total organic content, and others showed that sulfate levels in Martin Lake were high and fluctuated among different sampling sites. After extracting genomic DNA from sediment samples, we are also conducting detection and quantification experiments of Hg methylating genes *hgcA* in various sites around the lake. Detection results have shown the presence of the *hgcA* gene in most sampled sites around the lake. It is hypothesized that sampled areas surrounding the power plant will have higher concentrations of MeHg and more abundant *hgcA* genes due to atmospheric deposition and cooling water runoff from the power plant.

# Closing Keynote: ASM Distinguished Lecturer

**36.**
**The bacterial protein export zoo**
<u>**Miriam Braunstein**</u>, PhD
University of North Carolina Chapel Hill School of Medicine

All bacteria have pathways for exporting specific proteins out of the cytoplasm and across the inner membrane. The exported proteins may either remain in the cell envelope or be fully secreted into the extracellular environment, and they play critical roles in bacterial physiology and pathogenesis. There are numerous types of protein export pathways and new systems are being discovered all the time. However, functional similarities between diverse systems exist and there are examples of components that are shared between or co-opted from different systems. This lecture will highlight protein export pathways that the diderm bacteria and infamous bacterial pathogen *Mycobacterium tuberculosis* uses to transport effector proteins into the host environment for the purpose of evading the host immune response and causing disease. Focusing on the specialized SecA2 pathway, I will discuss how a second copy of the universally conserved SecA protein plays a central role in *M. tuberculosis* pathogenesis and our discovery of SatS, a novel chaperone protein, that functions in protecting and delivering proteins to the SecA2 pathway. I will also discuss our identification of the *M. tuberculosis* SapM phosphatase as an effector protein that is chaperoned by SatS and secreted by the SecA2 pathway. This ongoing project expands our appreciation of the array of export systems that exist in bacteria.

# POSTER PRESENTATIONS ABSTRACTS

## GENERAL MICROBIOLOGY - graduate students
## Samuel Kaplan Award Poster graduate student presentation

**GS P1.**

**Differential gene regulation of the iron transcriptome by nonsense-mediated mRNA decay in**
***Saccharomyces cerevisiae***

**Jacqueline Carroll**[1], Bessie W. Kebaara[1]
[1]Baylor University

The Nonsense-mediated mRNA decay pathway, otherwise known as NMD, is a highly conserved pathway within eukaryotic organisms. The NMD pathway was named after the function for which it was discovered, serving as a mode of mRNA surveillance and targeting mRNAs containing premature termination codons (PTCs) for degradation. However, the NMD pathway has also been shown to regulate gene expression by targeting fully functional, naturally occurring mRNAs. Though the mechanisms of NMD-mediated regulation of natural mRNAs is still largely unknown, varying factors have been shown to affect transcript sensitivity to NMD, including physical features of the transcripts and changes in environmental conditions. Previous research has found that NMD is implicated in maintaining copper and iron homeostasis. Iron is necessary for oxidative phosphorylation and respiration, though toxic in excess. Defects in iron homeostasis in humans are implicated in numerous cancers, developmental conditions, and anemias. The effects of differing environmental cues, such as varying levels of environmental iron, in relation to genetic regulation by NMD are not known. In exploring how NMD regulates the iron transcriptome to cope with iron-scarcity and iron starvation events, RNAseq analysis of wildtype and NMD mutant *Saccharomyces cerevisiae* was done. Both normal and iron-deplete conditions have shown differential gene expression regarding cell membrane components and mitochondrial function. Additionally, we found that the low-iron transcriptional regulators, *AFT1* and *AFT2*, as well as downstream targets belonging to the *FRE* family of membrane ferric and cupric reductases, are differentially regulated by NMD in normal and iron-deplete conditions.

**GS P2.**

**Targeted, high-throughput transcriptional activation via a CRISPR-associated transposon system**

**Andrea M. Garza Elizondo**[1], Daniel Haller[1], and James Chappell[1]
[1]Rice University

Understanding the relationship between genotype and phenotype is one of the grand challenges of biology and has motivated the development of molecular tools with the goal of deciphering gene function. Advances in these tools, such as the advent of CRISPR-based gene editors, have allowed researchers to assess gene expression on phenotypic outcomes at a larger scale than previously possible, providing new methods for interrogating an entire genome in a single experiment. However, state-of-the-art technologies have failed to provide a targeted, high-throughput transcription activation strategy for prokaryotes, limiting our ability to investigate otherwise silent or cryptic genes identified through bioinformatics but whose function remains unknown. Here, we adapt a CRISPR-associated transposon (CAST) system into a transcription activator by harnessing its ability to insert a DNA cargo into a host's genome and using it to insert promoter elements upstream of silent genes. To demonstrate this novel function, CAST is used to insert a strong, constitutive promoter as cargo upstream of fluorescent reporter genes, leading to robust transcription of the gene of interest. We demonstrate fine-tuning of transcription activation through the use of variable strength and inducible promoters. Finally, we demonstrate the utility of this approach in non-synthetic contexts by activating the transcription of endogenous *E. coli*

genes. This work sets the stage for the use of CAST as a tool to perform high-throughput transcriptional perturbations for studies that include in-depth analysis of gene regulatory networks, high-throughput mapping of phenotype-genotype underlying drug resistance, and activation of silent biosynthetic gene clusters.

**GS P3.**
**Secretion of heterologous peptides from Gram-negative bacteria**

**Sun-Young Kim[1]**, Jennifer K. Parker[1], Monica Gonzalez-Magaldi[1], Mady S. Telford[1], Daniel J. Leahy[1], and Bryan W. Davies[1,2].
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[2]John Ring LaMontagne Center for Infectious Diseases, The University of Texas at Austin, Austin, Texas

Peptides perform a limitless array of functions, from endocrine signaling, to regulating microbes, to building biomaterials. Microbial systems that can produce recombinant peptides enable discovery of new effectors, exploration of sequence activity relationships, and the potential for in vivo delivery. However, we lack simple systems for controlled peptide secretion from Gram-negative bacteria. This prevents the use of key research organisms, including Escherichia coli, as peptide secretion vehicles. Type I secretion systems are widespread in Gram-negative bacteria and allow for one-step export of peptides from the cytosol to the environment. However, little is known about the capacity of these systems and how export is influenced by the cargo peptide sequence. Here, we investigate the microcin V type I secretion system and show it can export a wide range of synthetic and natural peptide sequences. We demonstrate that secretion through this system is not affected by peptide charge or hydrophobicity and appears only constrained by peptide length. Bioactive peptides, including an antibiotic, a microbial signaling factor, a protease inhibitor, and a human hormone, can all be secreted and elicit their intended biological effect. Importantly, secretion through this system is not limited to E. coli, and we demonstrate its use in additional Gram-negative species that can inhabit the gastrointestinal tract. Our findings further our understanding of peptide export through microcin type I systems, as well as peptide discovery, study, and delivery using Gram-negative bacteria.

**GS P4.**
**Molecular interactions between peptidoglycan integrity maintenance and outer membrane lipid asymmetry in *Acinetobacter baumannii***

**Sinjini Nandy,** Misha I. Kazi, Joseph M. Boll
The University of Texas at Arlington

The Gram-negative cell envelope is essential because it provides mechanical strength to counter the turgor and acts as a barrier to restrict the entry of toxins and antibiotics. The outer membrane (OM) and peptidoglycan are tightly linked during growth, but the molecular factors and pathways that coordinate assembly are poorly understood. Identifying cooperative factors that promote assembly between cell envelope layers, may provide insights into how we can effectively target antimicrobials against Gram-negative pathogens. *Acinetobacter baumannii* is a nosocomial pathogen that has a high propensity to overcome antimicrobial treatment. LdtJ is a periplasmic LD-transpeptidase promoting fitness during growth in *A. baumannii*. However, it is unknown how LdtJ contributes to growth. Previous work in *Escherichia coli* showed that periplasmic tetrapeptide accumulation is toxic. Muropeptide analysis of Δ*ldtJ* showed increased tetrapeptide pools, which are LdtJ substrates, and this accumulation may also be toxic in *A. baumannii*. Transposon sequencing analysis suggested that deletion of *mlaE* in Δ*ldtJ* background restored the growth defect. *M*aintenance of *l*ipid *a*symmetry (Mla) retrograde phospholipid transport system removes mislocalized surface-exposed phospholipids to maintain OM asymmetry. When Mla is disrupted, accumulation of surface-exposed phospholipids induces OM vesicle formation. Here, we tested the hypothesis that periplasmic tetrapeptide toxicity can be relieved by *mlaE* disruption, where increased OM vesicle formation releases tetrapeptides into the environment to reduce cellular toxicity. Thus, *mlaE*

serves as a compensatory mutation to restore Δ*ldtJ* fitness defect. This study will provide insights into how OM asymmetry and peptidoglycan integrity maintenance pathways are coordinated to maintain cell envelope homeostasis.

**GS P5.**
### New insights into the regulation of methylotrophic growth in *Paracoccus denitrificans*

**Trusha Parekh**[1], Sneha Narvekar[1], Cheyenne Ziegler[1], Faruck Morcos[1] and Stephen Spiro[1]
[1]University of Texas at Dallas

*Paracoccus denitrificans* utilizes C1 compounds methanol and methylamine as sources of energy. The formaldehyde generated from both substrates is oxidized to formate and then to CO2, which is assimilated via the Rubisco pathway. Expression of the genes required for C1 metabolism is activated by a two-component regulatory system, FlhSR, in response to formaldehyde. Co-expressed with FlhSR is a FIST-domain family protein we designate FlhT. The only FIST-domain proteins previously characterized are nitric oxide sensing hemoproteins that regulate biofilm formation and quorum sensing. Assays of growth on C1 substrates and of promoter and enzyme activities demonstrated that FlhT is required alongside FlhS and FlhR for activation of expression of genes involved in the oxidation of methanol, methylamine and formaldehyde. Results from direct coupling analysis and structural modeling are consistent with the formation of a complex between FlhT and FlhS. We propose that FlhT binds formaldehyde and, in response, activates the autophosphorylation and/or phosphotransfer activities of the hybrid histidine kinase FlhS. Catabolism of choline is accompanied by the formation of formaldehyde; growth of *P. denitrificans* on choline requires FlhSR to up-regulate the expression of formaldehyde dehydrogenase that is required to remove the toxic formaldehyde. By assaying growth of mutants, and promoter and enzyme activities we show that the source of formaldehyde during choline catabolism is a glycine betaine monooxygenase that oxidatively demethylates glycine betaine producing dimethylglycine. Our work establishes a novel role for a FIST-domain protein and adds new complexity to the regulation of C1 metabolism and formaldehyde detoxification in *P. denitrificans*.

**GS P6.**
### Methyl halide transferase-based gas reporters for quantification of filamentous bacteria in microdroplet emulsions

**Xinhao Song**[1], Sarah Kong[1], Seokju Seo[1], Ramya Prabhakar[1], Heer Mehta[1], and Yousif Shamoo[1]
[1]Department of BioSciences, Rice University

Application of microfluidic techniques in experimental evolution is a rapidly emerging field. Water-in-oil microdroplets can serve as controllable micro-vessels for experimental evolution studies. To evaluate cell growth within microdroplets, various optical approaches including color, fluorescence, and luminescence-based reporters have been developed. However, optical reporters suffer from limitations when used for microdroplets with potentially strong background interference or when opaque culture media are required. Sensitivity can be limited during early growth when cell density is low. In addition, optical detection is typically not amenable to filamentous organisms or biofilm-producing organisms that have significant non-linear changes in opacity and light scattering during growth. In this study, we show that volatile methyl halide gases (MeX) produced by reporter cells expressing a methyl halide transferase (MHT) can serve as an alternative non-optical detection approach particularly suitable for microdroplet emulsions. In this study, an MHT-labeled *Streptomyces venezuelae* reporter strain was constructed and characterized. Protocols were developed for the encapsulation and incubation of *S. venezuelae* in microdroplets. Using *S. venezuelae* encapsulated in microdroplets, we observed a complete life cycle of *S. venezuelae* including mycelia extension, mycelial fragmentation, and late-stage sporulation. Methyl bromide (MeBr) production was detected and quantified by gas chromatography-mass spectrometry (GC-

MS) from *S. venezuelae* gas reporters incubated in either liquid suspension or emulsion microdroplets. Furthermore, MeBr production was used to estimate bacterial density in both environments. Overall, using MeBr production as a means of quantitating cellular growth provided a 100-1000 fold increase in sensitivity over the same strain expressing Red Fluorescent Protein.

## GS P7.
### Bacterial secretion of affibodies and other biologics.
### Mady Telford
University of Texas at Austin

Bacteria can be engineered to act as living therapeutics for treatment of a variety of human diseases. Antibodies are often used as treatment because of their ability to selectively bind to target antigens. Synthetic proteins called affibodies represent an antibody alternative that is much smaller (~7.5kDa) but retains the specific binding capability of antibodies. Affibodies can be engineered to bind many different ligand variants and are an attractive therapeutic candidate because of their small size and the ability to be fused together for bispecific targeting. Here we show that E. coli can be engineered to continuously produce and secrete affibodies into the supernatant via a non-canonical type 1 secretion system (T1SS). Secreted affibodies maintain their biologic viability, fold properly, and bind specifically to their intended target. This system can be easily modified to secrete a variety of proteins which are easily purifiable via affinity chromatography. This method of purifying biologics provides an alternative approach to protein purification that eliminates the need for cell lysis, thus reducing the amount of work and equipment needed in classical protein purification.

## GS P8.
### Development of a high-throughput minimum inhibitory concentration (HT-MIC) testing workflow
### Suman Tiwari[1], Nicholas Dillon[1], and Oliver Nizet[2]
[1]University of Texas at Dallas, [2] La Jolla Country Day School

The roots of the minimum inhibitory concentration (MIC) determination go back to the early 1900s. Since then, the test has undergone modifications and advancements in an effort to increase its dependability and accuracy. Although biological investigations use an ever-increasing number of samples, complicated processes and human error sometimes result in poor data quality, which makes it challenging to replicate scientific conclusions. The automation of the few manual steps using protocols decipherable by machine can ease some of the procedural difficulties. Originally relying on manual pipetting and human vision to determine the results, modern broth dilution MIC testing procedures have incorporated microplate readers to enhance sample analysis. However, current MIC testing procedures are unable to simultaneously evaluate a large number of samples efficiently. Here, we have created a workflow using the Opentrons OT-2 robot to enable high-throughput MIC testing. We have further optimized the analysis by incorporating Python programming for MIC assignment to streamline the automation. With this workflow, we conducted MIC testing on four different strains, each with three replicates and eight different antibiotics and examined a total of 1,152 wells. Comparing our workflow to a conventional plate MIC procedure, we find that the HT-MIC method is 630% faster while simultaneously boasting a 100% accuracy. Our high-throughput MIC workflow can be applied in both academic and clinical settings since it is faster, more efficient, and more accurate than many conventional methods.

**GS P9.**
**Assessing lysine-lipid asymmetry in the group B streptococcal membrane by lipid labeling**

**Aparna Uppuluri[1]**, Ziqiang Guan[2], and Kelli Palmer[1],
[1]University of Texas at Dallas, [2]Duke University

Group B *Streptococcus* (GBS) is a gram-positive bacterium that causes meningitis in neonates. MprF is an integral membrane protein that plays important roles in bacterial virulence. The GBS MprF synthesizes a novel, highly cationic glycolipid, lysyl-glucosyl-diacylglycerol (Lys-Glc-DAG) as well as lipid lysyl-phosphatidylglycerol (Lys-PG). The goal of this study is to investigate the Lys-lipid distributions in both the leaflets of the membrane to determine whether one or both lipids face the extracellular environment. This is important to understand the roles of these lipids in host-pathogen interactions. In our study, we used 2,4,6-trinitrobenzenesulfonic acid (TNBS) to derivatize Lys-lipids from GBS. TNBS cannot cross the membrane due to its water solubility and net negative charge and hence, it only derivatizes aminolipids in the outer leaflet. To test our assay conditions, we performed the lipid labeling experiment in *Bacillus subtilis* and found that TNBS labels both Lys-PG and Phosphatidylethanolamine. We have determined that TNBS labels both Lys-Glc-DAG as well as Lys-PG in GBS, indicating that these lipids are indeed flipped to the outer leaflet of the membrane. Ongoing studies include analysis of growth-phase dependent effects on Lys-lipid localization and identification of the mechanism for Lys-Glc-DAG flipping to the outer leaflet.

**GS P10.**
**Elucidating the function of an unusual hydrophobic peptide in *Pseudomonas aeruginosa***

Ziqiang Guan[1], **Fabiha Zaheen Khan[2]**, Kelli Palmer[2]
[1]Duke University, [2]University of Texas at Dallas

*Pseudomonas aeruginosa* is a Gram-negative bacterium that is the major cause of chronic infections in cystic fibrosis (CF) patients and causes a range of other opportunistic infections. *P. aeruginosa* produces a number of antimicrobial factors that allow it to effectively compete against other microbes. We identified a hydrophobic short peptide that co-fractionates with lipids in Bligh-Dyer extractions of *P. aeruginosa* stationary phase cultures. This unusual peptide has a disulfide bond, indicating post-translational modification. We used bioinformatics to identify the gene that encodes this peptide. Interestingly, the peptide appears to be a cleavage product of a Gly-Gly peptidase, which is encoded upstream of the putative peptide precursor gene. Based on literature analyses of existing knowledge of these genes, we present two models for the function of this short peptide and its linked peptidase: an immunity factor/toxin pair used by the type-6 secretion system (T6SS), or a bacteriocin-like antimicrobial factor secreted by a non-T6SS mechanism.

**GS P11.**
**Characterizing the autoregulation of Spo0A, the master regulator of biofilm and sporulation in *Bacillus subtilis***

**Brenda Zarazúa-Osorio[1]**, Anuradha Marathe[2], Priyanka Srivastava[3], Masaya Fujita[1]
[1]University of Houston, [2]Wadsworth Center, [3]Vanderbilt University Medical Center

In response to starvation, *Bacillus subtilis* cells differentiate into either biofilm formation or sporulation. These differentiation programs are triggered by the phosphorylated and active form of Spo0A transcription factor (Spo0A~P). Upon starvation, Spo0A is activated (phosphorylated) by a multicomponent phosphorelay, leading to a gradual increase in the cellular levels of Spo0A~P. This gradual increase successively activates a series of genes involved in biofilm formation and sporulation. The mechanisms of the gradual increase in Spo0A~P have been analyzed, revealing that the transcription of *spo0A* gene is controlled by two promoters (Pv and Ps) and autoregulated by binding of Spo0A~P to the

four cis elements (0A boxes). However, the regulatory mechanism of the transcription of *spo0A* gene is not well understood. Using Electrophoretic Mobility Shift Assay, we determined the relative affinities of Spo0A~P to the four 0A boxes. We then introduced systematic mutations into the 0A boxes and the promoters. Using *lacZ* gene reporter, we determined promoter activities of the systematically mutated 0A boxes and the promoters. Then, we examined the effects of the mutated 0A boxes and promoters on biofilm formation and sporulation. Our systematic approaches revealed that, with differential binding of Spo0A~P to each of the four 0A boxes, the Pv promoter plays an important role in properly achieving biofilm formation, while Ps promoter is essential for sporulation, but dispensable for biofilm formation. Our results reveal that sporulation and biofilm formation are induced by precise timing and level of the *spo0A* transcription through two promoters in an autoregulatory manner.

## PATHOGENIC MICROBIOLOGY - graduate students
## Samuel Kaplan Award Poster graduate student presentation

### GS P12.
### Expression of diverse streptococcal multiple peptide resistance factors and lipid hydrolase in *Streptococcus mitis*

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*Streptococcus agalactiae* (Group B *Streptococcus;* GBS) is a gram-positive pathogen that encodes multiple peptide resistance factor (MprF). GBS MprF synthesizes a novel lipid, lysyl-glucosyl-diacylglycerol (Lys-Glc-DAG), and the well-known lipid lysyl-phosphatidylglycerol (Lys-PG). Additionally, GBS encodes a predicted alpha-beta hydrolase upstream of *mprF*. In *Enterococcus faecium*, this hydrolase is responsible for the turnover of Lys-PG. This project has three aims: to determine whether other streptococcal MprF proteins synthesize Lys-Glc-DAG and/or Lys-PG; the impact of Lys-Glc-DAG and Lys-PG production on *Streptococcus mitis* survival in low pH; and whether the GBS hydrolase is responsible for turnover of both Lys-Glc-DAG and Lys-PG. *S. mitis* was chosen as a heterologous host for this study since it does not natively encode *mprF.* Candidate MprF proteins from other streptococci with high identity to GBS MprF were identified by BLASTp. We found that expression of *S. ferus mprF* and *S. downei mprF* in *S. mitis* conferred synthesis of Lys-Glc-DAG. Significantly, *S. ferus* MprF synthesized Lys-Glc-DAG at a similar level to GBS. Expression of *S. salivarius mprF* in *S. mitis* conferred synthesis of Lys-PG also at a similar level to GBS MprF. The production of Lys-Glc-DAG and/or Lys-PG in *S. mitis* through the utilization of plasmids expressing the different *mprFs* did not increase the survival of *S. mitis* in low pH. Finally, preliminary investigation of the GBS and *E. faecium* hydrolases through a cell lysate assay did not show turnover of Lys-Glc-DAG and/or Lys-PG, demonstrating that methods for investigating hydrolase activity require refinement.

### GS P13.
### *Candida albicans* biofilm development in urinary catheters

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The fungal opportunistic pathogen *Candida albicans* is the second most common species isolated from biofilms found on urinary catheters.  These biofilms may occlude the catheter, thus impacting patient quality of life.  Additionally, these biofilms serve as a reservoir for pathogens that may promote symptomatic infection.  Despite the frequency of colonizing and infecting urinary catheters, little is known

about the molecular basis of *C. albicans* biofilm formation in the unique urinary environment. *C. albicans* biofilms have been extensively studied in other infection models, identifying a complex transcriptional regulatory network. We are taking three approaches to extend the knowledge of *C. albicans* catheter-associated urinary tract infections (CAUTIs). First, we assessed biofilm growth of mutants lacking each of the major transcriptional biofilm regulators. We report the relative effect of these regulators is distinct in this environment. To further examine the mechanisms of biofilm development in urinary catheters, we will determine the transcriptional profile of *C. albicans* biofilms grown in artificial urine media. Second, we assessed biofilm formation in a panel of isolates collected from chronically catheterized individuals. We found these isolates varied in competency to form biofilms. We will compare the molecular mechanisms that dictate biofilm development in the historical lab strain to these clinical isolates. Lastly, we seek to determine the contribution of these molecular mechanisms in a preclinical CAUTI murine model. The completion of this study will elucidate the major determinants of *C. albicans* biofilm development in CAUTI, thus identifying potential targets for anti-biofilm drugs or catheter coatings.

## GS P14.
### The distinct transcriptome of virulence-associated phylogenetic group B2 *Escherichia coli*

Jacob Hogins[1], Zhenyu Xuan[1], Philippe Zimmern[2], Larry Reitzer[1]
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*Escherichia coli* and especially phylogenetic group B2 strains are associated with urinary tract infections (UTIs) and a variety of other diseases. The still unknown basis for virulence potential has been analyzed by numerous genomic and transcriptomic approaches which suggest complex host-pathogen interactions and multiple virulence strategies. We compared the core gene expression in 28 strains from nonpathogenic and UTI-associated *E. coli* (UTEC) and from groups A, B2, and D. The transcriptomes followed the phylogenetic grouping with few exceptions: group A and D transcriptomes were similar but differed substantially from group B2 transcriptomes. Some UTEC transcriptomes mapped outside the B2 and AD clusters and showed altered expression of previously described virulence genes from other pathogens.

Relative to B2, the differentially expressed genes suggest that UTEC strains have an upregulation of PhoP regulon genes and activation of the stringent response which downregulates the translational machinery in response to different stresses. During an infection, few bacteria are internalized into epithelial cells, and few of these bacteria replicate within these cells. We propose that the UTEC transcriptomic pattern describes the mechanism required to survive a viability-threatening environment that could include passage through and exit from intracellular vesicles such as lysosomes. The expressed genes suggest a basis for innate immunity evasion and antibiotic resistance. We note that the UTEC gene expression pattern has been observed for another intracellular pathogen, *Salmonella*. Our results suggest a distinct transcriptome for group B2 strains, transcriptome malleability, and the possibility of a changing transcriptome during an infection.

## GS P15.
### Development of a co-culture model for assessing competing mammalian host cell and bacterial attachment on dental biomaterials

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Dental prosthesis implantation occurs under complex conditions involving numerous oral bacterial species and host mammalian cells. Mammalian cell proliferation and oral bacterial adhesion have been previously

studied individually. However, the competition between mammalian cells and bacteria on dental biomaterials through an *in vitro* co-culture model has not been thoroughly studied. A major limitation of prior co-culture models is the lack of mammalian cell survival beyond short time periods (4-72 h) due to bacterial overgrowth. A previous study incorporated spectinomycin as a bacteriostatic agent to suppress bacterial growth in cell culture medium, allowing for mammalian cell survival up to 48 h. Nonetheless, no other studies have evaluated mammalian and bacterial co-culture growth for extended time points. Thus, the goal of the present study was to develop a co-culture model using a bacteriostatic agent that would allow for survival of both an early colonizing oral bacterial species (*Streptococcus mutans*) and host mammalian cells involved in acute inflammation (macrophages) and wound healing (fibroblasts) post implantation. Chloramphenicol (CAM) was found to exhibit bacteriostatic effect on *S. mutans* while allowing for mammalian cell growth. In this novel co-culture model, both mammalian cells and oral bacteria not only survived but also proliferated until at least 7 days while maintaining stable inoculum size and adhesion of *S. mutans*. In conclusion, no significant differences in *S. mutans* planktonic growth and adhesion or mammalian cell viability were observed on titanium vs. zirconia surfaces except for 7 days at which bacterial counts were marginally high on zirconia.

**GS P16.**
**A tractable nematode model for the emerging fungal pathogen, *Candida auris***
**Melissa Martinez**[1,2], Melissa Cruz[2], Pedro Miramón-Martinez[2], Danielle Garsin[2], Michael Lorenz[2]
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*Candida auris* is an emerging multidrug-resistant fungal pathogen associated with a mortality rate approaching 60%. Since its discovery in 2009, outbreaks of *C. auris* have been on the rise, particularly in long-term care facilities where it is readily passed from patient to patient. The genetic factors that contribute to the virulence, drug resistance, and stress-tolerant nature of *C. auris* are almost completely unknown. Adding to this complexity, there are five genetically distinct clades of *Candida auris* of different geographic origins, each of which is clinically relevant. Current murine models of *Candida auris* infections are not ideal. Mice must be immunosuppressed, and the long time-course of this model leaves animals susceptible to other infections that complicate analysis. Additional relevant animal models, especially ones amenable to high throughput analysis are needed. The nematode, *Caenorhabditis elegans,* is a tractable model organism that has been validated as a valuable tool for studying microbial pathogenesis. We have developed a *C. elegans* assay for *C. auris* virulence, showing that strains from at least three clades rapidly kill worms. Moreover, we show that this model can distinguish differences in virulence between strains. We are now developing a high-throughput version of this model using live-dead staining and automated image analysis to improve the utility of this assay. This will give us additional tools to investigate this pathogen of growing concern.

**GS P17.**
***Gardnerella vaginalis* growth is eliminated by a novel narrow-spectrum factor secreted by**
***Lactobacillus jensenii***
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Bacterial vaginosis (BV) is the most common vaginal infection in reproductive aged women. While the vaginal microbiota of health women is dominated by lactobacilli, it shifts to favor other bacteria, specifically *Gardnerella vaginalis* (Gv), in women with BV. Lactobacilli produce antimicrobial factors including hydrogen peroxide, lactic acid, and bacteriocins. We hypothesize that besides these factors, lactobacilli and Gv influence each other's growth within the vaginal environment through additional unidentified factors. To assess the interaction, we co-cultured Gv with *Lactobacillus jensenii* (Lj), in medium simulating vaginal fluid. Lj significantly reduced Gv growth at 20 h post inoculation (hpi) and eliminated Gv at 24 hpi. Cell-free supernatant (CFS) of Lj harvested at 16 hpi did not affect Gv growth while Lj CFS harvested at 24 hpi eliminated Gv. Growth of Lj was unaffected by either 16h- or 24h-CFS. Additionally, 24h-CFS did not affect growth of other vaginal lactobacilli, *Enterococcus faecalis*, *Staphylococcus epidermidis*, or *Escherichia coli*. Time course experiments using 24h-CFS showed elimination of Gv began at 2 hpi and was complete by 4 hpi. To rule out hydrogen peroxide and D-lactic acid as responsible agents, we tested concentrations double that produced by Lj; neither inhibited Gv growth. Fractionation of 24h-CFS by molecular weight revealed that the fraction containing ≤ 30-kDa proteins retained the inhibitory effect while the fraction containing less than ≤ 10-kDa proteins did not. These results suggest that: 1) a novel 10- to 30-kDa Lj secreted product eliminates Gv and 2) the effect of this factor is unique to Gv.

## GS P18.
## Role of borrelia unfed tick induced protein (BtiP) in the colonization of the Lyme disease agent within tick and mammalian hosts

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*Borrelia burgdorferi* (*Bb*), the agent of Lyme disease, is transmitted to humans and other vertebrate hosts following the bite of infected *Ixodes scapularis* tick. Regulation of gene expression influenced by signals prevalent in the tick vector and vertebrate hosts is key to the survival, transmission and colonization of *Bb* during it natural infectious cycle. Deletion of Borrelia host adaptation Regulator (BadR), a major regulator of gene expression in *Bb*, resulted in derepression of *rpoS* and the *rpoS*-dependent regulon critical for adaptation of *Bb* in mammals while several other determinants crucial for the tick-phase of infection were down-regulated. One down-regulated protein in the *badR* mutant was designated as Borrelia unfed tick induced Protein (BtiP) with no known function. Our central hypothesis is that BtiP is essential for the survival of *Bb* during the nutrient-depleted, unfed tick phase of its infectious cycle. Immunoblot analysis revealed that BtiP is induced when infectious *Bb* strain B31-A3 is propagated under conditions mimicking the unfed ticks (pH7.6/23˚C) compared to fed tick conditions (pH6.8/37˚C). Moreover, BtiP is downregulated in the *badR* mutant. Examination of upstream region of *btiP* revealed one BadR binding consensus motif ATTTTATA and several putative AAAATATT motifs that can presumably bind BadR suggesting the molecular basis of the regulation of *btiP* by BadR during the tick phase of infection. Through the use of luciferase assays, a functional connection between BadR and the promoter region of *btiP* will be investigated. Electrophoretic mobility shift assays and chromatin immunoprecipitation will determine if the connection between BadR and the promoter region of *btiP* occur *in vitro* and *in vivo*, respectively. The determination of a direct or indirect connection between BadR and *btiP* will aid in the characterization of BtiP during the mouse-tick-mouse cycle of infection. Disrupting regulatory and metabolic pathways/determinants of *Bb* offer novel strategies to develop vaccine- and antibiotic-independent strategies to reduce pathogen burden in the reservoir and transmission hosts leading to a reduction in the incidence of human Lyme disease.

## GS P19.
## Removal of phosphate from lysate protein by a recombinant phosphase from *Acinectobacter baumannii*

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*Acinetobacter baumannii* is an opportunistic pathogen associated with hospital-acquired and combat-related infections. Pathogens use a variety of strategies to interact with and manipulate host cell response to their advantage, allowing them to survive and propagate. One such response is phosphorylation/dephosphorylation of proteins, a complex process carried out by specific protein kinases and phosphatases, respectively. *Acinetobacter baumannii* (Clinical Isolate 79) histidine-tagged recombinant SurE 5'-nucleotidase was expressed in *E. coli* and purified to near homogeneity by nickel-affinity column chromatography, and shown to readily hydrolyze phosphate from a wide variety of nucleoside monophosphate and non-nucleoside compounds as well as artificial phosphorylated substrates. However, little information is available regarding removal of phosphate from phosphorylated protein substrates. Using this recombinant phosphatase from *Acinetobacter baumannii*, a phospho-tyrosine specific antibody, 2D-PAGE and Western Blotting, we investigated removal of phosphate from J774A.1 murine macrophage protein lysate to evaluate this enzyme's possible role in host defense evasion. Data presented here indicate phosphate removal from macrophage protein lysate, suggesting a broader substrate specificity for this enzyme. Although the identity of these phosphate-bearing protein substrates remains to be determined, such information could give insight into the possible role of this enzyme and dephosphorylation in the pathogenesis of this opportunistic pathogen.

## GS P20.
## A novel type of cytotoxic membrane vesicles produced by *Pseudomonas aeruginosa*

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*Pseudomonas aeruginosa* is a Gram-negative opportunistic human pathogen that frequently causes many nosocomial. This pathogen also frequently infects people with compromised lung functions. Over 30% patients diagnosed with cystic fibrosis (CF) have *P. aeruginosa* in their airways. Alarmingly, multidrug-resistant isolates are frequently observed and have been classified as a serious threat by the CDC and WHO. *P. aeruginosa* possesses a large armamentarium of acute and chronic virulence factors. Previously, our lab found that the bacteria-free spent media (filtrate) from *P. aeruginosa* showed toxicity towards murine macrophages. This cytotoxicity was independent of pyoverdine, a toxin commonly secreted by *P. aeruginosa* strains, but was associated with membrane vesicles (MVs).
Here, we developed a MV purification pipeline mainly based on macromolecule precipitation and density gradient ultracentrifugation and characterized MVs using biochemical and cell biological methods, like transmission electron microscopy. The micrographs showed a class of membrane vesicles, which were around 35 nm on average and had a single-layered membrane around them. MV toxicity was measured in mammalian cells, bacterial cells as well as non-cellular model, giant plasma membrane vesicles. In addition to the lab-adapted strain PA14, a panel of 69 clinical isolates from pediatric patients with CF was used, demonstrating a strong correlation between MV production and cytotoxicity of bacterial supernatant. Current results indicate that these vesicles belong to a novel type with unique characteristics and may be important determinants of the damage inflicted upon host cells during *P. aeruginosa* infection.

# AMR & MICROBIAL ECOLOGY  - graduate students
## Samuel Kaplan Award Poster graduate student presentation

### GS P21.
### Novel drug combinations to treat *Rhodococcus equi i*nfection

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*Rhodococcus equi* is a Gram-positive intracellular pathogen known to cause pneumonia in foals, and is considered opportunistic in humans. It is endemic to many horse breeding farms and infects neonatal foals with immature immune systems. The current standard of care (SoC) treatment, a macrolide and rifampin, is becoming ineffective due to the rise of multidrug resistant *R. equi,* sparking a need for new therapies. To address this need, we evaluated the efficacy of a novel compound, C58, in combination with gallium maltolate (GaM), which has previously shown antimicrobial activity against *R. equi in vivo.*

Using standard assays, we found a MIC of C58 of 1 µg/mL and MBC of 4-8 µg/mL demonstrating the antimicrobial efficacy of C58. An $LD_{50}$ of C58-treated J774.A1 murine macrophages of 40 µg/mL highlighted the low toxicity. Further, C58 and GaM were synergistic in combination as indicated by a fractional inhibitory concentration of 0.5 at a C58/GaM ratio of 0.25/32 µg/mL against *R. equi*. To improve solubility and promote uptake by macrophages, C58 was encapsulated within PLGA/PEG nanoparticles (C58-NPs). We observed more than a 2-log reduction of intracellular *R. equi* isolates in J774.A1 macrophages treated with C58-NPs/GaM.

These data indicate the potential for C58 and GaM to replace the current SoC treatment for *R. equi* pneumonia and quell the rise of multidrug resistant *R. equi*. Nanoparticle encapsulation will expand the therapeutic applications of this novel antimicrobial facilitating nebulized, local delivery to the lungs that may be investigated in future clinical studies in *R. equi* infected foals.

### GS P22.
### Antigen stabilized vaccines against recurrent urinary tract infection

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Recurrent urinary tract infection (rUTI) is most often caused by uropathogenic E. coli (UPEC). One reason for the high incidence of rUTI is that sterilizing immunity does not develop during UTI. Vaccination can enable the development of sterilizing immunity; however, the immune responses necessary to develop sterilizing immunity against UTI remain unclear. Recent work suggests that local antigen persistence is important for the development of tissue resident memory T cells (TRMs) within the bladder mucosa that may protect against rUTI1. TRM cells are produced in lymph tissue, enter circulation, and are then "homed" to mucosal sites acting as sentinels against infection. We hypothesize that encapsulating whole-cell UPEC within a biomimetic framework will create a slow-release inactivated antigen depot that will

stimulate bladder TRM cell recruitment and development. We used biomimetic encapsulation to encapsulate acute cystitis strain UTI89 in a zeolitic imidazole framework (ZIF-8) and create a slow-releasing inactivated whole-cell UPEC vaccine. Colony forming unit (CFU) assays showed that encapsulated UTI89 are inactivated within one hour. We observed that ZIF-8 encapsulated UTI89 was able to agglutinate yeast similarly to live UTI89 while UTI89 fixed in formalin was not. We also found that UTI89 encapsulated in ZIF-8 had a bladder residence time of 48 hours compared to 30 minutes for fixed UTI89. In conclusion, encapsulation of UTI89 in ZIF-8 is a safe and effective way to inactivate, preserve, and deliver a whole-cell bacterial vaccine that preserves the surface antigens of encapsulated bacteria and has increased residence time in the bladder.

## GS P23.
### Discovery of novel broad-spectrum antibiotics and inhibitors for β-lactamases using combinatorial chemistry approaches

**Jiayi Fan**, Jane Park, Wanzhi Huang, and Timothy Palzkill
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Antibiotic resistance due to the emergence, spread, and persistence of multidrug-resistant bacteria has become a rising threat to the public health. Currently, β-lactams are the most widely used class of antibiotics. Resistance to β-lactams is primarily caused by the bacterial production of β-lactamase enzymes, which hydrolyze and inactivate the drugs. Prevalent β-lactamases such as OXA-48 and NDM-1 are able to hydrolyze a broad set of substrates including carbapenems, the last resort β-lactam antibiotics. Discovery of β-lactamase inhibitors is one avenue to address the antibiotic resistance problem. Alternatively, finding a new drug target to screen for novel antibiotics is another strategy to combat drug resistance. The targets of β-lactams are penicillin-binding proteins (PBPs), which are involved in the bacterial cell wall formation. Gram-negative bacteria have an outer membrane outside the drug target that can decrease antibiotic penetration, making Gram-negatives less susceptible to many β-lactams. In the outer membrane of Gram-negative bacteria, a β-barrel assembly machine (BAM) catalyzes the integration of β-barrel proteins into the outer membrane. The BAM subunit A (BamA) is conserved in all Gram-negative species and is essential for cell viability. Since BamA is exposed at the surface of outer membrane, we can bypass inhibitors penetration problem. Therefore, BamA is an excellent target for the development of new antibiotics.
In this study, we are using combinatorial approaches including established DNA-encoded small molecule libraries and a focused combinatorial peptide library to discover, produce, and validate new inhibitors against β-lactamases and novel antibiotics that act on BamA.

## GS P24.
### Novel *Streptomyces* bacteriophage endolysins: isolation, purification, and functional domain testing.

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As the post-antibiotic era arises, alternative strategies for control of bacterial infections are needed. Endolysin, a protein encoded by a phage gene, can lyse the peptidoglycan (PG). It has been reported that endolysins from phages infecting Gram-positive bacteria can lyse bacterial cells when added externally. We, thus, are interested in exploring novel endolysins from Streptomyces phages as only a few of them have been experimentally characterized. Based on our bioinformatics results, we have selected three putative endolysins, including those from Streptomyces phage NootNoot gp34 (predicted SAR endolysin – transglycosylase), Streptomyces phage Nabi gp26 (amidase), and Streptomyces phage Araceli gp35 (CHAP). We hypothesize that [1] the putative genes of interest may encode for novel endolysins that have the ability to degrade *Streptomyces* and ESKAPE pathogen cell walls, and [2] each endolysin can degrade

PG at a different site. To verify that the three putative proteins are endolysins with different catalytic domain, we plan to isolate, purify, and test for their PG-degrading activity. As well, a comparison of the lytic activity of these proteins may provide some insight into their bactericidal effectiveness. So far, we have successfully cloned and expressed the Nabi gp26 gene product. Zymogram and colorimetric PG hydrolysis assay will be used to test for their ability to degrade PG. Lastly, mass spectrometry will be used to identify and analyze the degraded PG which will result in protein domain analysis.

## GS P25.
### Nanobodies: overcoming the outer membrane barrier with small, charged proteins

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At the interface of every interaction between a Gram-negative bacterium and its environment is the outer membrane. The asymmetrical architecture of the outer membrane layer includes an outer leaflet composed of lipopolysaccharide molecules that create a net negatively-charged, nearly impermeable barrier. Although protection provided by this barrier confers a benefit to bacteria, membrane characteristics that inhibit access to the cell can limit the efficacy of existing antimicrobials and introduce a critical challenge to antimicrobial design. The implications of this challenge have never been more imminent, as multi-drug resistance continues to rise and the discovery of new antibiotics continues to decline, thereby further depleting the global arsenal of effective antimicrobial treatments. Introducing new approaches for gaining access to the cell beyond its surface is key to mitigating the growing threat of pathogenic Gram-negative bacteria. Our recent work addresses this by leveraging the electrostatics of the outer membrane to move engineered proteins beyond the cell surface. To accomplish this, we have investigated how nanobodies, the isolated ~15kDa variable domains of heavy-chain-only antibodies, can be engineered to overcome the Gram-negative outer membrane. Here, we show that nanobodies can be modified to carry net positive charges, and that such modifications grant these molecules the acquired ability to perturb and bind the outer membrane. Based on our work, nanobodies may introduce a novel avenue for moving beyond the outer membrane and gaining access to Gram-negative cells.

## GS P26.
### Effects of a drought-tolerant *Bradyrhizobium* isolate on soybean growth in Arkansas

**Angelica Ponce**, Christian Peterson, Camile Cordon, Spencer Reiling, and Woo-Suk Chang
University of Texas-Arlington

The soil bacterium *Bradyrhizobium japonicum* is essential in agriculture because of its potential to reduce synthetic nitrogen fertilizers. The symbiotic relationship with leguminous plants like soybeans (*Glycine max*) results in root nodules responsible for nitrogen fixation. *B. japonicum* converts atmospheric nitrogen into ammonia, which the plant utilizes for growth. Drought is an unfavorable effect on plants inhibiting the symbiotic relationship. Our lab previously isolated a Texas-native drought-resistant strain, *B. japonicum* TXVA, and optimized the application for inoculation. This study was conducted at the Pine Tree station in Colt, Arkansas using various cultivars to measure the efficacy of the TXVA strain versus a non-inoculated control and a commercial inoculant. The analysis evaluated included root nodule comparisons, soil physio-chemical, plant biomass, and plant tissue analysis. We hypothesize that this first-time application of TXVA in this field will positively impact plant growth, nutrient availability, and yields.

**GS P27.**
**Microbial source tracking in ambient waters**

**Ariel Robles**[1], Gloria Rodriguez[2], Davida Smyth[3]
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Microbial source tracking (MST) is a technique that aims to determine the sources of microbial contamination usually in waterway by tracing biological and chemical indicators to the source of contamination. Rivers and the soil surrounding them face potential contamination with microbes from fecal matter causing a major threat to human health because it can harbor a variety of dangerous human and animal pathogens. The use of MST can allow for corrective action to be taken to help prevent further contamination and potential transmission of pathogenic microbes, reducing the incidence of illness associated with contaminated food and water. While MST has focused on the use of culture and molecular-based methods of microbial identification, recent advances in sequencing technology have opened new avenues of exploration in the field. Our project aims to develop molecular and sequence-based tools and techniques, that combine sequencing with assays of viability that can be used to identify the presence of viable bacteria and viruses in water and soil and to quantify and trace the identity of the microbes found to the precise source. While much of these techniques can be developed in the lab, access to real-world settings and diverse ecosystems allows us not only to test our method but also train our method to identify microbes of relevance to San Antonio. We aim to sample water from several water bodies across the region monthly, from rural to urban settings, to quantify the microbes found and to test our molecular methods with real-world samples to determine efficacy.

**GS P28.**
**Developing a faster, inexpensive, accessible, microbial detection method for wastewater surveillance**

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In the 19th century, wastewater surveillance was used to monitor and track diseases within our communities. During the COVID-19 pandemic, wastewater surveillance predicted the emergence of the Omicron variant of the SAR-CoV-2 virus before it was identified clinically. SARS-CoV-2 is spread via contaminated aerosols and droplets. People infected can have no symptoms, or mild to moderate symptoms, or become severely ill, requiring hospitalization. Wastewater surveillance can detect virus levels, or new variants early on at a community level, before these variants start to spread and can help alert public health authorities to investigate potential outbreaks within our communities and develop any countermeasures to prevent further spread. As humans shed other microbes beyond SARS-CoV-2 in their urine and feces daily, testing wastewater is easier, faster at scale and inexpensive to collect, detect, and analyze. This additional public health tool will allow us to see trends on a larger scale than testing individuals and can be applied to other emerging microbes, such as monkeypox. Our goal is to develop a wastewater surveillance program in the San Antonio region, using faster and relatively inexpensive detection methods, with the potential to detect every infected individual within our community and allow public health authorities to determine appropriate actions. Our program will build upon the existing infrastructure at the wastewater plants and expand our surveillance to include rivers, lakes, and the aquifer so that we have an expansive network of water bodies in which to survey for emerging pathogens closer to the source.

## GS P29.
**A ribozyme for non-destructive reporting of gene transfer within a soil consortium.**
**Malyn Selinidis[1]**, James Chappell[1], Joff Silberg[1]
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The metabolic capabilities of soil microbial consortia dynamically change due to Horizontal Gene Transfer (HGT), a process whereby cells acquire genetic information through conjugation, transformation, transduction, or vesicle intake. the major role microbes play in ecosystem-scale processes, such as biogeochemical cycling, our understanding of HGT is limited in soil communities since traditional methods, such as fluorescent reporters, are ineffective for dynamic studies in these opaque matrices. To provide a robust tool for monitoring HGT in soils, we designed a novel reporter based upon a methyl halide transferase (MHT), an enzyme that produces a volatile methyl halide, that allows gene expression to be monitored in soil without disruption. Specifically, we split MHT into two non-functional fragments and code these fragments into two bacteria: (1) a donor strain capable of sharing genes and (2) a recipient strain that accepts this gene through HGT and produces MHT in response. Our split protein system combines MHT fragments using a catalytic RNA called a splicing ribozyme so that reporter gas is only produced when the recipient cell takes up a gene via HGT and thus becomes a transconjugant. We show that our tool successfully regulates gas production with minimal background following conjugation in Escherichia coli. Preliminary results show that our tool will allow for robust monitoring of intraspecies conjugation rates. In the future, we will use this tool to investigate how physicochemical properties of soil matrices influence HGT rates.

## GS P30.
**Agent-based modeling to establish a protocol for sampling DNA from the air**
**Lyndsy Stacy[1]**, Ashley Teufel[2], Davida Smyth[3]
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It has become painfully apparent in the last two years the efficiency in which viruses can spread through aerosols. While this is not new information, we did not yet have the tools to create effective safety measures. Our team is working on a method of measuring and modeling how aerosols containing viral particles suspend and move throughout the air. We have previously used a nebulizer to simulate the spread of aerosols filled with a detectable bacteriophage and were able to observe how viable particles spread and factors such as humidity that can affect this. We will expand on previous work by using a new air sampler to quantify DNA. We will also be introducing more variables such as airflow and movement of the source to see how these changes may affect how much the aerosols spread. Using an agent-based model we will further explain the phenomenon of aerosol travel and potentially use this as a future guideline for social distancing or other safety protocols. An issue with current models is that little experimental data is collected and much of the outcomes are hypothetical. With experimental data used to create the baseline for our model, we anticipate that it will be more accurate and applicable to those trying to create safety protocols while keeping aerosols in mind.

## GS P31.
**Activated charcoal as a sink for diffusing AHL molecules in the microdroplets system**
**Adeline Supandy[1]**, Seokju Seo[1], Yousif Shamoo[1]
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Multidrug-resistant pathogens has become one of the fastest growing problems of this century. Unfortunately, with the lack of robust antibiotic discovery pipeline, the number of effective antibiotics has dwindled. Microfluidic generated microdroplets has emerged as a method to discover new antimicrobials. These microdroplets impose spatial structure and create separate spatial

microenvironments. This is especially beneficial in isolating slower-growing, often antimicrobial-producing, bacterial populations. While cells are well separated in these microdroplets, small molecules, such as the quorum sensing signal AHL, were able to diffuse freely. The diffusion of these small molecules poses a problem as it promotes the growth of 'cheater' cells, which receive benefits without paying the molecule production cost. One way to control this diffusion problem is by surrounding the droplets with an adsorbent material that act as sink to bind the diffusing small molecules. Activated charcoal (AC) is an example of adsorbent material and has been used previously to segregate toxic substances and drugs in both humans and water. In minimal media, suppression of AHL was only observed after incubation with high AC concentration, at least 50 mg/mL AC. This is likely due to the difference in affinity for AHL between AC and the synthetic *E. coli* reporter strain. We postulate that the *E. coli* reporter strain has a much higher affinity for AHL, likely higher than the affinity of most antibiotics for their target. As such, a less sensitive reporter strain may help in reducing the amount of AC needed and mimic a more common situation.

## GS P32.
### Characterizing putative DD-carboxypeptidases that promote outer membrane integrity in *Acinetobacter baumannii*

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The Gram-negative cell envelope is an essential structure that provides structural integrity to protect against turgor and a permeability barrier to restrict entry of toxins and antibiotics. The outer membrane (OM) and peptidoglycan (PG) layers both contribute to cell envelope rigidity to maintain homeostasis. Specific enzyme complexes coordinate their assembly during growth. PG assembly is dependent on modification of stem peptides to crosslink adjacent strands. Crosslinking enzymes, including penicillin-binding proteins, are validated targets for antimicrobial treatment. *Acinetobacter baumannii* is a Gram-negative nosocomial pathogen that is associated with hospital-acquired infections and is oftentimes resistant to antimicrobials/antibiotics. Antibiotic susceptible strains can quickly develop tolerance or even resistance which leads to treatment failure. Physical tethering of the PG and OM layers is required for survival during antibiotic treatment in *Escherichia coli*; however, links between the two layers are not well-understood. A recent study showed that modification of PG stem pentapeptides into tetrapeptides was required to link the PG layer to OM proteins. Our preliminary data indicates that putative DD-carboxypeptidases act on *de novo* stem pentapeptides and cleave the terminal D-alanine to form tetrapeptide substrates that form links with OM proteins. Here, we show that PBP5, PBP6, and PBP7 are DD-carboxypeptidases, and their inactivation induces morphological defects in *A. baumannii*. We would like to further understand and characterize these DD-carboxypeptidases to determine their physiological role in the cell. We hypothesize that DD-carboxypeptidase-dependent tetrapeptide formation is required for links between PG and OM proteins to increase mechanical cell envelope rigidity to protect against the turgor.

## GS P33.
### Bacteriophage resistance associated with reduced bacterial fitness in the urinary environment

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The estimated 7 million urinary tract infections (UTI) occurring annually in the United States represent the most common cause of outpatient antibiotic prescriptions. These infections are primarily caused by uropathogenic *E. coli* (UPEC). Due to growing antibiotic resistance and negative impact of antibiotics on the healthy microbiota, alternative UTI treatments are needed. Bacteriophages (phages), viruses that

infect bacteria, are appealing alternatives due to their specificity for bacterial hosts and ubiquity. Yet, as with antibiotics, bacteria can become resistant to phage.

Our group hypothesized UPEC would become resistant to phage ES17 through genomic changes, but resistance would negatively impact bacterial fitness in the urinary environment. We isolated phage-resistant UPEC by repeatedly challenging strains UTI89 and DS566 with phage ES17. Through sequencing, we identified resistance to ES17 in both strains associated with mutations in lipopolysaccharide (LPS) biosynthesis. These phage-resistant bacteria display attenuated growth in urine and may be sensitized to membrane-interacting antibiotics. Importantly, while these bacteria had variable attenuations to adherence, invasion, and biofilm formation *in vitro*, this phenotype does not result in successful bladder colonization *in vivo*. While resistance to phages such as ES17 may arise during treatment, the incurred fitness costs may render UPEC more susceptible to environmental conditions or antibiotics. This susceptibility could be exploited to develop novel antibiotic-phage combinations for treating UTI. Future work seeks to identify mechanistic causes underlying this decreased fitness, assess the utility of bacteriophages targeting multiple distinct molecular targets for treating UTI, and identify the immune response to phage and phage-resistant bacteria.

## MICROBIOME & COMPUTATIONAL BIOLOGY - graduate students
## Samuel Kaplan Award Poster graduate student presentation

### GS P34.
**Stability of honey bee gut symbiont *S. alvi* traits during laboratory propagation**

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The western honey bee (*Apis Mellifera*) is an indispensable pollinator in natural ecosystems and agriculture. Bees have a co-evolved gut microbiome dominated by approximately 5 microbes. *Snodgrassela alvi*, a core member of the bee gut microbiome, robustly colonizes the ileum. Previously, we showed that it can be engineered to express double-stranded RNA to induce a host RNAi response that can protect bees from pathogens or be used to study bee gene function. We investigated whether passaging *S. alvi* in lab would eventually compromise its ability to recolonize the bee gut. *Objective:* Determine the stability of *S. alvi* traits during laboratory propagation. *Experimental design:* 12 populations of *S. alvi* were propagated on Columbia blood agar for 10 passages. Genome sequencing was performed on evolved end-point strains. Colonization of microbiota-deficient bees by evolved clones was measured by performing CFU counts on gut homogenates. *Results*: Three out of 12 *S. alvi* populations evolved phenotypic changes during passaging. They appeared lighter, less sticky, and would readily resuspend in liquid. Both the ancestral and three lab evolved strains that we tested robustly colonized the bee ileum with an average of $10^7$ CFU/bee after 10 days. Genome sequencing data is currently being analyzed to identify new mutations that affected adhesion or other traits. Conclusion: Short-term lab adaptation did not appear to impair *S. alvi* colonization of the bee ileum. In the future, we will test if there are changes in the competitive ability of evolved strains to colonize bees by coinoculating with wild-type *S. alvi*.

### GS P35.
**DL-ARG: leveraging deep learning to predict and classify antimicrobial resistance from long and short-sequence reads**

**Tallon Coxe**[1], Xiaoqiong Liu[2], Pranathi Vasireddy[2], Aashish Pandi[2] and Rajeev Azad[3]
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Antimicrobial resistance (AMR) is a global health crisis and remains a challenging problem for clinicians and researchers alike. As traditional methods for AMR detection are often time-consuming and costly, we leverage state-of-the-art computational methodology with the hope to combat the rising mortality rates caused by drug-resistant pathogens. The development of next-generation sequencing technologies, along with the rapidly evolving field of deep learning (DL) has innovated the way researchers may investigate genomic data to detect the presence of AMR. We employ DL-ARG, a deep neural network to predict and classify antibiotic resistance genes (ARGs) while also detecting novel ARG sequences from user-inputted read sequences. Trained using known resistance genes from the Comprehensive Antibiotic Resistance Database (CARD) and MEGARes, we test our model using microbial surface data from the International Space Station available in the NASA GeneLab repository. We hypothesize that DL-ARG will outperform existing AMR detection models by utilizing a deep latent space to predict both known and potentially novel ARGs.

## GS P36.
### The effect of inoculation of beneficial bacteria on microbial diversity in soils infected with a pathogenic fungus

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The rhizosphere presents a dynamic microbial population due to direct influences from plant root exudates, soil type, structure, texture, plant species and growth stages, leading to changes in plant-microbe interactions. Therefore, it is crucial to monitor the corresponding fluctuation in the microbial composition of the rhizosphere following biotic or abiotic perturbation. This study focuses on the effect of *Bacillus* as a biocontrol agent against *Colletotrichum* and *Phytophthora* infection in the pepper rhizosphere. Soil samples were collected from the infected field. The biocontrol treatment and maintenance of the treated soil were done in the greenhouse. The microbial community analysis was performed using the V3-V4 hypervariable region of 16S rRNA. In addition, the soil physicochemical analysis was carried out to collect information about the corresponding effect on soil macro- and micronutrients, organic matter, pH, and electric conductivity. Our results showed that *Proteobacteria* dominate the healthy and infected soil. However, *Proteobacteria* content is much higher in healthy soil (52-58%) compared to infected soil (34-44%), regardless of treatment. Nevertheless, *Verrucomicrobia* and *Acidobacteria* are present in higher frequencies in infected soil. Notwithstanding, *Pseudomonas*, a common pathogen for several diseases in pepper, was observed to be decreased in the biocontrol-treated soil for both healthy and infected soil samples. In conclusion, this study presents a piece of collective knowledge on the impact of bacterial inoculation on soil microbial populations infected with the pathogenic fungus.

## GS P37.
### CAFÉ_GI: A tool for identification of genomic islands in bacterial genomes

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One of the evolutionary forces driving the evolution of bacterial genomes is the acquisition of clusters of genes called genomic island (GI) through horizontal gene transfer (HGT). These GIs have been known to confer versatile traits to the recipient bacteria, such as, resistance against antibiotics, virulence or hypervirulence, and ability to metabolize nutrients. Thus, it is of great significance to identify the coordinates and composition of GIs because such findings will be of immense benefit to biomedical research. Due to an immense growth of genome sequence data in recent years, there is an unmet need

to elucidate genomic structure information. Unfortunately, biological experiments may contribute only a small fraction of the information on GIs in all sequenced genomes. Therefore, computational methods and tools for the identification and characterization of bacterial GIs are greatly needed. Here, we focused on developing a tool, CAFÉ_GI, for identification of GIs using composition bias, aberrant phyletic pattern, and marker gene enrichment as features to detect GIs in bacterial genomes. We generated chimeras by inserting core (backbone) genomic segments from donors into the core genome of a recipient to simulate horizontal transfer event. We used our tool to identify GIs in real and synthetic datasets. The real test dataset comprised of GIs from 104 representative bacterial genomes. Comparative assessment of out GI detection tool with other commonly used tools showed that CAFÉ_GI outperformed other commonly used GI detection tools. The performance of CAFÉ_GI will be further assessed on additional genuine genomes.

**GS P38.**
**Assembly of quality genomes from metagenomic reads generated from the rhizoplane of wheat**
**Ken P. Dickson** and Michael G. LaMontagne
University of Houston – Clear Lake

Microbes associated with plant roots can increase the yield and sustainability of agricultural systems; however, despite the recent explosion of microbiome research, the vast majority of rhizobacteria remain known only through fragments of their genome. Draft genomes from these microbes would describe many rhizobacteria for the first time but the high diversity of soil systems makes the assembly of genomes from metagenomic read sets generated from plant microbiomes challenging. To address this, we applied a gnotobiotic plant system to simplify the microbial community prior to metagenomic analysis. This approach involved inoculating surface-sterilized wheat seeds with dilute slurries generated from the rhizoplane of wheat seedlings. These seeds were sown in sterile soil and grown, under gnotobiotic conditions, to the seedling state. At harvest rhizoplane samples were collected for metagenomic analysis by shotgun sequencing with an Illumina MiSeq run and Oxford Nanopore Technologies (ONT) sequencer. The Illumina run produced 14,685,437 reads. Hybrid assembly of Illumina and ONT reads produced 680 contiguous units (contigs) that were at least 10 kbp. These contigs were binned into metagenome-assembled genomes (MAGs) using metaWRAP. This MAG assembly pipeline produced six medium-quality MAG bins and one high-quality MAG. This suggests an efficient approach to generating MAGs from the rhizoplane microbiome.

**GS P39.**
**Multi-species housing impacts: overlapping microbiomes – preliminary data**
**Kaelyn Dobson**[1], Camila Carlos-Shanley[1,] Christina Burges[2], and Patti Clark[3]
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Heterospecific species housing is implemented in zoological settings to create a multi-species habitat for viewer education, animal engagement, and optimal space use. These heterospecific housing protocols are suggested to influence the gut microbiome of individuals similarly to those seen in the wild. A preliminary comparison of squirrel monkey (*Saimiri*) microbiomes housed with a heterospecific tamandua (*Tamandua tetradactyla*) and those only housed with conspecifics is presented. 43 primate and 15 tamandua fecal samples were collected from Texan zoological facilities (Austin Zoo and San Antonio Zoo), stored in 95% ethanol, processed through DNA extraction kits for 16SrRNA sequencing and analyzed using the dada2 pipeline with a microbiome analyst analysis. The presence of a sympatric *Tamandua* is statistically significant with housing influencing the Campylobacterota ($p=0.004$) and Desulfobacterota ($p=0.007$) phyla in the microbiome of the *Saimiri* individuals. These two microbe phyla are associated with gastrointestinal illness (Campylobacterota) and diabetic gut dysbiosis (Desulfobacterota). These alterations to the gut microbiome may be detrimental and it is key for further research to identify the

impacts of such changes to the composition and biodiversity. Facilities are recommended to do extensive research on natural sympatric species with considerations of the extent of heterospecific housing, as these changes may be natural.

**GS P40.**

**The second mouse gets the cheese: how the field of reproductive tract microbiology benefits off the generation of a humanized gut-microbiota mouse model**

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Vaginal dysbiosis, characterized by a vaginal microflora deficient in *Lactobacillus spp,* affects about 25% of women at any point in time and is associated with increased susceptibility to urogenital infections. To date, non-translational, conventional murine models have been used to test causality of the vaginal microbiota in vaginal pathogen colonization. To enhance the clinical relevance of mouse models, we characterized the vaginal microbiota of gnotobiotic mice colonized with human fecal microbial communities (Human microbiota, or [HMb]mice). Using 16S rRNA amplicon sequencing, we found that the [HMb]mice vaginal microbiome clustered into five discrete humanized murine community state types ([h]mCST) dominated by multiple *Lactobacilli* (CST I), *Staphylococcus succinus* (CST II), *Enterobacteriaceae* (CST III), an even consortium of microbes (CST IV), or a collection of other dominant microbes (CST V). We then challenged the model with human pathogen Group B *Streptococcus* (GBS) which vaginally colonizes approximately 1 in 4 women and is associated with vaginal dysbiosis and maternal and fetal sepsis during pregnancy. We found that [HMb]mice had decreased GBS vaginal burden and uterine ascension when compared to conventional mice seven days post infection ($p$ = 0.0035 and $p$ = 0.0230, respectively). ANCOM analysis indicated *Pseudomonadaceae* (specifically *Pseudomonas*), *Comamonadaceae, Lactobacillus, Enterobacteriaceae,* and *Acinetobacter* as differentially abundant in the vaginal swabs of mice with undetectable uterine GBS. We conclude that endogenous microbiota play a role in conferring protection against GBS colonization. Furthermore, the [HMb]mouse model is an improved model that will help elucidate microbe-microbe interactions in future colonization and infection studies.

**GS P41.**

**Effects of a drought-tolerant *Bradyrhizobium* isolate on soybean yield and the soybean rhizosphere microbiome**

**Christian Peterson**[1], Sarbjeet Niruala[2], and Woo-Suk Chang[1]
University of Texas at Arlington[1], Northwestern University Feinberg School of Medicine[2]

The soil bacterium *Bradyrhizobium japonicum* is an agriculturally important microbe because of its symbiotic nitrogen fixation ability associated with the soybean plant. Due to the increased global demand for this crop, breeders and farmers have developed various ways to maximize the yield such as applying *B. japonicum* as a biofertilizer, which forms nodules on the roots of the soybean plant and provides nitrogen. Optimizing this process will benefit soil health by not only reducing the use of chemical nitrogen fertilizers, but also attracting beneficial microorganisms to the rhizosphere. However, drought is the major constraint for survival of inoculants and the maintenance of their symbiotic efficacy in soybean fields. Thus, we have developed a molecular marker system to select for drought tolerance, which resulted in identifying a rhizobial strain (i.e., *Bradyrhizobium* sp. TXVA) with the highest drought tolerance. The objectives of this research are i) to evaluate the isolate by comparing soybean yield and root nodulation among TXVA and the non-inoculated control under non-irrigated conditions at drought prone sites, and

ii) to analyze the metagenomic profiles of rhizosphere soils treated with this inoculant in South Texas. By analyzing nodulation and the final soybean yield along with the microbiome data, we conclude that TXVA shows more positive effects on soybean growth and production compared to the non-inoculated control.

**GS P42.**

**Benchmarking metagenomic classifiers on simulated ancient and modern metagenomic data**

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Ancient metagenomics refers to the analysis of complex genomic content which is retrieved from degraded biological material of ancient or extinct organisms. Research in this field is commonly focused on adding a new perspective to our understanding of the past and providing exciting sources of information for modern metagenomics studies. However, metagenomic profiling of the ancient microbial DNA samples is challenging due to the accumulation of specific damage patterns on DNA over time. To date, the comparative assessment of metagenomic profilers at different depths of degradation of ancient metagenomes is not yet accounted for. We performed a comprehensive evaluation of popularly used metagenomic profilers at all the various levels of simulated DNA damage (from modern to high-level metagenomic damage) targeting different damage patterns (deamination, fragmentation, and modern DNA contamination). All three types of alignment-based profilers- DNA-to-DNA, DNA-to-protein, and DNA-marker based profilers were included in this study. Following taxonomic assignment, the profiles were evaluated based on the F1 score (harmonic mean of precision and recall) for species presence/absence, relative abundance, and alpha diversity. As compared to deamination and fragment length, contamination of ancient DNA (with modern DNA sequences) was found to have the most prominent effect on the performance of each profiler. Further, DNA-to-DNA and DNA-to-markers based profiling approaches were seen to have complementary strengths which could be combined to expand the classification of ancient metagenomic samples.

**GS P43.**

**Modelling the transmission of COVID-19 during the first wave in India using a data driven SEIRD model**

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The global pandemic, COVID-19, caused by SARS-CoV-2 has affected every nation and has caused approximately 6.6 million deaths till date. COVID-19 has been extensively investigated to understand different aspects of this pandemic, including the spatiotemporal dynamics of the disease, in concerted efforts to develop strategies and tools to mitigate its effects. Epidemiological modelling helps in understanding the dynamics of an epidemic that is an interplay of several factors, including those related to pathogen, environmental and social conditions. Predictions from these models inform governments to develop strategies, policies and response plans for effective control of the disease. In this study, we applied an extended version of SIR(Susceptible-Infected-Recovered) model that incorporate exposed and death compartments in addition to susceptible, infected, and recovered compartments to study the first

COVID-19 wave in India. This study focuses on role of non-pharmaceutical interventions (NPIs) on transmission dynamics of the disease in India. For this purpose, the time during the first wave is divided into multiple sub-periods based on stringency index, which is a composite measure of nine different intervention measures. The parameters, effective contact rate ($\beta$) and basic reproduction number $R_0$, for the sub-periods are calculated to assess effectiveness of the non-pharmaceutical interventions. Our analysis shows that the NPIs were successful in reducing $\beta$ and $R_0$ and were thus effective in controlling the dynamics of disease during the time period when COVID-19 vaccines were not available.

## GS P44.
### Defining the evolutionary framework of colistin resistance In *Acinetobacter baumannii*

**Muneer Yaqub**[1], Chinmai Rudra[1], Zoe Du[1], Reagan Crow[1], and Nicholas Dillon[1]
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*Acinetobacter baumannii* is considered a high priority pathogen with serious public health threat, due to its notoriety in conferring resistance to multiple antibiotics. The antibiotic colistin is currently the last line of defense for the treatment of multidrug-resistant *A. baumannii* infections. Unfortunately, colistin resistance has begun to emerge in the clinic, threatening our capability to successfully treat *A. baumannii* infections in patients. The genetic basis for colistin resistance in *A. baumannii* is unknown. In this study, we used a machine learning model to predict mutations associated with colistin resistance from clinical strains of *A. baumannii.* We selected 31 predicted mutations that were correlated with colistin resistant strains for analysis via standard antibiotic susceptibility testing. Although all 31 mutations were associated with colistin resistant strains, only 1/31 mutations directly conferred colistin resistance. Surprisingly, we found 11/31 of the mutations instead conferred a growth advantage when grown in sub-inhibitory concentrations of colistin. While some of these mutations directly influenced *A. baumannii's* colistin resistance, others instead provided a fitness advantage that is not captured by standard antibiotic susceptibility testing. We are now examining if the predicted mutations influence virulence and exploring the epistatic interactions between these various mutations. This study seeks to provide a holistic framework for the evolution of colistin resistance in *A. baumannii,* with a view to improving clinical therapeutic strategies for combating MDR *A. baumannii* infections.

## GENERAL MICROBIOLOGY - undergraduate students
### Joan Abramowitz Award - Poster undergraduate student presentation

## UP1.
### Use of *S. Aureus* to study airflow and filtration in a collegiate environment

Gregory Kasriel[1], **Stephanie Davidson**[1], Lyndsy Stacy B.S.[1], Dr. Davida Smyth Ph.D.[1]
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As SARS-CoV-2 continues to mutate and spread, it is imperative that the scientific community continue research on reducing exposure and transmission in the public. The Centers for Disease Control and Prevention (CDC) have clear guidelines for vaccines, masks, social distancing, and personal hygiene. Unfortunately, the guidelines regarding air filtration are not as expounded upon. *Staphylococcus Aureus* behaves similarly to SARS-CoV-2 in the air and can be utilized as a safe means of implementing testing in real world applications, as roughly 30% of the population at any given time has *S. aureus* in their nasal passage. Undergraduates on the A&M San Antonio campus are using air sampling instrumentation to ultimately correlate time in location, occupancy of location, and activity in location to determine whether current air filtration systems are sufficiently removing *S. aureus* in the air, or whether updated systems and protocols are required. A protocol was developed, and baseline unoccupied data was collected, to

begin the study. Environmental factors including humidity, temperature, and CO2 levels were collected as well as they provide valuable information pertaining to occupancy and *S. aureus* behavior in the air. This research is expected to highlight and stress the importance of looking at HEPA filtration systems and protocols, and possibly expanding on current CDC guidelines to include time, occupancy, and activity in tandem, and its application to SARS-CoV-2.

## UP 2.
### Models for cellular aging in yeast
**Taylor Holly**, Zitlaly Gutierrez, and Todd P. Primm
Department of Biological Sciences, Sam Houston State University

*Saccharomyces cerevisiae*, more commonly known as budding yeast, were used to look at the effects of aging on the cellular level. Yeast was used in our study due to its short lifespan and ease of manipulation. To study aging, the cells have to be in a non-replicating state. We reproduced a previous starvation model, with yeast suspended in a simple buffer. We compared this control to our own novel model, where replication in typical YPD media is blocked by 3-hydroxy-4-methoxyacetophenone (3H4MoAP). This compound was discovered during a CURE project at SHSU. Aging over time is tracked by optical absorbance, viability staining with methylene blue, and plating for CFU. Initial results suggest that at 30 C, yeast in both conditions age very little (small loss of viability and CFU) over a week. At 35 C, yeast appear to lose viability at a faster rate in the 3H4MoAP condition that the starvation condition. This suggests that the dormancy condition without nutrients is more complete than when replication is blocked by the acetophenone. Future studies will examine other temperatures and conditions and to include comparison of yeast strains which are mutants in suspected aging-related genes.

## UP 3.

### Examining the effect of antibiotics on CRISPR-Cas defense efficacy against conjugative plasmids
**Pranav Kumar**[1], Chahat Upreti[1], Kelli L. Palmer[1]
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The discovery of antibiotics led to the Antibiotic Era, responsible for global increase in life expectancy and quality of life. However, the rise of antibiotic resistance, perpetuated by horizontal gene transfer within bacteria, threatens to put us in a post-antibiotic era. CRISPR-Cas is a native bacterial defense mechanism which could be a possible tool to address this trend. *Enterococcus faecalis* is an opportunistic pathogen that colonizes humans and other animals, and several agricultural settings. *E. faecalis* natively encodes Type II CRISPR-Cas systems. It has been previously shown that CRISPR-Cas can act as a barrier to transfer of resistance-encoding plasmids in *E. faecalis*, both *in vitro* and in the mouse intestine. However, these experiments have been performed in antibiotic-free conditions. Since antibiotics are a ubiquitous presence in most bacterial niches, it is crucial to test CRISPR-Cas activity against conjugative plasmids in their presence. A previous study has shown that bacteriostatic antibiotics enhance the ability of CRISPR-Cas against bacteriophages by delaying viral development. We hypothesize that antibiotics (bactericidal and bacteriostatic) affect the efficacy of CRISPR-Cas to block resistance encoding conjugative plasmids. For this, we have measured the minimum inhibitory concentrations of several antibiotics on strains that either possess or lack CRISPR-Cas. Next, we are measuring the minimum bactericidal concentrations for these antibiotics to categorize them as bactericidal or bacteriostatic, followed by testing their effect on CRISPR-Cas defense efficacy. We envision that our work will be an important addition toward the effort of utilizing CRISPRCas as an antimicrobial to tackle antibiotic resistance.

**UP 4.**

**Nodule formation inhibited by *Paenibacillus sp*. isolated from Texas native *Aeschynomene indica* plants**

**Jenny Le**, Meghan Rose, Woo-Suk Chang
University of Texas at Arlington

Nodule Formation Inhibited by *Paenibacillus* sp. Isolated from Texas native *Aeschynomene indica* Plants Jenny Le, Meaghan Rose, and Woo-Suk Chang University of Texas at Arlington Leguminous plants have beneficial symbiotic relationships with nitrogen-fixing bacteria that arose as a result of an immune response by the host plant to the invading bacteria. This causes the formation of nodules and produces usable forms of nitrogen (i.e., ammonia) for the host plant. Most legume plants form only root nodules; however, *Aeschynomene indica* can form both root and stem nodules. This is done independently of the typical signal molecules called Nodfactor. The aim of this work was to identify bacteria from Texas A. indica stem nodules that are NF-independent. In observing stem isolates for nodulation capabilities, one isolate was found to inhibit nodule formation upon application to the host plant. This isolate, named "TSN7", was identified as a *Paenibacillus* species. Growth chamber experiments were conducted to assay TSN7 nodulation abilities. A. indica seeds were inoculated with TSN7, (+) controls BTAil or RDA-1, and (-) controls E. coli DH5a and non-inoculated. The plants were harvested after 40 days of growth and nodules as well as plant mass were observed. TSN7 did not form true nodules, and only formed a few small pseudonodules. However, non-inoculated plants formed functioning nodules, suggesting that TSN7 was able to inhibit nodule formation in the host plant despite being identified as a plant growth promoting endophyte previously. These results suggest that plant growth promoting bacteria such as *Paenibacillus* species may host immune response and avoid nodule formation.

**UP 5.**

**Characterization of antibiotic production and microbial diversity in the soils of San Antonio**

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This study aims to characterize the differences in soils sampled from highly developed urbanized areas with predominately non-native vegetation and undeveloped land with native vegetation. San Antonio, located in south Texas, is a rapidly expanding city with over 13,000 people moving to the city from 2020 to 2021. The increase in population has led to mass development on the city's north and south sides. New construction has destroyed native plants and topsoil. Microbes from soil are noted for their capacity to produce antibiotics. However, the widespread overuse of antibiotics in humans and animal agriculture has led to the prevalence of antibiotic resistance/multi-drug resistant bacterial strains. Staphylococcus aureus and Staphylococcus hemolyticus have multi-drug-resistant strains found in hospital and community gathering points.

Soil samples collected in and around San Antonio, Texas, were tested for Antibiotic production against Staphylococcus aureus and Staphylococcus hemolyticus. Soil samples were diluted with PBS and then grown on LB agar. Single colonies were chosen based on morphological differences and screened for antibiotic production. Colonies that produced zones of inhibition were then selected for further analysis. We have identified >40 colonies with antimicrobial activity so far. This study begins to establish a sampling gradient across the San Antonio area for microbiological soil diversity and antibiotic production capabilities of the soil microbes.

**UP 6.**
**Mercury reduction gene merA detection in martin lake**
**Kyren Miller**, Riqing Yu
Department of Biology, The University of Texas at Tyler

Martin Lake, which holds Luminant's Martin Lake Steam Station in Tatum, Texas as the largest mercury (Hg) emitter nationally, is a recreational lake ecosystem and also provides cooling water to the power plant. Atmospheric Hg which settles into lake sediment undergoes complex microbial transformations. Hg(II) and methylmercury can accumulate through aquatic food chains and biomagnified in fish, which causes health risk to humans if consumed. In order to understand the Hg contamination and cycling in this lake ecosystem, we took sediment and pore water samples in Martin Lake over five seasons from 2019 to 2020, did basic chemical analyzes, and extracted microbial genomic DNA to target the microbial Hg reduction genes (merA). Microbial merA genes encode enzyme MerA which catalyzes the reduction of Hg(II) to volatile Hg(0). By reducing toxic Hg(II), microbes could remove Hg(II) from habitats and detoxify this metal. Thus detection of microbial merA genes could analyze Hg(II) reduction potential and evaluate Hg cycling. We have used seven sets of primers to detect different groups of microbes which could reduce Hg(II). The Hg analyses in the sediment samples indicated the total Hg levels were not very high, so we hypothesize that Hg(II) reduction in the lake might be active and play a large role in removing Hg. The initial results show the merA genes occurred within at least one sampled area. Further studies are underway to explore Hg(II) reduction and its relationship with Hg recycling and contamination.

**UP 7.**
**Antimicrobial effects of human metabolite lysophosphatidylcholine**
**Julie Nguyen**[1], Savannah Pas[1], Ziqiang Guan[2], Kelli L. Palmer[1], Yahan Wei[1]
[1]Department of Biological Sciences, The University of Texas at Dallas
[2]Department of Chemistry, Duke University Medical Center

Lysophosphatidylcholine (LPC) is a human metabolite with immunogenic functions. It has been implicated in a variety of human diseases, such as cardiovascular and neurological diseases, and bacterial infections such as pneumonia. Research into this molecule by others has indicated potential antibacterial properties, as it sensitizes bacteria to antibiotics and confers therapeutic effects against sepsis in mouse models. However, the direct effects of LPC on bacterial growth have never been characterized. In this study, we report antimicrobial effects of LPC at physiologically relevant concentrations (30 µM to 1 mM) towards several Gram-positive bacterial pathogens, including Staphylococcus aureus. Spontaneous LPC-resistant mutants arose in S. aureus after 4 days exposure to LPC. Whole genome sequencing of these mutants identified mutations in genes involved in lipid biosynthesis and odd-numbered fatty acid export. Lipidomic analyses confirmed the lack of odd-numbered fatty acids in the resistant mutants. Additionally, when grown in chemically defined medium, these resistant mutants demonstrate a growth advantage against the wild-type (WT) strain with shorter generation times. One of the mutants, SA-B6, has decreased susceptibility to daptomycin compared to the WT. Future work is required to reveal the detailed molecular mechanisms underlying resistance against LPC and to evaluate whether adaptation to LPC affects S. aureus virulence.

**UP 8.**
**Standardization of *Saccharomyces cerevisiae* microplate reader covering parameters**
<u>Catherine E. Nickel</u>, K. O'Keefe, and M. Claire Edwards
St. Edward's University

Plate-reader-derived growth curves are commonly used in various fields of research, but researchers often fail to report critical experimental settings which can be associated with inconsistencies and reproducibility issues. To increase reproducibility in future experiments and generate a set of standard parameters for 96-well plates, the optimal plate covering conditions for generating yeast growth curves was determined. Yeast growth at 37C was measured at OD600 every 15 minutes for 24 hours using a BioTek Synergy H1 microplate reader. Four lid coverings were tested for evaporation under growth conditions previously stated with no shaking. Of the four lid coverings tested, the sticker and MicroClime Environmental lid coverings were the most effective at reducing evaporation. Not using a lid resulted in high levels of evaporation with nearly 100% evaporation seen on the bottom edge of the plate and using a standard plastic lid resulted in a prominent edge effect. The effects of evaporation on the growth of yeast were studied using the sticker and standard plastic lid. These results display that it is crucial to standardize procedures and methods to obtain consistent results for future research.

**UP 9.**
**Prevalence of antibiotic-resistant *Vibrio* strains in oysters harvested from Galveston Bay**
<u>Heather K. Nolte</u>, Hannah R. Vaughan, Jocelyn P. Mendoza, Michael G. LaMontagn

Food poisoning from the consumption of seafood contaminated with *Vibrio* species causes thousands of illnesses and dozens of deaths each year in the United States. *Vibrio* species are sensitive to temperature and salinity.  Consequently, as the temperature and salinity of coastal waters changes with climate the prevalence of *Vibrio* species could change. Additionally, antibiotic-resistant genes are spreading in estuaries and many antibiotic-resistant *Vibrio* species have been recently detected in shellfish worldwide. Galveston Bay is a major estuary with a thriving oyster fishery; however, little is known about the prevalence of these "superbugs" in Galveston Bay. To address this, fresh oysters were purchased from local markets in Galveston Bay over the course of a year.  Isolates were identified with a matrix-assisted – time of flight mass spectrometry system and a dozen representative isolates were screened for antibiotic sensitivity with a disc diffusion assay. *Vibrio* strains were isolated from all ten oysters sampled. *Vibrio* species identified included *V. brasiliensis, V. alginolyticus, V. vulnificus, V. parahaemolyticus, V. pectenicida, V. natriegens, V. pacinii, V. orientalis V. fluvialis, V. harveyi, V. diazotrophicus* and *V. anguillarum.* Several isolates (3/12) showed resistance to streptomycin. This suggests that antibiotic resistant *Vibrio* bacteria are common in oysters harvested from Galveston Bay.

**UP 10.**
**Developing a cellular aging model in yeast**
<u>Myka Mccraw</u>, **Madison Wolfrom**, and Todd P. Primm
Department of Biological Sciences, Sam Houston State University

In order to support extensive research on organism aging, we have a model system to look at aging at the cellular level, involving the tractable single-celled eukaryote Saccharomyces cerevisiae (budding yeast). One way that aging is monitored is loss of plating CFU over time. This method assumes that after growth, colonies represent individual cells. If yeast cells were clumping together or sticking to containers, then the CFU method will be inaccurate. Since some of our results were inconsistent, we needed to confirm that our plating protocol was accurate. Based on Thomas et al. 2015, we compared four diluents of YPD, sterile water, peptone-salt solution, and phosphate-buffer saline with Tween-80.

Serial dilutions at 10^-4, 10^-5, and 10^-6 were performed after one, two and three days of incubation from yeast in two aging models. Preliminary results suggest that there is no significant difference between the diluents in the CFU counts. In future work we plan to test other diluents, as well as compare a spot-plating method from the same publication with our current whole plate CFU method to potentially make our experiments more efficient.

**UP 11.**
**Prevalence of antibiotic resistant bacteria on microplastics in Galveston bay**
**Allison M. Wyrick**, Alex J. Estala, Michael G. LaMontagne
University of Houston – Clear Lake

Microplastics (μP) are ubiquitous in the marine environment. These particles include polymer beads used as feedstocks in manufacturing, fragments of consumer waste, and lost fishing gear. These pollutants are highly abundant in the Gulf of Mexico and estuaries connected to it and can serve as reservoirs for antibiotic resistant bacteria (ARB). This could accelerate the spread of antibiotic resistance genes in the bay; however, little is known about the microbes associated with μPs in Galveston Bay. Further, the heterogeneity of μPs makes monitoring the microbes associated with them challenging. To address this, we suspended an aquarium filter bag containing μPs off a pier in Galveston Bay. At harvest, bulk water was also collected to sample free-living bacteria. Libraries of readily-culturable, aerobic, heterotrophic bacteria were generated from these samples by cultivation on marine agar and identified with a Matrix Assisted Laser Desorption/Ionization – Time of Flight Mass Spectrometry system. Representative isolates were then screened for antibiotic resistance and susceptibility with a disc diffusion assay. All 37 bacteria isolated from μPs were identified as *Vibrio* species. Almost all of them (93%) showed resistance to at least one antibiotic and the majority (53%) were multi-drug resistant. Only a few bacteria were isolated from the bulk water. These isolates were not successfully identified with the MALDI-TOF system. This suggests that μPs in Galveston Bay could be a hot spot for ARB.

**PATHOGENIC MICROBIOLOGY - undergraduate students**
**Joan Abramowitz Award - Poster undergraduate student presentation**

**UP 12.**
**Withdrawn**

**UP 13.**
**Characterization of antibacterial mechanisms of zinc oxide in *Staphylococcus aureus***
**Alexander Caron[1]**, John Reeks[2], Dustin Johnson[1], Iman Ali[1], Michael Delgado[1], Shauna M. McGillivray[1]
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Rising precedence of antibiotic resistance has increased interest in nontraditional antibacterial agents such as zinc oxide nanoparticles (ZnO NPs). Although the anti-microbial activity of ZnO NPs is well established, the mechanism of this activity is unknown. Current literature hypothesizes that ZnO NP cytotoxicity could be mediated through one or multiple proposed mechanisms including production of reactive oxygen species (ROS), release of toxic $ZnO^{2+}$ ions, and charged interactions that disrupt the cell wall and cause osmotic stress. Literature also suggests bacteria may be unable to gain resistance to ZnO because antibacterial action occurs through multiple mechanisms. To illuminate the properties of ZnO and determine which of the proposed mechanisms occur, ZnO susceptibility was assessed in *Staphylococcus aureus*. To determine if bacteria gain resistance to ZnO, *S. aureus* was passed in ZnO at

sublethal doses. We find that *S. aureus* swiftly gain antibiotic resistance, suggesting ZnO antibacterial activity may operate through a single mechanism. To determine the predominant mechanism, susceptibility assays were performed in *S. aureus* mutants with deletions in the catalase gene, *katA*, which is important for defense against $H_2O_2$, and *mprf*, a gene important to cell membrane charge. We find that production of $H_2O_2$ and charged interactions with the cell envelope are not significant in ZnO susceptibility. Lastly, we find that media conditioned with ZnO effectively inhibits bacterial growth even in the absence of ZnO particles. We conclude that physical contact with *S. aureus* is not necessary for ZnO activity, although the precise mechanism by which bacterial growth is inhibited is not yet elucidated. Future work will investigate the role of other soluble species in the as well as continue to investigate how the type of media affects the conditioned media.

## UP 14.
### The role of glycolipids in *Streptococcus* sp. 1643

**Guan H. Chen**[1], Kelli L. Palmer[1], and Yahan Wei[1], and Ziqiang Guan[1]
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The emergence of superbugs spurs a necessity to explore novel mechanisms for controlling populations of pathogenic bacteria. This study aims to identify the functions of glycolipids in *Streptococcus* sp. 1643 (SM43), a mitis group *Streptococcus* isolated from an endocarditis patient. Glycolipids anchor lipoteichoic acid (LTA) to the cell membrane in addition to facilitating cell to cell interactions. LTA is a cell surface polymer that plays roles in regulation of cell division and cell shape among other physiological processes. In SM43, the glycolipid anchor is predicted to be synthesized through the functions of two glycosyltransferase genes, *cpoA* (FDR735_RS04120) and *cpoC* (FDR735_RS04125). To confirm their roles, these genes were deleted individually through homologous recombination with DNA fragments generated through SOEing PCR. Successfully generated mutants were confirmed through Sanger sequencing and lipidomic analyses, confirming the physiological roles of the genes. Compared to the wild-type strain, both mutants have significant growth deficiencies when cultured in Todd Hewitt broth, chemically defined medium, and human serum. Dramatic changes in cellular morphology are observed through bright-field confocal microscopy. Additionally, the mutants were found to have increased susceptibility to daptomycin and vancomycin through Etest strip analyses. This suggests that the glycolipids and/or properly anchored LTA have major roles in repelling antibiotics that target the cell envelope. In general, our study indicated that glycolipid anchors play critical roles for efficient growth and drug resistance. Better understanding of glycolipid anchors may yield answers in restoring and improving antibiotic potency.

## UP 15.
### Identifying novel mutants with increased susceptibility to hydrogen peroxide and reduced virulence in *Bacillus anthracis* tterne

**Luke Hamilton**, Victoria Adeleke, Zach Rouseau, Lauren Callaghan, Taylor Kelly, and Shauna M. McGillivray
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*Bacillus anthracis* is a gram-positive bacterial pathogen that causes the deadly infectious disease anthrax. *B. anthracis* contains two plasmids, pX01, and pX02. These plasmids were found to be necessary for the virulence of *B. anthracis.* However, *B. anthracis* contains over 5,000 chromosomal genes and we believe that there are additional virulence genes that have yet to be discovered. Our lab constructed a transposon mutant library with random disruptions in the *B. anthracis* Sterne genome to screen for novel virulence factors. This library has been successfully used to identify the chromosomal genes *clpX* and *yceGH* and show their importance for *B. anthracis* virulence. Using this library, we screened around 1,000 mutants using hydrogen peroxide, a reactive oxygen species involved in innate immune defence. We obtained

three mutants, 11F11, LV1 and LV2, that were susceptible to hydrogen peroxide *in vitro*. We then performed *Galleria mellonella* injection assays to observe their virulence in an invertebrate animal infection model. We observed reduced virulence in LV2 *in vivo* in a survival model of infection. We are currently investigating virulence using a competition model. We are also currently working to determine the transposon insertion site and discover which genes are disrupted. Once we have identified the interrupted genes, we will construct independent mutants using insertional mutagenesis to confirm the observed phenotypes. The findings of this research could be used as potential therapeutic drug targets and could offer insight into the mechanisms that *B. anthracis* uses for its pathogenesis.

**UP 16.**
**Role of <u>*Borrelia*</u> <u>su</u>gar phosphorylation <u>P</u>rotein (BsuP) in the patho-physiology of Lyme disease agent.**
**Alexis Ho[1]**, Jessica O'Berry[2], and J. Seshu[3]
[1]Department of Molecular Microbiology and Immunology, [2]South Texas Center for Emerging Infectious Diseases (STCEID), [3]The University of Texas at San Antonio, San Antonio, Texas

*Borrelia burgdorferi (Bb)*, the causative agent of Lyme disease, undergoes adaptive gene expression in response to environmental signals during different stages of its life cycle in ticks and vertebrate hosts. Gene regulation is mediated by proteins that transduce signals from the environment to cytosol of *Bb* and influence levels of gene expression critical for pathogen colonization in different hosts. After blood meal ingestion by ticks feeding on vertebrate hosts, *Bb* is exposed to nutrients such as sugars, fatty acids, and peptides. The increase in growth rate of *Bb* after ingestion that influence gene expression in response to substrate level phosphorylation are not well defined. We hypothesize that histidine-containing phosphocarrier protein designated as <u>B</u>orrelia <u>su</u>gar phosphorylation <u>P</u>rotein (BsuP) is essential for survival of *Bb* in ticks and vertebrate hosts in phosphorylation of sugar substrates during translocation across the cell membrane. The presence of a conserved histidine at position 15 ($H_{15}$) from the N-termimus could serve as an acceptor of phosphoryl group from Enzyme 1 provides significant bioinformatic basis to determine the role of BsuP in the transfer of phosphoryl group to sugar substrates. To test this, we have overexpressed and purified recombinant BsuP in *E. coli* and in the process of generating antisera to determine the growth conditions of *Bb* that results in maximal levels of BsuP. Mutants lacking *bsuP* or with replacement of $His_{15}$ with alanine will allow us to determine the physiological role of BsuP and open a novel regulatory mechanism impacting the virulence potential of Lyme disease agent*.*

**UP 17.**
**Bacteriophage-containing biodegradable microsphere technology to treat osteomyelitis**
**Jerril Jacob[1]**; Vishal Ubha[2]; Heidi B. Kaplan[1]; Catherine G. Ambrose[1]
[1] McGovern Medical School, University of Texas Health Science Center at Houston, TX, [2] Texas Tech University at Lubbock, TX

The increasing incidence of antimicrobial resistant (AMR) infections is a growing concern within the healthcare and scientific communities. One novel approach to address AMR osteomyelitis, bone infections, is to use bacteriophage (phage) to target and kill the bacterial pathogen. We have previously demonstrated that biodegradable microsphere technology can be used to encapsulate bacteriophages. Here, we investigated the effect of three parameters on the entrapment and elution of phage in these poly(lactic-co-glycolic acid) (PLGA) microspheres: the effect of varying PLGA polymer formulation, the effect of shelf life, and the effect of sterilization. The microspheres were manufactured according to a water-oil-water protocol that incorporates $3 \times 10^9$ *S. aureus*-specific phage K into a 1-ml solution of PLGA (250 mg) with varying PLGA/PEG (polyethylene glycol) compositions. The shelf life of phage-containing microspheres after 2 or 4 weeks of storage at 4°C or 25°C and the effect of ethylene oxide (EtO) sterilization were also investigated by measuring elution before and after storage or treatment. All three

factors were evaluated using an elution assay. The eluent was collected after 24hr, 72hr, and 7 days. The microspheres made with 10% PEG eluted significantly more phage than spheres without PEG (p<0.001). The shelf life experiment showed that immediate elution was significantly higher than 2 weeks (p=0.018) and 4 weeks (p=0.01); but temperature had no significant effect. All EtO sterilized samples eluted phage at below the limit of detection (P=0.039). As a result, further studies are required to investigate how to manufacture microspheres sterilely.

## UP 18.
### Establishing an invertebrate infection model for *Staphylococcus hemolyticus*
**Rebecca McGehee**, Davida Smyth, and Chris Mares.
Texas A&M University at San Antonio

The goal of this project was to establish an invertebrate infection model for Staphylococcus hemolyticus infections using the wax moth larva (Galleria mellonella). The first portion of this project was to establish growth patterns of S. hemolyticus, this was done by establishing a 24-hour growth curve. We next started to grow, harvest, and collect highly concentrated quantities of S. hemolyticus in order to obtain high inoculums that would be used for injection into the wax worm larva. The high dose inoculums were then prepared, and larvae were ordered. We have successfully established a high dose infection model in wax worm larvae. We are still working toward establishing other parts of the model. For example, we would like to include a mid-range dose to see if we can generate an intermediate survival curve. We are also currently trying to establish our own colony of wax worm larvae so we can grow and maintain our own stock as needed for future experiments. This is important to help us control the life cycle stage at which we can infect larvae as this could have a major impact on the outcome of infection. From the several rounds of wax moth larva we have infected with S. hemolyticus we observed a consistent immune response. When infected the larva become melanated, with the melanization rate matching the potency of the given S. hemolyticus dosage. This unique and easily observable immune response show potential for future projects and could be used as a module in a course.

## UP 19.
### Transcriptional regulation of lipoproteins Lpp1 and Lpp2 in the nosocomial pathogen, *Acinetobacter baumannii*
**Christina Nguyen**, Joseph M. Boll
The University of Texas at Arlington

The lipid A precursor of LPS/LOS was canonically thought to be essential for Gram-negative survival. However, *Acinetobacter baumannii* inactivates lipid A biosynthesis to gain resistance to the last line antimicrobial, colistin. We do not understand how *A. baumannii* survives without LOS (LOS$^-$) to develop colistin resistance but found that lipoproteins are enriched in the LOS$^-$ outer membrane of LOS$^-$ cells. In Escherichia coli, physical tethering the OM to peptidoglycan via the lipoprotein, Lpp, provide mechanical stability to the cell envelope when LOS is not produced. We found that two putative Lpp proteins are present in *A. baumannii*. Lpp1 is expressed in both growth and stationary phase, while Lpp2 is only expressed in stationary phase , suggesting separate roles in OM assembly. Furthermore, Lpp2 appears to be express in stress. Here, we have created a screen to identify regulatory mechanisms that control Lpp1 and Lpp2 transcription during growth and stationary phase. Together, our studies can show that in response to OM defects, *A. baumannii* lipoproteins increase cell envelope stability.

## UP 20.
### Exploring host-pathogen interactions in the liquid killing assay
**Nikita Singh**, Donghoon Kang, Lois Armendariz, Natalia V. Kirienko

Department of BioSciences, Rice University, Houston, Texas, United States

The Gram-negative pathogen *Pseudomonas aeruginosa*, with its propensity to cause severe disease within immunocompromised patients and its ever-expanding range of resistance to drugs, is a critical target of study in today's medicinal world. Previously, the Kirienko lab identified the siderophore pyoverdine as a major virulence factor during *P. aeruginosa* pathogenesis against *Caenorhabditis elegans* in liquid (termed Liquid Killing, LK). Pyoverdine translocation into the host disrupts iron homeostasis, resulting in mitochondrial damage. In addition to pyoverdine, *P. aeruginosa* secretes other unknown virulence factors that also directly impact host survival. Similarly, the full extent of host defense activation in *C. elegans* during LK is undetermined, with research pointing to lipid metabolism being involved in host defense. To identify relevant virulence and host defense genes for LK, I examined a collection of deletion mutants for the *P. aeruginosa* reference strain PA14, along with several gene knockdowns of *C. elegans* lipid metabolism genes. We observed that *pqsA*, involved with quorum sensing, and *pilY1*, which is responsible for type IV pili biogenesis, were required for *P. aeruginosa* virulence. In *C. elegans*, several lipid metabolism genes appeared to play a role in survival during LK. Based on these findings, additional PA14 deletion mutants can be tested to elucidate the mechanism of *pqsA* and *pilY1*-dependent virulence against *C. elegans*. Additionally, lipid metabolism genes shown to be significant in LK survival will be tested for activation of mitochondrial surveillance pathways.

## UP 21.
### Genotypic and phenotypic association of antibiotic resistance in *Pseudomonas aeruginosa*
__Justin Wright__ and Ali Azghani
University of Texas at Tyler

*Pseudomonas aeruginosa* is a Gram negative, opportunistic pathogen capable of causing a number of acute illnesses. *P. aeruginosa* readily evolves resistance to virtually all antibiotic classes in a multifactorial nature that makes this pathogen difficult to treat. The relationships between different levels of expression for resistance genes and the effect on the resistance phenotype of a given organism is poorly understood, hence the focus of this research.

We hypothesized that trade-offs in resistance arising from regulation and overexpression of one or two resistance genes exist which might cause under expression of other resistance genes. This may lead to relative susceptibilities to other antibiotics and we intend to elucidate these relationships.

We used clinical samples of *P. aeruginosa*, basic bacterial culture protocols, RNA extraction, and RT-qPCR for six chromosomally expressed genes, ampC, mexR, mexT, mexZ, nfxB, and oprD. ANOVA and Levene's post hoc testing indicated general expression trends and antibiotic efficacy in this population.

ANOVA analysis indicated that among fourteen strains tested Tobramycin was the most effective drug treatment while Aztreonam was the least effective choice. Among resistance genes tested, ampC was overexpressed in all strains and showed the highest average overexpression. Conversely MexR, an upstream repressor for the mexAB-oprM efflux pump, showed no overexpression in any strain.

We are in the process of analyzing more samples and collecting quantitative minimum inhibitory concentration data for assessing phenotype-genotype association in an effort to gain insight into the molecular mechanisms of multi drug resistance.

## POSTDOCTORAL FELLOW POSTERS
### Samuel Kaplan Award - Poster graduate student presentation

## PDP 1.
### The role and dynamics of ethanolamine-utilizing bacterial microcompartments

**Ayan Chatterjee[1]**, Karan Gautam Kaval[2] and Danielle A Garsin[1]
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Bacterial pathogens face many challenges within the host environment including nutrient starvation. Therefore, finding alternative nutrient and fuel sources is of key importance. Ethanolamine (EA) is a potential source of carbon, nitrogen and/or energy in host environments for many bacteria including species of *Enterococcus*, *Escherichia*, *Clostridium*, *Listeria*, *Klebsiella*, and *Salmonella*. In common, these bacteria have a *eut* (ethanolamine utilization) locus that consists of the genes required for EA metabolism. Catabolism of EA takes place inside special microcompartments known as bacterial microcompartments (BMC), which are viral capsid like proteinaceous icosahedral structures. In this study using *Listeria monocytogenes* as a model organism, the importance and dynamics of BMC formation was examined with the hypothesis that it is a nutrient source that contributes to intracellular replication.

A nitrogen free minimal media (ACMM) was optimised for this study. *L. monocytogenes* was able to grow on a nitrogen-free minimal media (ACMM) only when EA or glutamine was added as a nitrogen source. On the other hand, *eutV* and *eutB* knockout strains were not able to utilize EA as a nitrogen source and did not grow. The addition of glutamine as a nitrogen source rescued the growth defect in the knockout strains. qRT PCR analysis showed that the *eut* genes were upregulated upon addition of EA to the ACMM media. A recombinant *L. monocytogenes* was generated that expressed the BMC structural gene *eutK* as a mNeongreen (mNG) fusion protein. Confocal imaging of the recombinant bacteria expressing *eutK*-mNG showed the formation of BMC-like structures in presence of EA, which was greatly reduced in the *eutV* knockout strain. The *eutV* and *eutB* knockout strains were defective in intracellular replication in BMDM. The study confirms the hypothesis that in the model organism *L. monocytogenes*, EA can be used as a nutrient source, specifically of nitrogen, and that this metabolism contributes to optimal intracellular replication. Additionally, it provides preliminary evidence that the Eut BMCs of *L. monocytogenes* can be fluorescently tagged, providing a tool for future studies in host cells.


**PDP 2.**
**CDC-48 influences SKN-1 activity in response to pathogen infection**
**Carolaing Gabaldón** and Danielle A. Garsin
Department of Microbiology and Molecular Genetics, The University of Texas Health Science Center at Houston, Texas

In Caenorhabditis elegans, bacterial infections can lead to an imbalance in the amount of ROS produced by the cell, causing oxidative damage to a variety of molecules (DNA, RNA, lipids and proteins). Attempts to counteract the damage occur by transcriptional activation of detoxification programs in response to high levels of oxidative stress. In our lab, we observe the effects of infection on the host by exposing C. elegans to the human pathogens Enterococcus faecalis and/or Pseudomonas aeruginosa, which are ingested and colonize the lumen of the intestine. The infection triggers the expression of the transcription factor SKN-1, a protein that is activated by ROS and is involved in the activation of detoxification genes such as gst-4 (glutathione Stransferase 4) and gcs-1 (glutamate-cysteine ligase), which encode proteins that promote the survival of the animal.

An RNAi screen looking for genes whose loss prevented SKN-1 activation on pathogen discovered cdc-48. CDC-48 is involved in targeting ubiquitinated substrates for proteolysis and is thought to help maintain cellular proteostasis. Specifically, loss of cdc-48 by RNAi failed to cause the activation of SKN-1 reporter genes following infection with E. faecalis or P. aeruginosa. Congruently, the levels of SKN-1 in the nucleus were observed to be significantly decreased. Finally, the absence of cdc-48 during infection renders C. elegans significantly more susceptible to the pathogen.

My current focus is to understand the mechanism by which CDC-48 influences SKN-1 activity, and this is an active area of ongoing investigation. In conclusion, CDC-48 affects the activation and nuclear localization of SKN-1 to affect survival on human pathogens such as E. faecalis and P. aeruginosa.

**PDP 3.**

**Development of novel antifungals against *Candida b*ased on an antifungal peptide produced by *E. faecalis***

**Shantanu Guha**[1], Melissa R. Cruz[1], Giuseppe Buda De Cesare[1], Shane Christy[1], Michael C. Lorenz[1], Danielle A. Garsin[1]
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Fungal resistance to commonly used medicines is a growing public health threat. The most common cause of dangerous, bloodstream, fungal infections are from *Candida* species, and there are emergent strains of *Candida* resistant to all current antifungals. To increase the probability of successfully treating *Candida* infections, novel antifungals must be developed. The basis of our project in developing a novel antifungal agent is a secreted bacterial peptide, EntV, which is produced by *Enterococcus faecalis* and restricts *C. albicans* to a non-virulent form*.* By targeting virulence rather than viability, the chances of developing resistance to EntV may be less than traditional antifungals. Our investigation aims to identify the minimal structural features necessary for EntV activity, generate a combinatorial peptide library using the truncated peptide as a template, conduct high-throughput screening to determine gain-of-function peptide variants, and test EntV and its variants in preclinical models to determine its effectiveness and potential usage. We hypothesize that by rationally varying specific residues in combination, we will generate more potent antifungal peptides than the template sequence through synthetic molecular evolution. Thus far, we have used *C. albicans* to screen the novel antifungal peptides that generated from the library, and we have identified five gain-of-function mutant peptides which are now being more stringently tested against several fungal species, both *in vitro* and *in vivo*. We expect that our discoveries will contribute to the development of novel antifungals in the fight against antimicrobial resistant fungi.

**PDP 4.**

**Cellular and transcriptional signatures of innate immune response following *Borrelia burgdorferi* infection of murine splenocytes unveiled by single cell RNA-Seq (scRNA-Seq)**

**Venkatesh Kumaresan**, Nathan Kilgore, Taylor MacMackin-Ingle and Janakiram Seshu
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Lyme disease is the most common tick-borne infectious disease in the US caused by a spirochetal pathogen *Borrelia burgdorferi* (*Bb*). *Bb* establishes *a long-lasting avirulent infection in reservoir hosts such as rodents facilitating its transmission to uninfected hosts including humans via Ixodes scapularis ticks. Despite the established roles of immune response on the survival of spirochetes in the rodent hosts, the cellular and molecular background of the early immunological response against Bb infection remains unknown. We used single-cell RNA-seq to unveil the cellular heterogeneity and molecular signatures of the splenic immune cells,* the largest secondary lymphoid organ comprising majority of the innate and adaptive immune cells that primarily respond to pathogens. Single cell cluster analysis decoded the heterogeneity of neutrophils, macrophages, B cells and T cells in the splenocytes. Comparison of cellular subsets between *Bb* infected and bystander cells revealed that *Bb* infected neutrophils are enriched with apoptosis-related genes and demonstrated the activation of caspase3-mediated apoptosis in neutrophils during *Bb* infection. In addition, we identified a cluster of pro-inflammatory macrophages in *Bb* infected sample enriched with complement mediated phagocytosis related genes, c1q and Ficolin and

demonstrated their role in uptake of *Bb* infected neutrophils. Further, we identified that the *Bb* infected neutrophils and macrophages exhibit unique molecular pattern involving upregulation of Cxcl1/Cxcl2/Il1b axis. The findings of this study provide novel information on the immunological response of neutrophils and macrophages against *Bb* infection in their natural reservoir host.

**PDP 5.**
**Toxic mechanisms of STM3845 in *Salmonella Typhimurium***
**Joana Rocha[1]**, Haley Boyd[2], Aravindh Nagarajan[1], Helene Andrews-Polymenis[1]
[1] Texas A&M University, School of Medicine, [2] Texas A&M University, School of Veterinary Medicine and Biomedical Sciences

Bacterial retrons are used in molecular engineering to encode reverse transcriptases that produce high quantities of multi-copy single-stranded DNA (msDNA). In *Salmonella* Typhimurium (STm), retron SEN2 is critical for growth at ambient temperature, in anaerobic conditions, and for gut colonization during STm infection. Furthermore, retron SEN-2 constitutes a toxin-anti-toxin system in which an intervening ORF, STM3845, acts as a toxin when not bound to its cognate reverse transcriptase and/or msDNA. Thus, the reverse transcriptase and msDNA together form the antitoxin unit for the STM3845 toxin. To understand how STM3845 acts as a toxin, we attempted to identify STM3845 binding partners during STm growth without oxygen and at ambient temperature. We cloned STM3845 under the control of an IPTG-inducible promoter bearing a 3xFlag epitope tag. The plasmid bearing this construct was transformed into STm deleted for the SEN2 retron, and into *E. coli*. Strains containing this plasmid were grown in anaerobic and ambient temperature conditions, and production of STM3845-3xflag was induced with IPTG. Co-immunoprecipitation was used to capture STM3845-3xflag and associated proteins. The resulting captured material was analyzed using mass spectrometry and analyses are underway to identify proteins targeted by toxin STM3845. These protein-protein interactions will help us better understand the mechanisms of how retron SEN-2 regulates STm growth in low temperature and anaerobic conditions.

## STAFF POSTERS

**SP 1.**
**Uncovering the mechanism behind metronidazole inactivation in *Enterococcus faecalis* and its role in protecting metronidazole-susceptible bacteria**
**Muqaddas Amer[1]**, Jacob Rappazzo[1], Kezia Phillip[1], Michael Neugent[1], Jordan Owen[2], Ziqiang Guan[2], Julian Hurdle[3], and Kelli Palmer[1]
[1]University of Texas at Dallas, [2]Duke University, [3]Texas A&M Institute of Biosciences and Technology

The nitroimidazole antibiotic metronidazole is used for the treatment of obligate anaerobic bacterial infections. *Enterococcus faecalis* is a Gram-positive commensal bacterium and opportunistic pathogen that typically resides in the gastrointestinal tract and is intrinsically resistant to metronidazole. We have determined that *E. faecalis* and some other enterococcal species inactivate metronidazole. While the main goal of this study is to elucidate the molecular mechanism by which *E. faecalis* and other enterococci can inactivate metronidazole, we also aim to verify if *E. faecalis* can contribute to the proliferation of metronidazole-susceptible bacteria by protecting them from metronidazole. By screening of transposon mutants, we found that the Extracellular Electron Transfer (EET) pathway is required for metronidazole inactivation by *E. faecalis*. We further developed an efficient anaerobic co-culture assay to provide us with reliable and valid assessments of if *E. faecalis* can protect metronidazole-susceptible bacteria, in this case *Escherichia coli*, *in vitro*. The results from our assay confirm that *E. faecalis* can protect *E. coli* from metronidazole *in vitro*, whereas transposon mutants with insertions in certain EET genes, as well as some other enterococci, i.e. *E. faecium* and *E. dispar*, have a diminished ability or are unable to protect. Our

results suggest that *E. faecalis* in the human intestine may protect pathogens from killing by metronidazole.

## SP 2.
### Putrescine as a requirement for pili-mediated surface motility in *Escherichia Coli*
<u>Sydney Hall</u>[1], Jacob Hogins[1], Larry Reitzer[1]
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Urinary tract infections, one of the most common types of bacterial infections in women in the US, are frequently caused by *Escherichia coli*. As an established virulence factor in such infections, type I fimbriae/pili promote pathogenicity by mediating adhesion to urothelial cells lining the inner surface of the bladder (1). Our lab also found that pili can contribute to surface motility in commensal *E. coli*. Pili-mediated surface motility of W3110 (a laboratory strain of *E. coli*) is abolished when *speB*, the primary biosynthetic gene for the polyamine putrescine, is removed. A transcriptomic analysis of this mutant showed that loss of the gene did not affect the transcription of other *spe* genes, other motility genes, or any transcriptional regulator.

Because polyamines often affect translation, we assessed whether putrescine controlled the translation of *fimA* which codes for the major pilus subunit. We constructed a strain lacking *speB* and *fliC* and contained a plasmid with a *fimA*-GFP translational fusion. In this strain, movement absolutely requires putrescine and pili. Motility assays, enzyme linked immunosorbent assays (ELISAs)—to measure FimA protein, and electron microscopy showed that 1 mM putrescine is optimal for pili production and motility, but higher concentrations are inhibitory. These results show complex putrescine-dependent, pili-mediated motility by translational control of at least one gene required for pili synthesis. We propose that putrescine affects translation of key motility components in other organisms with putrescine-dependent motility.

## SP 3.
### LD-Transpeptidase regulatory elements promote the viability of lipooligosaccharide deficient *Acinetobacter baumannii*
<u>Deborah Omoregie</u>, Hannah Bovermann, Joseph Boll
The University of Texas at Arlington

*Acinetobacter baumannii* is an emerging nosocomial pathogen with a high propensity to develop resistance to commonly prescribed antibiotics. Carbapenem resistant *A. baumannii* is a high-level threat to public health as reported by the Center for Disease Control (CDC). Due to the increasing threat, Polymyxin E (colistin) is a last-resort prescription to treat carbapenem resistant *A. baumannii* infections; however, the continuous use of colistin has led to its resistance. Colistin resistance in *A. baumannii* is mediated by inactivation of lipooligosaccharide (LOS) biosynthesis. This is unexpected because LOS was canonically thought to be essential for Gram-negative viability yet, the underlying molecular mechanisms that promote *A. baumannii* survival without LOS are not well understood. Previous work showed that two putative LD-transpeptidases (LDTs), denoted as LdtJ and LdtK, are essential for survival without LOS. Here, we engineered *ldtJ-lacZ* and *ldtK-lacZ* transcriptional reporter fusions to measure gene expression. A blue/white screen was utilized to discover transcriptional regulatory elements with the use of a transposon mutant library. Whereas each putative LDT was expressed in wild type, which formed blue colonies, several white colonies were isolated. The transposon junctions in the white colonies were sequenced and putative regulatory elements were found. Next, we plan to use mutagenesis to characterize the regulatory elements to understand how they contribute to antibiotic resistance in a variety of environmental conditions. These studies could show new targets to improve antibiotic treatment against *A. baumannii*.

# FACULTY POSTERS

## FP 1.
## Linking community structure to ecosystem functioning – specific plankton and interactions are good predictors of carbon export at the Western Antarctic Peninsula

### Yajuan Lin[1]
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The Southern Ocean plays an important role in global oceanic productivity and carbon cycling. Within the Southern Ocean, the Western Antarctic Peninsula (WAP) is a region of particular interest due to rapid changes associated with climate change, including significant loss of sea-ice, warming and freshening of the surface ocean, as well as shifting of the marine ecosystem and food web. As part of the Palmer Long Term Ecological Research program (PAL-LTER), we have collected five years of WAP data on 1) microbial community structure (18S and 16S rDNA metabarcoding) and 2) high-resolution net community production (NCP, $O_2$/Ar based), as a proxy for surface ocean carbon export. Our results showed that the community structure, interactions and NCP were strongly influenced by sea-ice extent. Using genetic programming, a machine-learning approach, we constructed statistic models to predict NCP, with explanation power up to 80% of the NCP variability. Among the top performing models, an ice-associated plankton assemblage emerged as the best biological predictor for NCP. This assemblage was mainly dominated by a diverse array of polar diatoms and dinoflagellates, with central taxa identified as *Thalassiosira, Odontella, Porosira, Actinocyclus, Proboscia, Chaetoceros* and *Gyrodinium*. Our interdisciplinary approach reveals that the coastal Antarctic system is highly sensitive to climate change in the aspects of microbial community structure and biological carbon drawdown.

**FP 2.**

**The fitness effects of antimicrobial resistance mutations in *E. coli* are modulated by strong genotype by environment interactions**

Aaron Hinz[1,2], André Amado[3,4], Claudia Bank[3,4], Rees Kassen[2], and **Alex Wong[1,5]**

[1]Carleton University, [2]University of Ottawa, [3]Instituto Gulbenkian de Ciência, [4]Universitat Bern, [5]Texas A&M AgriLife

Antimicrobial resistance poses a serious challenge to health care worldwide. Attempts to control resistance by stopping antimicrobial use have met with mixed success. Failures of a critical assumption underlying such strategies – that resistant strains suffer a disadvantage in the absence of drug (the "cost of resistance") – may be responsible for difficulties in controlling resistance by cessation of drug use. In particular, resistance mutations may be cost free, and hence persist, in some environments or on some genetic backgrounds. We investigated the contributions of genetics and environment to variation in the costs of antimicrobial resistance in *Escherichia coli*. We found that the costs of antimicrobial resistance vary substantially in different growth conditions, on different genetic backgrounds, and for different resistance mutations. Attempts to predictively model the fitness consequences of AMR mutations largely failed, owing to these strong effects of environment and genetic background. The unpredictability of the costs of resistance implies that the outcomes of restriction protocols will be difficult to foresee.